Journal of Natural Products
Vol. 53, No. 3, pp. 603-608, May-Jun 1990

BIOACTIVE SESQUITERPENE POLYOL ESTERS FROM *EUONYMUS BUNGEANUS*

Y.Q. TU,*

Department of Chemistry, Lanzhou University, People's Republic of China

D.G. WU, J. ZHOU,

Kunming Institute of Botany, the Academy of Science of China

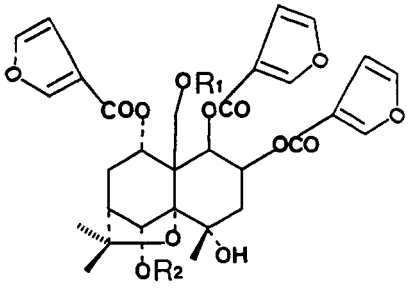
Y.Z. CHEN, and X.F. PAN

Department of Chemistry, Lanzhou University, People's Republic of China

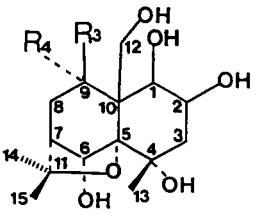
ABSTRACT.—Three new sesquiterpene polyol esters were isolated from the seed oil of *Euonymus bungeanus*. Their structures were elucidated on the basis of chemical reactions and spectral analysis as 6α,12-diacetoxy-1β, 2β, 9α-tri(β-furancarbonyloxy)-4α-hydroxy-β-dihydroagarofuran [**1**], 6α,12-diacetoxy-1β, 9α-di(β-furancarbonyloxy)-4α-hydroxy-2β-2-methylbutanoyloxy-β-dihydroagarofuran [**2**], and 6α, 12-diacetoxy-2β, 9α-di(β-furancarbonyloxy)-4α-hydroxy-1β-2-methylbutanoyloxy-β-dihydroagarofuran [**3**].

Some species of plants of the Celastraceae, especially the powdered root bark of Chinese bittersweet, *Celastrus angulatus* Max., were traditionally used in China to protect plants from insect damage (1); the chemistry of this family of plants has been reviewed (2). Insecticidal alkaloids with a β-dihydroagarofuran skeleton, such as wilfordine and wilforine from *Tripterygium wilfordii* Hook (3) and an insect antifeedant alkaloid from *Maytenus rigida* (4), have been isolated. However, Wakabayashi *et al.* (1) reported that a non-alkaloid sesquiterpene polyol ester, celangulin, also exhibits insect antifeedant activity. In this paper, the isolation and structure elucidation of three new sesquiterpene polyol esters **1**, **2**, and **3** from the seed oil of *Euonymus bungeanus* Max. are presented. Although the use of *E. bungeanus* as a natural insecticide has not been reported, preliminary insecticidal tests of compounds **1**, **2**, and **3** against the insects *Pieris rapae* and *Ostrina furnacolis* indicated that compounds **1** and **3** exhibited insect antifeedant effect against *P. rapae* and compounds **2** and **3** exhibited an insecticidal effect against *O. furnacolis*.

Compound **1** analyzed for $C_{34}H_{36}O_{15}$ by hrms. Its ir revealed the characteristic absorption of an ester group at 1730 cm^{-1}. In good agreement with that, its eims and nmr suggested the presence of two acetate esters [ms *m/z* 43 (Ac); ^{13}C nmr δ 21.3 and 21.5 (2×Me), δ 170.3 and 170.9 (2×-COO-); ^{1}H nmr δ 2.14 and 2.33 (2×s, 2×3H)] and three β-furancarboxylate esters [ms *m/z* 95 (furancarbonyl); ^{13}C nmr δ



1 $R_1=R_2=Ac$
4 $R_1=Ac$, $R_2=H$
5 $R_1=R_2=H$



6 $R_3=H$, $R_4=OH$
7 $R_3=OH$, $R_4=H$

109.3, 109.5, and 109.9 (3 × CH), δ 118.4, 118.6, and 118.9 (3 quaternary carbons), δ 143.6, 143.7, and 143.8 (3 × CH), δ 147.7, 148.0, and 148.6 (3 × CH), δ 161.5, 161.6, and 161.7 (3 × -COO-); ^{1}H nmr δ 6.3–6.8, 7.2–7.4, and 7.6–8.2 (3 × m, 3 × 3H)].

Full hydrolysis of compound **1** with NaOMe/MeOH gave a parent alcohol **6** (5), which was identified with 1,2,4,6,9,12-hexahydroxy-β-dihydroagarofuran by a comparison of its ^{13}C-nmr chemical shifts (Table 1) with those of a known polyol **7** (6). The molecular composition of **1** showed a remaining free hydroxy whose presence was confirmed by its characteristic ir absorption at 3550 cm^{-1}. This free hydroxy was placed at C-4 because the 4-OH is not esterified in all other compounds of this class (2). Therefore, five esters were located at C-1, C-2, C-6, C-9, and C-12 of 4α-hydroxy-β-dihydroagarofuran, respectively.

Generally, H-1 and H-6 had an unexceptionally axial configuration (2). The configuration of H-2 could not be deduced from the coupling constant $J_{1,2}$ of compound **1** itself, due to the presence of overlapping multiplets of H-1 and H-2 in the ^{1}H nmr. However, the ^{1}H nmr of **5**, derived from **1**, exhibited a doublet ($J_{1,2}$ = 3.5 Hz) of H-1, suggesting the configuration of an equatorial H-2 (1,2). Furthermore, H-9 was assigned an equatorial configuration on the basis of the presence of one doublet ($J_{8,9}$ = 7.2 Hz) of H-9 (2,7). Inspection of a molecular model showed that the double-faced angle between Heq-8 and Heq-9 was near 90°.

The location of five esters in **1** was based on partial hydrolysis (8). Compound **1**, when subjected to hydrolysis with Et_2NH/MeOH at 5°, gave a major product **4**, whose ^{1}H nmr (Table 2) showed one fewer acetate ester than that of **1**, and showed an upfield chemical shift of H-6 to 4.85 ppm as compared with **1** (6.19 ppm). Thus, one acetate ester in **1** was determined to be at C-6. Further hydrolysis of compound **4** with Et_2NH/MeOH at room temperature produced a major product **5**, whose ^{1}H nmr exhibited one fewer acetate esters than that of **4**, and exhibited upfield chemical shifts (4.43 and 4.33) as compared with **4** (5.15 and 4.55). Therefore, the second acetate ester in **1** was placed at C-12. Based on the evidence above, compound **1** was elucidated as 6α,12-diacetoxy-1β,2β,9α-tri(β-furancarbonyloxy)-4α-hydroxy-β-dihydroagarofuran.

TABLE 1. ^{13}C nmr (400 MHz) Data in $CDCl_3$.

Carbon	Compound						
	1	2	3	4	5	6[b]	7[b]
C-1[a]	68.6	68.1	68.4	68.1	68.6	69.0	69.3
C-2[a]	68.8	68.6	69.1	68.7	69.2	71.3	71.6
C-6[a]	70.6	70.7	69.6	70.5	71.6	73.4	72.9
C-9[a]	78.1	78.3	78.0	78.8	78.7	79.6	80.1
C-3	42.0	42.3	41.9	41.0	41.1	44.8	43.8
C-4	69.6	69.7	69.7	72.1	72.2	73.6	73.9
C-5	91.2	91.2	91.2	91.0	91.4	92.2	92.4
C-7	49.2	49.2	49.3	50.2	50.2	51.7	52.0
C-8	34.8	34.8	34.6	34.6	34.2	34.8	35.2
C-10	55.1	55.2	54.9	53.8	55.5	55.6	56.2
C-11	84.6	84.5	84.6	84.9	84.5	83.5	84.4
C-12	66.1	65.9	66.1	66.0	63.5	66.0	66.1
C-13[a]	25.3	25.1	25.3	24.9	25.1	27.3	27.3
C-14[a]	25.6	25.6	25.6	26.4	26.4	27.7	27.3
C-15[a]	29.4	29.4	29.4	30.0	30.0	30.4	30.2

[a]The data were not assigned.

[b]The data were obtained on 100 MHz spectrometer [7] in C_5H_5N.

TABLE 2. ^{1}H nmr (400 MHz) Data in $CDCl_3$.[a]

Proton	Compound					
	1[c]	**2**	**3**[c]	**4**[c]	**5**	**6**
H-1	5.78	5.71 d (3.2)	5.69	5.80	5.76 d (3.5)	5.07 d (3)
H-2	5.78	5.66 m	5.69	5.80	5.80 m	4.92 m
H-3	2.1–2.3	2.0–2.3	2.1–2.4	2.1–2.4	2.1–2.3	2.3–2.6
H-4	—	—	—	—	—	—
H-6	6.19 s	6.19 s	6.19 s	4.85 d (5.4)	5.19 s	5.23 s
H-7	2.6 m	2.6 m	2.6 m	2.1–2.4	2.5 m	2.3–2.6
H-8	2.1–2.3	2.0–2.3	2.1–2.4	2.1–2.4	2.1–2.3	2.3–2.6
H-9	5.42 d (7.2)	5.34 d (7.2)	5.32 d (6.9)	5.35 d (7.2)	5.60 d (7.2)	4.65 m
H-12	5.32 4.49 AB (13)	5.06 4.53 AB (13)	5.26 4.45 AB (13)	5.15 4.55 AB (13)	4.43 4.33 AB (11.8)	5.22 4.50 AB (11)
H-13[b]	1.52 s	1.51 s	1.51 s	1.57 s	1.57 s	1.78 s
H-14[b]	1.56 s	1.54 s	1.53 s	1.63 s	1.62 s	2.02 s
H-15[b]	1.60 s	1.58 s	1.60 s	1.80 s	1.86 s	2.34 s

[a]Coupling constants in parentheses.
[b]The data were not assigned.
[c]H-1 and H-2 showed overlapping multiplets.

Compound **2** was very similar to compound **3**. Their ^{1}H-nmr and mass spectra indicated that they both contained the same ester substituents, namely two acetates, two β-furancarboxylates, and one 2-methylbutanoate (2). Full hydrolysis of these two compounds with NaOMe/MeOH afforded their corresponding parent alcohols, which were both identified as **6** on the basis of tlc. Characteristic absorptions at 3560 cm^{-1} in the ir spectrum of **2** and 3530 cm^{-1} in that of **3** indicated the presence of free hydroxy groups in both compounds. As with compound **1**, the free hydroxy group in **2** and **3** was placed at C-4. Therefore, both compound **2** and **3** possessed five esters located at C-1, C-2, C-6, C-9, and C-12. However, compound **2** was not the same as **3** because they had different R_f values on tlc.

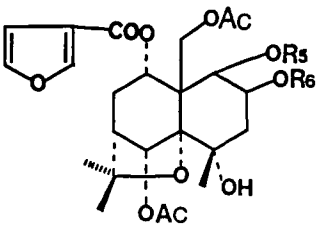
A careful comparison of mass and ^{1}H-nmr spectra of **2** and **3** with those of **1** showed that these three compounds all exhibited the same intense fragment ion at *m/z* 192, which analyzed for $C_{11}H_{12}O_3$ by hrms and was indicative of substitution by a 9-furancarbonyloxy group. In addition, all three compounds gave the same ^{1}H-nmr chemical shift at 6.19 ppm for H-6, suggesting that compounds **2** and **3** also had a 6-acetoxy substituent. Therefore, either compound **2** or **3** had the three remaining ester groups (one acetate ester, one β-furancarboxylate ester, and one 2-methylbutanoate ester) located at C-1, C-2, and C-12, respectively.

In this class of compounds, if an acetate ester and an aromatic acid ester are placed at C-1 and C-9 (or at C-9 and C-1), respectively, the acetate methyl group exhibits a higher field ^{1}H-nmr chemical shift than normal (1). However, it is possible that not only acetate esters but other aliphatic acid esters, such as 2-methylbutanoate esters, could give a similar result. Generally, the normal ^{1}H-nmr chemical shift for acetate esters is 1.9–2.7 (1), while the normal ^{1}H-nmr chemical shifts for 2-methylbutanoate esters are 0.8–0.9 (t, 7 Hz, Me), 1.0–1.3 (d, 7 Hz, Me), 1.3–1.5 (m, CH_2), and 2.3–2.5 (m, CH) (1,2).

Compound **2** had normal ^{1}H-nmr δ values of 2.13 and 2.30 for two acetate esters and normal ^{1}H-nmr δ values, 0.82 (t, 7.3 Hz, Me), 1.14 (d, 7.2 Hz, Me), 1.50 (m, CH_2), and 2.40 (m, CH), for a 2-methylbutanoate ester. Thus the ester group at C-1 was not an acetate ester nor a 2-methylbutanoate ester but a β-furancarboxylate ester. Furthermore, the intensive McLafferty rearrangement fragment *m/z* 512 (15%)

$[M-60-102]^+$ suggested that the ester group at C-12 was not a 2-methylbutanoate but an acetate because of the unfavorable McLafferty rearrangement of 2-methylbutanoate ester at C-12. As a result, compound **2** was elucidated as 6α,12-diacetoxy-1β, 9α-di(β-furancarbonyloxy)-4α-hydroxy-2β-2-methylbutanoyloxy-β-dihydroagarofuran.

Compound **3** gave normal ^{1}H-nmr δ values of 2.13 and 2.32 for two acetate esters, but the high field ^{1}H-nmr δ values, 0.61 (t, 7.4 Hz, Me), 0.84 (d, 6.8 Hz, Me), 1.19 (m, CH_2), and 2.00 (m, CH), for the 2-methylbutanoate ester, suggesting that the 2-methylbutanoate group in **3** was placed at C-1 and two esters (one acetate and one β-furancarboxylate) were placed at C-2 and C-12. Further investigation of the eims of **2** and **3** showed that compound **2** gave an abundant rearrangement ion at *m/z* 512 (15%) $[M-60-102]^+$ and a weak rearrangement ion at *m/z* 502 (<1%) $[M-60-112]^+$. Conversely, compound **3** gave the weak rearrangement ion at *m/z* 512 (<1%) $[M-60-102]^+$ and the abundant rearrangement ion at *m/z* 502 (15%) $[M-60-112]^+$. These facts indicated that the 2-methylbutanoate ester in **2** and the unplaced β-furancarboxylate ester in **3** were esterified on the C-2 carbon at which the McLafferty rearrangement of the ester group was favorable. Compound **3** was thus elucidated as 6α,12-diacetoxy-2β,9α-di(β-furancarbonyloxy)-4α-hydroxy-1β-2-methylbutanoyloxy-β-dihydroagarofuran.



2 R_5= CO  , R_6= COCHC$_2$H$_5$ (CH$_3$)

3 R_5= COCHC$_2$H$_5$ (CH$_3$) , R_6= CO 

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting point was determined on a Kofler apparatus. ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Bruker AM-400 nmr spectrometer with TMS as internal standard. Uv spectra in EtOH were obtained on a UV-210A spectrophotometer. Eims were obtained on VG ZAB-HS mass spectrometer operating at 70 eV ionizing energy. Ir spectra were determined on a Perkin-Elmer 577 instrument. Cc was carried out on Si gel (200–300 mesh) column with petroleum ether/EtOAc as eluent. Hplc was carried out on a Merck RP-8 column with MeOH/H_2O as eluent. Preparative tlc was carried out on Merck Si gel 60 F254 plates with C_6H_6/EtOAc as eluent. Detection of components was with a uv lamp. *E. bungeanus* was collected in Yunnan Province, China, and authenticated by the faculty of Department of Systematic Botany. Kunming Institute of Botany, the Academy of Science of China. Voucher specimens have been deposited at the Botanical Garden of Kunming Institute of Botany (Academy of Science of China). The bioactive test for compounds **1**, **2**, and **3** was performed with the larvae of *P. rapae* and *O. furnacolis*; Me_2CO was used for the control group.

BIOACTIVE EXPERIMENTS.—Leaves were macerated with Me_2CO solution of test sample (200 ppm) for 1 min. After the leaves were dried in air, the larvae of *P. rapae* were fed with them for 48 h. The leaf area which was eaten out, the weight of the larvae, and the number of dead larvae were recorded, and the results were calculated. Antifeedant rates (%) were 42 [**1**], 31 [**2**], and 67 [**3**]. Corrected death rates (%) were 0 [**1**], 0 [**2**], and 22 [**3**].

The Me_2CO solution of test sample (500 ppm) and the larvae of *P. rapae* which had been starved for 3 h were used for insecticidal test in the same method as above. Antifeedant rates (%) were 22 [**1**], 28 [**2**], and 33 [**3**]. Death rates (%) were 12.5 [**1**], 25 [**2**], and 75 [**3**]. Corrected death rates (%) were 0 [**1**], 14 [**2**], and 71 [**3**].

The Me_2CO solution of test sample (300 ppm) and the larvae of *O. furnacolis* were used for an insecticidal test by the same method as above. Corrected death rates (%) were 12.5 [**1**], 50.0 [**2**], and 70 [**3**].

EXTRACTION AND ISOLATION.—Dried and pulverized seeds (3 kg) were extracted with petroleum ether (5 liters) at room temperature for 6 days. Removal of solvent under reduced pressure left a reddish-brown oil (533 g). After extraction of oil with MeOH-H_2O (90:10) (3 × 500 ml) and concentration of the MeOH/H_2O solution under reduced pressure, a crude extract (110 g) was obtained as reddish-brown semisolid. A portion (20 g) of the crude extract was chromatographed on a Si gel column (200–300 mesh, 400 g) with petroleum ether-EtOAc (80:20, 70:30, 20:80) as eluent to give 86 fractions, which were combined on detection of tlc to give 11 groups of combined fractions. The largest amount of three groups was subjected to reversed-phase hplc on a Merck RP-8 column with MeOH-H_2O (80:20) as eluent to give compounds **1** (380 mg), **2** (185 mg), and **3** (113 mg).

COMPOUND **1**.—Compound **1** was obtained as a white amorphous powder: $[\alpha]^{14}D$ 83.94 ($c = 0.554$, $CHCl_3$); uv λ max nm (log ε) 241 (1.945), 201.5 (2.318); ir ν cm^{-1} (KBr) 3550, 2960 (br s), 1730, 1570, 1500, 1395, 1365; eims *m/z* $[M]^+$ 684, $[M-15]^+$ 669, $[M-112]^+$ 572, $[M-60-112]^+$ 512, 192, 95 (100%), 43; hrms *m/z* 684.2008 (calcd for $C_{34}H_{36}O_{15}$, 684.2043), 192.0755 (calcd for $C_{11}H_{12}O_3$, 192.0783); ^{13}C nmr see Table 1; ^1H nmr see Table 2.

COMPOUND **2**.—Compound **2** was obtained as colorless crystals: mp 145–146° from petroleum ether/EtOAc; $[\alpha]^{14}D$ 43.07 ($c = 0.534$, $CHCl_3$); uv λ max nm (log ε) 240 (1.729), 201 (2.202); ir ν cm^{-1} (KBr) 3560, 2980, 2940, 1735, 1575, 1505, 1376, 1365; eims *m/z* $[M]^+$ 674, $[M-15]^+$ 659, $[M-42]^+$ 632, $[M-60-102]^+$ 512, $[M-60-112]^+$ 502, 192, 95 (100%), 85, 43; hrms *m/z* 674.2551 (calcd for $C_{34}H_{42}O_{14}$, 674.2562), 192.0754 (calcd for $C_{11}H_{12}O_3$, 192.0783); ^{13}C nmr see Table 1; ^1H nmr see Table 2.

COMPOUND **3**.—Compound **3** was obtained as a white amorphous powder: $[\alpha]^{14}D$ 70.85 ($c = 0.494$, $CHCl_3$); uv λ max nm (log ε) 240 (1.916), 201 (2.296); ir ν cm^{-1} (KBr) 3530, 2980, 2940, 1740, 1720, 1570, 1500, 1380, 1360; eims *m/z* $[M-15]^+$ 659, $[M-15-42]^+$ 617, $[M-60-102]^+$ 512, $[M-60-112]^+$ 502, 192, 95 (100%), 85, 43; hrms *m/z* 659.2313 (calcd for $C_{33}H_{39}O_{14}$, 659.2328), 192.0770 (calcd for $C_{11}H_{12}O_3$, 192.0783); ^{13}C nmr see Table 1; ^1H nmr see Table 2.

COMPOUND **4**.—A solution of 100 mg of compound **1** and MeOH (2 ml) was mixed with a solution of 2.4 ml MeOH and 0.04 ml Et_2NH at 5° and then set aside at 5° for 2 days. Concentration of the reaction mixture under reduced pressure left a light yellow solid, which was subjected to preparative tlc on a Si gel plate with C_6H_6-EtOAc (80:20) as eluent to give the major partial hydrolysis product **4** (56 mg, 60%) as a white amorphous powder. The ^{13}C- and ^1H-nmr values are given in Tables 1 and 2, respectively.

COMPOUND **5**.—A solution of 50 mg of compound **4** and 1 ml MeOH was mixed with a solution of 1.2 ml MeOH and 0.02 ml Et_2NH and then set aside at room temperature for 2.5 days. Removal of solvent under reduced pressure left a light yellow solid, which was purified on a preparative Si gel plate with C_6H_6-EtOAc (80:20) as eluent to give the major partial hydrolysis product **5** (32 mg, 68%) as a white amorphous powder. ^{13}C- and ^1H-nmr values are given in Tables 1 and 2, respectively.

COMPOUND **6**.—Compound **1** (98 mg) was dissolved in 4 ml NaOMe/MeOH solution (0.1 M) and then set aside overnight at room temperature. Concentration of the reaction solution under reduced pressure afforded a light yellow solid, which was subjected to preparative tlc on a Si gel plate with $CHCl_3$-MeOH (90:10) as eluent to give the full hydrolysis product **6** (42 mg, 92%) as a white amorphous powder. ^{13}C- and ^1H-nmr values are given in Tables 1 and 2, respectively.

ACKNOWLEDGMENTS

We thank all of the members of instrument group of Kunming Institute of Botany, The Academy of Science of China, for helpful measurement of nmr, uv, ir, and optical rotation data, and all of the members of ms group of Analysis and Measurement Center of Lanzhou University, for helpful measurement of ms data. We also thank Prof. Huan Duanping, Department of Plant Protection, The South Agriculture University of China, Guanzhou, for helpful tests of biological activity.

LITERATURE CITED

1. N. Wakabayashi, W.J. Wu, R.M. Waters, R.E. Redfern, G.D. Mills Jr., A.B. DeMilo, W.R. Lusby, and D. Andrzejewski, *J. Nat. Prod.*, **51**, 537 (1988).

2. R. Brüning and H. Wagner, *Phytochemistry*, **17**, 1821 (1978).
3. K. Yamada, Y. Shizuri, and Y. Hirata, *Tetrahedron*, **34**, 1915 (1978).
4. F. Delle Monache, G.B. Marini Bettolo, and E.A. Bernays, *Z. Angew. Entomol.*, **97**, 406 (1984).
5. S.M. Kupchan, R.M. Smith, and R.F. Bryan, *J. Am. Chem. Soc.*, **92**, 6667 (1970).
6. H. Budzikiewicz and A. Römer, *Tetrahedron*, **31**, 1761 (1975).
7. G. Baudouin, F. Tillequin, and M. Koch, *Heterocycles*, **22**, 2221 (1984).
8. R.L. Baxter, L. Crombie, D.J. Simmonds, and D.A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 2972 (1979).

Received 2 October 1989