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A new limonoid from the fruits of *Evodia rutaecarpa* (Juss.) Benth.

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Four limonoids have been isolated from the unripe fruits of *Evodia rutaecarpa* (Juss.) Benth., one of which is a new compound (**1**), identified as 23-oxo-21ξ-hydroxy-21,23-dihydroevodol (named evodirutaenin), the known compounds were identified as evodol (**2**), limonin (**3**) and shihulimonin A (**4**), on the basis of the analysis of ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, IR and MS spectral data. Shihulimonin A of known structure was also isolated from the unripe fruits of *Evodia rutaecarpa* for the first time.

1. Introduction

The dried unripe fruits of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae), popularly known in China as Wu-zhu-yu, has been prescribed for the treatment of gastrointestinal disorders, headache, abdominal pain, dysentery and postpartum haemorrhage (Kiangsu Institute of Morden Medicine 1977). It contains alkaloids and limonoids as its main components (Sugimoto et al. 1988a, 1988b; Shoji et al. 1988; Tang et al. 1996; Zhang et al. 1999; Zuo et al. 2003; Wang et al. 2004). The present paper describes the isolation and structural elucidation of a new limonoid named evodirutaenin.

2. Investigations, results and discussion

The 95% aqueous ethanolic extract of the unripe fruits of *E. rutaecarpa* was acidified by HCl after suspended in water and then extracted with cyclohexane and EtOAc to obtain cyclohexane extract, EtOAc extract and residual water layer. The EtOAc extract was applied on silica gel column chromatography and then purified further to give compounds **1**–**4**. Compounds **2**–**4** were identified as evodol (**2**), limonin (**3**) (Sugimoto et al. 1988a 1988b), which had previously been isolated from the unripe fruits of *E. rutaecarpa*, and shihulimonin **A** (= limonexic acid) (**4**), which had previously been isolated from the *Valencia* orange seeds (Emerson 1948), *Citrus* seeds (Dreyer 1965), bark of *Phellodendron amurense* (Kondo et al. 1985) and *Evodia rutaecarpa* (Juss.) Benth. var. *bodinieri* (Dode) Huang (Gai et al. 2001). Compounds were identified by comparison of their spectral data with those reported in literature.

Compound **1** was isolated as an amorphous solid. Its molecular formula was deduced to be $C_{26}H_{28}O_{11}$; the ESI-TOF-MS measured with negative ion mode gave a parent ion peak at m/z 515.1357 $[M-H]^-$ ($C_{26}H_{27}O_{11}$ requires 515.1553) and HR-SI-MS measured with positive ion mode gave a parent ion peak at m/z 517.1703 ($[M+H]^+$ calc. for $C_{26}H_{29}O_{11}$ requires 517.1704), and was consistent with NMR data. It had 13 degrees of unsaturation. The IR spectrum displayed characteristic absorption for hydroxyl groups (3424 cm^{-1}), a lactone carbonyl (1758 cm^{-1}) and a ketone carbonyl (1687 cm^{-1}) band. Its ^{1}H NMR (Table 1) spectrum showed four singlet groups [δ 1.09 (3 H, s), 1.20 (3 H, s), 1.22 (3 H, s), 1.76 (3 H, s)], four methines attached to the oxygen function [δ 4.34 (1 H, br s), 4.59 (1 H, s), 5.82 (1 H, br s), 6.54 (1 H, br s)]. The ^{13}C NMR spectrum (Table 2) of **1** contained resonance signals corresponding to four carbonyl carbons [δ 196.2, 170.1, 170.0, 166.2], two double bonds [δ 164.4, 142.5, 141.9, 121.7], one acetal carbon signal [δ 99.2], and three oxygenated methine carbon signals [δ 52.6, 78.7, 80.0]. Based on the above observations and comparison of ^{1}H and ^{13}C NMR spectral data (Table 1 and 2) with those of compounds **2**–**4**, **1** was identified as a limonoid-like compound. These spectra also suggested the presence of a trisubstituted expoxide (^{1}H NMR: δ 4.59; ^{13}C NMR: δ 66.1, 52.6). Limonoids isolated from Rutaceae commonly have a ketone oxygen at C_7 and a δ-lactone in ring D (Dreyer 1983). The ^{1}H and ^{13}C NMR spectral features of **1** closely resembled those of evodol (**2**) except for the presence of the signals due to an α-hydroxy dihydrofuran lactone moiety [^{1}H NMR: δ 6.54 (1 H, br s, H-21), 6.58 (1 H, br s, H-22), 9.57 (1 H, br s, C_{21}-OH); ^{13}C NMR: δ 164.4 (C-20), 99.2 (C-21), 121.7 (C-22), 170.0 (C-23)] and those of shihulimonin A (**4**) except for the signals due to a 6-hydroxy-5,6-dehydro-substituent moiety [^{1}H NMR: δ 6.38 (1 H, br s, C_6-OH); ^{13}C NMR: δ 141.9 (C-5), 142.5 (C-6)]. A comparison of the ^{1}H and ^{13}C NMR spectral data of **1** and **4** showed the same chemical shift values of an α-hydroxy dihydrofuran lactone group, with signal splitting showing that the hydroxyl was existing as a mixture of both epimers. The major epimer showed two furan protons resonating at δ 6.54 (1 H, br s, H-21), 6.58 (1 H, br s, H-22) and bonded to carbons resonating at δ 99.2 (C-21), 121.7 (C-22) which the latter being typical for an acetal, respectively, from the HMQC and HMBC experiments, and reported data (Nakagawa et al. 2001; Wattanapiromsakul et al. 2003). The stereochemistry of α-hydroxy dihydrofuran lactone group in **1** was obtained from an analysis of NMR signals and nuclear Overhauser effect (NOE) experiment (Fig.), which is similar to those of **4**. The carbon C-20 resonated at δ

Table 1: ^{1}H NMR data of compounds 1–4 (δ_H, 499.89 MHz; in C_5D_5N)

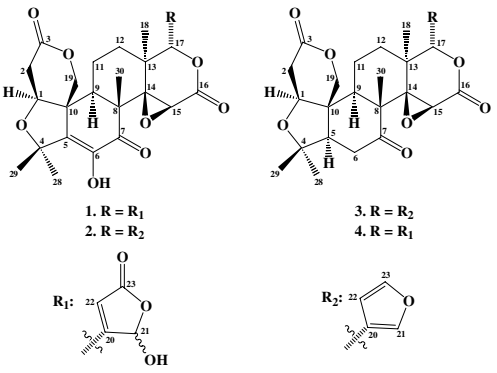
H	1	2	3	4
1	4.34 (1 H, br s)	4.38 (1 H, t, 2.5)	4.29 (1 H, d, 3.5)	4.33 (1 H, br s)
2	2.97 (2 H, dd, 2.5, 12.5)	2.92 (2 H, dd, 2.5, 12.5)	3.05 (1 H, d,16.5)	3.04 (1 H, d, 16.5)
			3.19 (1 H, d, 16.5)	3.18 (1 H, dd, 16.5)
5			2.63 (1 H, dd, 15.0, 3.0)	2.65 (1 H, d, 15.0)
6			2.54 (1 H, t,15.0)	2.54 (1 H, d,15.0)
			3.24 (1 H, dd, 15.0, 3.5)	3.29 (1 H, t, 15.0),
9	3.14 (1 H, dd, 3.0, 13.0)	3.19 (1 H, 3.0, 13.0)	2.78 (1 H, dd, 10.0, 2.5)	2.83 (1 H, d, 11.5)
11	1.95 (1 H, m)	1.89 (1 H, m)	1.82 (1 H, m)	1.98 (1 H, m)
	1.87 (1 H, m)	1.83 (1 H, m)	1.98 (1 H, m)	2.03 (1 H, m)
12	2.05 (1 H, m)	1.90 (1 H, m)	1.83 (1 H, m)	1.86 (1 H, m)
	1.78 (1 H, m)	1.79 (1 H, m)	2.00 (1 H, m)	2.26 (1 H, m)
15	4.59 (1 H, s)	4.60 (1 H, s)	4.63 (1 H, s)	4.48 (1 H, s)
17	5.82/5.74 (1 H, br s)	5.68 (1 H, s)	5.73 (1 H, s)	5.81/5.73 (1 H, br s)
18	1.09 (3 H, s)	1.24 (3 H, s)	1.26 (3 H, s)	1.36 (3 H, s)
19	4.79 (1 H, d, 13.0)	4.81 (1 H, d, 12.5)	4.69 (1 H, d, 13.0)	4.69 (1 H, d, 13.0)
	4.83 (1 H, d, 13.0)	4.88 (1 H, d, 12.5)	5.21 (1 H, d, 13.0)	5.25 (1 H, d, 13.0)
21	6.54/6.69 (1 H, br s)	7.72 (1 H, d, 1.0)	7.71 (1 H, d, 1.0)	6.50/6.71 (1 H, br s)
22	6.58/6.60 (1 H, br s)	6.52 (1 H, dd, 1.0, 2.0)	6.52 (1 H, dd,1.5, 1.0)	6.59/6.61 (1 H, br s)
23		7.62 (1 H, dd, 1.0, 2.0)	7.63 (1 H, dd,1.5, 1.0)	
28	1.76 (3 H, s)	1.78 (3 H, s)	1.18 (3 H, s)	1.15 (3 H, s)
29	1.22 (3 H, s)	1.21 (3 H, s)	1.24 (3 H, s)	1.23 (3 H, s)
30	1.20 (3 H, s)	1.24 (3 H, s)	1.26 (3 H, s)	1.24 (3 H, s)

Table 2: ^{13}C NMR data of compounds 1–4 (δ_C, 125.71 MHz; in C_5D_5N)

C	1	2	3	4
1	80.0	80.1	79.8	79.7
2	35.8	35.9	36.9	36.4
3	170.1	170.1	170.2	170.1
4	82.3	82.3	80.4	80.3
5	141.9	141.9	60.6	60.3
6	142.5	142.3	36.5	36.4
7	196.2	196.4	207.8	207.7
8	48.4	48.3	51.5	52.0
9	47.2	47.3	48.1	48.2
10	48.1	47.9	46.3	46.4
11	20.2	20.1	18.7	18.8
12	31.0	31.2	30.1	30.4
13	38.1	38.3	38.5	38.7
14	66.1	66.5	67.1	66.4
15	52.6	52.4	54.8	54.0
16	166.2	167.3	167.6	166.4
17	78.7/78.8	78.0	78.2	78.5/78.8
18	20.6	20.3	20.2	20.7
19	69.9	70.1	65.7	65.8
20	164.4/161.3	120.9	121.1	164.5/161.3
21	99.2/99.7	141.9	142.0	99.3/99.7
22	121.7/123.0	110.7	110.6	121.7/123.0
23	170.0/169.7	143.8	143.7	170.1/169.8
28	25.9	25.9	29.9	29.7
29	25.6	25.6	21.7	21.6
30	17.9	18.2	17.6	16.9

164.4, which, because it is highly deshielded, must be β to the lactone carbonyl requiring that the lactone carbonyl and acetal carbons be assigned to C-23 (δ 170.0) and C-21 (δ 99.2), respectively. This stereochemistry is also corroborated by the NOE correlation between H-22 at δ 6.58 and H-18 at δ 1.09, H-12α at δ 1.78 and H-18 at δ 1.09, H-12β at δ 2.05 and H-17 at δ 5.82. Thus the configuration of the α-hydroxy dihydrofuran lactone group at C-17 is presumed to be α, as in the case in all limonoids of the Rutaceae (Dreyer 1983; Nakagawa et al. 2001). Compound **1** can thus be defined as 23-oxo-21ξ-hydroxy-21,23-dihydroevodol. It is a novel compound which was named evodirutaenin.

Until now, all limonoids of the Rutaeceae, Meliaceae and Cneoranceae are with α-configuration of the furan at C-17 (Dreyer 1983; Wattanapiromsakul et al. 2003). The novel compound is not an exceptional case. *Evodia* species are good natural sources of limonoids, and most of the limonoids from *Evodia* genus are sharing the characteristics of occulting as β-substituted furan rings, as in **2** and **3** (Sugimoto et al. 1988a, 1988b). Limonoids with oxidized furan rings are relatively rare in *Evodia* genus.



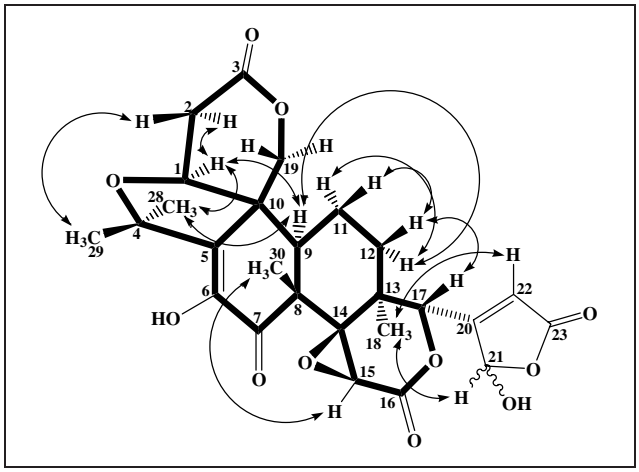


Fig.: The key NOESY correlations of compound **1**

3. Experimental

3.1. General

CC: silica gel (Tsingtao Marine Chemistry Co. Ltd, 200–300 mesh), the eluents were cyclohexane – EtOAc (2 : 1 → 1 : 1), EtOA, EtOAc – MeOH (3 : 1 → 1 : 1), $CHCl_3$-MeOH (150 : 1 → 8 : 1), and MeOH. IR: KBr disc. ESI-TOF-MS and FT-ICR-HR-MS were performed on MDS SCIEX API QSTAR and APEX FT-ICR (Bruker Daltonics) mass spectrometer, respectively. NMR spectra were performed on a Varian INOVA 500 spectrometer. All the NMR experiments were recorded at room temperature, operating at 499.89 MHz for ^1H and 125.71 MHz for ^{13}C with TMS as int. standard in C_5D_5N.

3.2. Plant material

The dried unripe fruits of *Evodia rutaecarpa* (Juss.) Benth. were obtained in Xiangtan City, Hunan Province of CHINA, in December 2000, and authenticated by Professor Xui-wei Yang. The voucher specimen of this plant is deposited in the Herbarium of School of Pharmaceutical Sciences, Peking University.

3.3. Extraction and isolation

The dried and powdered fruits of *Evodia rutaecarpa* (11 kg) were extracted with 95% EtOH (4.4 L) under reflux for 1 h × 4 to give ethanolic extracts 2.3 kg. The ethanolic extract (1.5 kg) was further suspended in water (1.5 L) and acidified then by HCl to pH 3, and extracted successively with cyclohexane (3.0 L × 4 times) and EtOAc (3.0 L × 4 times) to obtain cyclohexane extracts (150 g), EtOAc extracts (150 g) and residual water layer, respectively. The EtOAc extract was applied on silica gel column chromatography eluted with cyclohexane – EtOAc (2 : 1, 1 : 1 and 0 : 1, 300 mL each) and EtOAc – MeOH (3 : 1, 1 : 1, and 0 : 1, 300 mL each) to eight major fractions (Fr.1–Fr.8). Fr.4 (20.0 g) was subjected to silica gel column chromatography using a cyclohexane – EtOAc gradient system (3 : 1, 2 : 1, and 1 : 1, 800 mL each) following MeOH to give seven fractions (Fr. 4-I–Fr. 4-VII). Fr. 4-V (4.6 g) was further chromatographed over a silica gel column using $CHCl_3$-MeOH gradient system (150 : 1, 99 : 1, 9 : 1, 7 : 3 and 3 : 2, 50 mL each) to give 10 fractions (Fr. 4-V-1 – Fr. 4-V-10). Fr. 4-V-4 (1.5 g) was applied to a silica gel column eluted with $CHCl_3$-MeOH (99 : 1, 95 : 5, 9 : 1, 8 : 1 and 0 : 1, 100 mL each) to yield compounds **1** (8 mg) and **2** (12 mg). Compound **3** (120 mg) and **4** (80 mg) were deposited from Fr. 4-V-10 (0.5 g) and Fr. 4-VII using MeOH, respectively.

3.4. 23-Oxo-21ξ-hydroxy-21,23- dihydroevodol (evodirutaenin, 1)

White amorphous solid; IR ν_{max} (KBr) cm^{-1}: 3424, 2973, 2934, 1758, 1687, 1620, 1359, 1304, 1286, 1130, 1051, 1036, 988, 899, 600, 536; UV λ_{max} (MeOH) nm: 200, 249, 277. ESI-TOF-MS (negative) m/z 515.1357 $[M-H]^-$ ($C_{26}H_{27}O_{11}$ requires 515.1553). HR-SI-MS (positive) m/z 517.1703 $[M+H]^+$ ($C_{26}H_{29}O_{11}$ requires 517.1704). ^1H and ^{13}C NMR data see Tables 1 and 2.

3.5. Evodol (2)

White amorphous solid; IR ν_{max} (KBr) cm^{-1}: 3428, 2930, 2874, 1745, 1687, 1657, 1537, 1450, 1287, 1167, 1125, 1031, 956, 914, 876, 754, 602; UV λ_{max} (MeOH) nm: 200, 276. ESI-TOF-MS (negative) m/z 483.1361 $[M-H]^-$ ($C_{26}H_{27}O_9$ requires 483.1655). ^1H and ^{13}C NMR data (Tables 1 and 2) were in agreement with the data reported for evodol (Sugimoto et al. 1988a).

3.6. Limonin (3)

Colorless needles (CH_2Cl_2-isopropanol), m.p. 197–198 °C; IRν_{max} (KBr) cm^{-1}: 3488, 2967, 2939, 1757, 1708, 1285, 1165, 891, 875, 798, 600; UV λ_{max} (MeOH) nm: 202. ESI-TOF-MS (negative) m/z 469.1854 $[M-H]^-$ ($C_{26}H_{29}O_8$ requires 469.1862). ^1H and ^{13}C NMR data (Tables 1 and 2) were in agreement with the reported data for limonin (Sugimoto et al. 1988a).

3.7. Shihulimonin A (4)

White amorphous solid; IR ν_{max} (KBr) cm^{-1}: 3560, 3435, 2927, 1749, 1453, 1415, 1366, 1286, 1165, 1130, 1042, 1019, 964, 894, 815, 699, 598; UV λ_{max} (MeOH) nm: 206. HR-SI-MS (negative) m/z 501.1767 $[M-H]^-$ ($C_{26}H_{29}O_{10}$ requires 501.1766). ^1H and ^{13}C NMR data (Tables 1 and 2) were in agreement with the data reported for shihulimonin A (Gai et al. 2001).

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