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Two new non-steroidal constituents from *Dioscorea futschauensis* R. Kunth

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Dioscorone A (**1**) and a new isocoumarin derivative (**2**) were isolated from the rhizome of *Dioscorea futschauensis* R. Kunth. The structures of **1** and **2** were elucidated on the basis of detailed analysis of NMR spectra. Their anti-fungal activity against *Pyricularia oryzae* and cytotoxic activity on K562 and HCT-15 cell lines were evaluated *in vitro*.

1. Introduction

The rhizome of *Dioscorea futschauensis* R. Kunth (Dioscoreaceae) is used as the traditional Chinese medicine "Mian Bi Xie" for the treatment of rheumatism, and urinary tract disease. It is widely distributed in southeast China and is included in the Pharmacopoeia of the People's Republic of China (2000). In our on-going program of screening the bioactive agents from Traditional Chinese Medicine [1, 2], the ethanol extract of this plant showed a strong activity against the growth of *Pyricularia oryzae* P-2b, which stimulated us to investigate its chemical constituents. In a previous paper, we have reported four active steroidal saponins isolated from this plant [3]. Here, we wish to report the isolation and structure elucidation of two non-steroidal constituents, dioscorone A (**1**) and a new isocoumarin derivative (**2**), from the rhizomes of this plant.

2. Investigations, results and discussion

Dioscorone A (**1**) was obtained as an amorphous powder and was found to be a racemate by rotation measurement. The molecular formula was determined to be $C_{16}H_{12}O_6$ by high-resolution mass spectroscopic measurement. The UV spectrum of **1** showed absorptions at 203, 267, 308 nm (log ε 4.00, 3.65, 3.09). In its IR spectrum, absorption bands attributable to hydroxyl (3421 cm^{-1}) and carbonyl (1744 cm^{-1}) groups were observed. The presence of a lactone group in structure was also ascertained by its EIMS (m/z 257 $[M + H\text{-}44]^+$) spectrum. The ^1H NMR spectrum of **1** showed two methoxy signals at δ 3.91(CH_3-7) and δ 4.12 (CH_3 -3), two coupled aromatic protons at δ 7.74 (d, J = 8.7 Hz, H-9) and δ 7.87 (d, J = 8.7 Hz, H-10), two meta-coupled aromatic protons appearing at δ 6.87 (d, J = 2.1 Hz, H-6) and δ 7.35 (d, J = 2.1 Hz, H-8), a hydroxyl proton signal at δ 8.10 (d, J = 6.0 Hz, disappeared on deuterium exchange) and a oxymethine proton signal at δ 6.00 (d, J = 6.0 Hz) that indicated the existence of an −OCH(OH)-fraction in the structure. The ^{13}C NMR and DEPT spectra of **1** revealed 16 carbon signals including twelve aromatic carbon signals (8s + 4d), one carbonyl signal (δ 165.9), one oxymethine carbon signal (δ 94.4) and two methoxy carbon signals. The data above suggested that the structure of **1** was very similar to phenanthropyran derivatives [4, 5].

Combining data from HMQC and HMBC experiments, all the proton and carbon signals were carefully assigned. The sequential correlations of HMBC were successfully established (Fig.). To solve the relative stereochemistry, a NEOSY study was carried out. The NOE effect observed between the oxymethine proton (δ 6.00) and the methoxy proton (δ 4.12, CH_3-3) revealed that the oxymethine proton was equatorially oriented. The hydroxyl group takes an axial orientation, far away from the aromatic methoxy group at C-3, avoiding the severe steric interaction. From the foregoing evidence, the complete structure of compound **1** was established, and it was named as dioscorone A (**1**). Dioscorone A has a unique structure, introducing a lactone group into the 1 and 2 position of a phenanthropyran skeleton. It is the first example of this kind of compound.

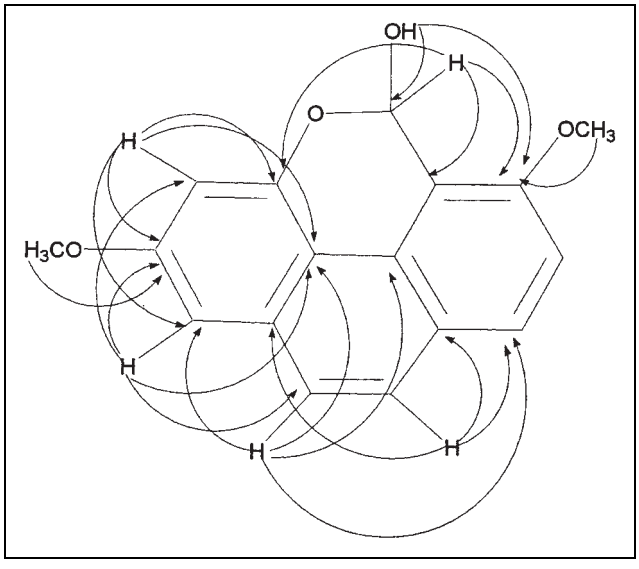
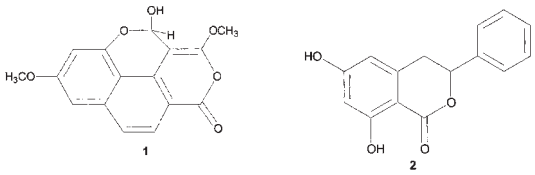


Fig.: HMBC correlation observed for compound **1**

Table: ^{13}C NMR (75 MHz) and ^{1}H NMR (300 MHz) data for 1 and 2[a]

Position	1		Position	2	
	δ_H	δ_C		δ_H	δ_C
1		165.9	1		169.3
2			2		
3		142.9	3	5.65, (dd, 3.3 11.4)	79.5
4		129.7	4	3.07 (dd, 3.3, 16.4)	34.1
4a		114.5		3.22 (dd, 11.4, 16.4)	
4b		115.8	4a		141.8
5		151.2	5	6.22 (d, 1.8)	107.8
6	6.87 (d, 2.1[b])	110.6	6		167.0
7		160.9	7	6.13 (d, 1.8)	101.3
8	7.35 (d, 2.1)	103.8	8		163.6
8a		138.2	8a		98.9
9	7.87 (d, 8.7)	127.9	1′		138.8
10	7.74 (d, 8.7)	120.9	2′	7.50 (m)	126.5
10a		136.5	3′	7.40 (m)	128.6
3-OCH_3	4.12	55.8	4′	7.40 (m)	128.6
7-OCH_3	3.91	60.1	5′	7.40 (m)	128.6
−O−CH(OH)−	6.00 (d, 6.0) 8.01(d, 6.0)	94.4	6′	7.50 (m)	126.5

[a] All of the signals were assigned by HMQC and HMBC spectral analysis (**1**, DMSO-d_6; **2**, methanol-d_4)
[b] J values (in parentheses) are reported in Hz



Compound **2** was obtained as colorless needles from methanol and it was also found to be a racemate by rotation measurement. Its molecular formular $C_{15}H_{10}O_4$ was determined from high-resolution mass spectroscopic measurements and NMR data. The IR spectrum of **2** showed absorption bands due to phenolic hydroxyl, a chelated δ-lactone, and an aromatic ring. The UV spectrum of **2** showed absorptions at 220, 276, and 300 nm (log ε 3.76, 4.05, 4.30). The ^{1}H NMR and ^{13}C NMR (Table) spectra of **2**, which were completely assigned on the basis of 2D NMR experiments, showed signals attributable to a monosubstituted bezene ring [7.40 (3 H, m), 7.50 (2 H, m)], a tetrasubstituted benzene ring [6.13 (d, J = 1.8 Hz), 6.22 (d, J = 1.8 Hz)], and a chelated δ-lactone [5.65, (dd, J = 3.3 11.4 Hz), 3.07 (dd, J = 3.3, 16.4 Hz), 3.22 (dd, J = 11.4, 16.4 Hz)]. On the basis of those findings and comparison of the ^{1}H and ^{13}C NMR data with that of thunberginol C [6], the structure of **2** was determined to be 3-phenyl-6,8-dihydroxydihydroisocoumarin.

Compounds **1** and **2** did not show any activity against the phytopathogenic fungus *Pyricularia oryzae.* In an *in vitro* cytotoxicity assay against cultured K562 (leukemia) and HCT-15 (colon) cell lines, compound **1** exhibited cytotoxicity on the HCT-15 cell line with an IC_{50} value of 2×10^{-2} mM, but no effect on the K562 cell line. Compound **2** was inactive in both cell lines.

3. Experimental

3.1. Instrumentation

The UV spectrum were determined on a Shimadzu UV-2201 UV-VIS recording spectrophotometer and the IR spectra were recorded on a Bruker IFS-55 (KBr). The optical rotation was measured on a Perkin-Elmer 241 polarimeter. NMR data were obtained using a JEOL JNM-GX400 (^{1}H 300 MHz, ^{13}C 75 MHz). EIMS and HRMS data was recorded on a VG Autospec-3000 mass spectrometer. Silica gel H (10–40 μm, Qingdao Haiyang Chemical Factory). TLC: silica gel G (10–40 μm, Qingdao Haiyang Chemical Factory).

3.2. Plant material, extraction and isolation

Rhizomes of *Dioscorea futschauensis* R. Kunth (Dioscoreaceae) were collected in 1999 from Fujian Province (China), and identified by Prof. Qishi Sun (Division of Pharmacognosy, Shenyang Pharmaceutical University). A voucher specimen is deposited at the herbarium of Shenyang Pharmaceutical University, Liaoning Province.

Air-dried powdered rhizomes (3000 g) of *D. futschauensis* were extracted with 75% EtOH (5000 ml × 2). The ethanol was evaporated and the residue was partitioned between water and n-BuOH to yield an n-butanol soluble fraction DB (100 g). Fraction DB (50 g) was subjected to CC on silica gel H (500 g) and eluted stepwise by $CHCl_3$−MeOH (100 : 1, 100 : 2, 97 : 3, 95 : 5, 90 : 10, 85 : 15, 80 : 20, 70 : 30 and 60 : 40, each 5000 ml) to give 27 corresponding fractions. Fraction 5 (0.5 g) was further purified by preparative TLC (benzene-acetone, 9 : 1, $R_f = 0.55$) to yield compounds **1** (5 mg) and **2** (4 mg).

Dioscorone A (**1**) was obtained as an amorphous powder; $[\alpha]_D^{24}$ 0° (c 0.15, $CHCl_3$). UV(EtOH) λ_{max} 203, 267, 308 nm (log ε 4.00, 3.65, 3.09); IR(Nujol) ν_{max} 3421, 2923, 2852, 1744, 1683, 1627, 1458, 1248, 1156, 1050 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6) in the Table; EIMS m/z (rel int) 300 [M^+] (100), 272 (25), 257 (12), 243 (10), 229 (34), 200 (5); HREIMS m/z (meas. 300.0657, calcd 300.0633).

Compound (**2**) was obtained as colorless needles from methanol; $[\alpha]_D^{24}$ 0° (c 0.2, ethanol). UV(EtOH) λ_{max} 220, 276, and 300 nm (log ε 3.76, 4.05, 4.30); IR(Nujol) ν_{max} 3370–2550 (br.), 1650, 1520, 1150 cm^{-1}; ^{1}H and ^{13}C NMR (methanol-d_4) in the Table; HREIMS m/z (meas. 254.0345, calcd 254.0335).

3.3. Bioassay

Antifungal assay against the phytopathogenic fungus *Pyricularia oryzae* and a cytotoxicity assay on cultured K562 (leukemia) and HCT-15 (colon) cell lines were carried out as previously reported [7].

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