Fitoterapia 95 (2014) 147–153

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Flavonoids and stilbenoids from *Derris eriocarpa*

Hong-Xia Zhang [a], Paul-Keilah Lunga [a,b], Zhi-Jian Li [a,c], Qin Dai [a], Zhi-Zhi Du [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China
[b] Department of Biochemistry, Laboratory of Phytobiochemistry and Medicinal Plants Study, Faculty of Science, University of Yaoundé 1, P.O. Box 812, Yaoundé, Cameroon
[c] School of Life Sciences, Yunnan University, Kunming 650091, PR China

ARTICLE INFO

Article history:
Received 20 January 2014
Accepted in revised form 14 March 2014
Available online 29 March 2014

Keywords:
Derris eriocarpa
Prenylisoflavone
Resveratrol analogue
Antimicrobial activity

ABSTRACT

One new resveratrol analogue, 1-(3′,4′,5′-trimethoxyphenyl)-2-methoxy-2-(4″-methoxyphenyl)-ethane-1-ol (**1**), and two new prenylisoflavones, 4′-hydroxy-5,7-dimethoxy-6-(3-methyl-2-butenyl)-isoflavone (**2**), and derrubon 5-methyl ether (**3**), together with 17 known compounds including one new natural product, 5,7-dihydroxy-3-[4′-*O*-(3-methyl-2-butenyl)-phenyl]-isoflavone (**4**), were isolated from the stems of ethnomedicinal plant *Derris eriocarpa* How. (Leguminosae). Their structures were elucidated based on chemical evidence and spectroscopic techniques including two-dimensional NMR methods. All compounds are reported from this species for the first time. Antimicrobial activities of the new compounds were evaluated. Compound **2** exhibited good inhibitory activities against *Candida guilliermondii*, *C. albicans* and *Microsporium gypseum* with the minimal inhibitory concentration (MIC) values of 12.5 μg/ml.

1. Introduction

Derris eriocarpa How, belonging to the Leguminosae family, is commonly known as 'Tugancao' in 'Zhuang' and 'Dai' ethnopharmacy in Guangxi and Yunnan Province of China [1]. The dried stems of the plant are used to relieve cough and reduce sputum, as well as in the treatment of nephritis, cystitis, urethritis, beriberi edema and cough [2]. The aqueous, ethanol and n-butanol extracts of *D. eriocarpa* have been reported to possess cough-relieving, sputum-reducing, anti-inflammatory and analgesic effect; meanwhile the aqueous and ethanol extracts showed no acute toxic response in mice with maximal tolerance dose of 62.8 and 96 g/kg [3–7]. Despite its long time use in folk medicine and diverse activities, there are no systematic chemical and bioactivity studies on *D. eriocarpa*. Only one preliminary chemical test reported that the plant may contain the components such as amino acid, polypeptide, protein, saccharide, polysaccharide, glycoside, saponin, organic acid, flavone, anthraquinone, cardiac glycoside, triterpene and phenols [8]. During our research on unique ethnomedicinal materials from Yunnan province, the plant was selected for further investigation on its chemical constituents and bioactivity. In this study, one new resveratrol analogue (**1**) and two new prenylisoflavones (**2**,**3**) together with 17 known compounds (**4**–**20**), including one new natural product, 5,7-dihydroxy-3-[4″- *O*-(3- methyl-2-butenyl)-phenyl]-isoflavone (**4**), (Fig. 2), have been isolated from the stems of *D. eriocarpa*, and reported here for the first time. In addition, the antimicrobial activities of the new compounds were evaluated against bacteria, yeasts and dermatophytes. Herein, we describe the isolation, structure elucidation and the antimicrobial activities of the new compounds.

2. Experiment part

2.1. General experiment procedure

Optical rotations were measured with a Horiba Sepa-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A tensor 27 spectrophotometer was used for scanning IR spectroscopy using KBr pellets. 1D and 2D NMR spectra were measured on Brucker AM-400 and DRX-500, Avance III 600 with tetramethylsilane (TMS) as internal standard. Unless otherwise specified, chemical shifts

* Corresponding author. Tel.: +86 871 65223224.
E-mail address: duzhizhi@mail.kib.ac.cn (Z.-Z. Du).

http://dx.doi.org/10.1016/j.fitote.2014.03.015
0367-326X/

(δ) were expressed in ppm with reference to the solvent signals. ESIMS was obtained on an API-Qstar-Pulsar-1 spectrometer. HREIMS was recorded on a Waters AutoSpec Premier P776 spectrometer.

Column chromatography (CC) was done using silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemical Ltd. Co., China) and Lichroprep RP-18 gel (40–63 μm, Merch, Germany), TLC was performed on silica gel GF254 (Qingdao Marine Chemical Led. Co., China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4.

2.2. Plant material

The fresh stems of *D. eriocarpa* were collected in Jingxi, Guangxi Province, China and identified by Prof. Wang Lisong. A voucher specimen (No.11-32219) has been deposited in the Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

2.3. Extraction and isolation

The air-dried and powdered stems of *D. eriocarpa* (6 kg) were extracted 3 times with 80% aqueous MeOH at room temperature. After evaporation of MeOH under reduced pressure, the aqueous residue was partitioned with petroleum ether, $CHCl_3$, EtOAc and n-BuOH to yield petroleum portion (150 g), EtOAc portion (12 g), n-BuOH portion (80 g). The $CHCl_3$ portion (150 g) was absorbed on silica gel and chromatographed on silica gel column, eluted with a gradient system of petroleum ether–acetone (1:0, 50:1, 25:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give 10 fractions (CP1–10); CP4 afforded **5** (2 g) after repeated crystallizing. CP6 (26 g) was absorbed on silica gel and chromatographed on silica gel column, eluted with a gradient system of petroleum ether–ethyl acetate (15:1, 5:1, 3:1) to give 10 fractions ($CP6_{a-j}$); $CP6_e$ (300 mg) was applied repeatedly to CC over silica gel to afford **4** (20 mg), **7** (17 mg), **10** (25 mg), **11** (15 mg); $CP6_g$ (3 g) afforded **2** (20 mg), **3** (15 mg), **6** (2 g), **8** (10 mg), **16** (5 mg). $CP6_i$ (2 g) afforded **1** (50 mg), **9** (5 mg), **13** (40 mg). CP8 (10 g) was absorbed on silica gel and chromatographed on silica gel column, eluted with a gradient system of chloroform–acetone (1:0, 30:1, 10:1, 4:1, 1:1, 0:1) to give 7 fractions ($CP8_{a-g}$); $CP8_c$ (50 mg) was applied repeatedly to CC over silica gel to afford **12** (30 mg), and $CP8_f$ (600 mg) afforded **14** (140 mg). The EtOAc portion (12 g) was absorbed on silica gel and chromatographed on a silica gel column, and eluted with a gradient system of chloroform–acetone (1:0, 60:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1) to give 10 fractions (EP1–10). EP6 (100 mg) afforded **15** (60 mg) and EP9 (300 mg) afforded **20** (35 mg) after applied repeatedly to CC over silica gel. The n-BuOH portion (80 g) was subjected to a Diaion D101 column eluted with 30%, 50%, 70%, and 100% EtOH. The 30% EtOH fraction (recorded as BP1, 15 g) was then absorbed on silica gel and chromatographed on silica gel column, eluted with a gradient system of $CHCl_3$–MeOH (15:1, 10:1, 5:1, 3:1, 1:1, 0:1) to give 7 fractions ($BP1_{a-g}$). After applied repeatedly to CC over silica gel, $BP1_c$ afforded **19** (120 mg), $BP1_d$ afforded **17** (25 mg), and $BP1_e$ afforded **18** (100 mg).

1-(3′,4′,5′-trimethoxyphenyl)-2-methoxy-2-(4″-methoxyphenyl)-ethane-1-ol (**1**): Gray oil, UV (MeOH) λ_{max}(nm) (log ε): 274 (3.02), 204(4.25); IR (KBr) ν_{max} cm^{-1}: 3455 (OH), 2938, 1592, 1511, 1461, 1420, 1243, 1126, 833; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESI-MS *m/z* 371 $[M + Na]^+$; HR-EI-MS *m/z* 348.1582, $[M]^+$ (calcd for $C_{19}H_{24}O_6$, 348.1582).

4′-hydroxy-5,7-dimethoxy-6-(3-methyl-2-butenyl)-isoflavone (**2**): White amorphous powder, UV (MeOH) λ_{max}(nm) (log ε): 260 (4.15), 203 (4.19); IR (KBr) ν_{max} cm^{-1}: 3415 (OH), 2916, 1629, 1610,1515, 1453, 1435, 1250. ^{1}H NMR, see Table 2 and ^{13}C NMR data, see Table 3; ESI-MS *m/z* 389, $[M + Na]^+$; HR-EI-MS *m/z* 366.1440 $[M]^+$ (calcd for $C_{22}H_{22}O_5$, 366.1440).

Derrubon 5-methyl ether (**3**): White amorphous powder, UV (MeOH) λ_{max}(nm) (log ε): 293 (3.77), 258 (3.93), 204 (4.13); IR (KBr) ν_{max} cm^{-1}: 3222(OH), 1632, 1582, 1489, 1433, 1243, ^{1}H NMR, see Table 2 and ^{13}C NMR data, see Table 3; ESI-MS *m/z* 403, $[M + Na]^+$; HR-EI-MS *m/z* 380.1269 $[M]^+$ (calcd for $C_{22}H_{20}O_6$, 380.1269).

5,7-Dihydroxy-3-[4′-*O*-(3-methyl-2-butenyl)-phenyl]-isoflavone (**4**): White amorphous powder, UV (MeOH) λ_{max}(nm) (log ε): 264 (4.33), 202 (4.32); IR (KBr) ν_{max} cm^{-1}: 3423, 1655, 1572, 1513, 1287, 1246, 1176, 1044, 1004, 836; ^{1}HNMR see Table 2 and ^{13}CNMR data see Table 3; ESI-MS (*m/z* 338, $[M + H]^+$; *m/z* 361, $[M + Na]^+$).

2.4. Antimicrobial assay

Microorganisms and culture media: The microorganisms used in this study included three bacteria strains: *Enterococcus faecalis* ATCC 10541, *Staphylococcus aureus* ATCC 25922 and *Providensia smartii* ATCC29916; three yeasts: *Candida albicans* ATCC 2091, *Cryptococcus neoformans* IP 90526 and *Candida guilimondis* (clinical isolate) and three dermatophytes: *Trichophyton mentagrophytes* E1425, *Microsporium gypseum* E1420 and *Trichophyton terrestre* E1422. They were obtained from the American Type Culture Collection (ATCC), "Ecole Nationale Vétérinaire d'Alfort" (E), "centre Pasteur" of Yaounde Cameroon and "Institut Pasteur de Paris" (IP). The culture media, Nutrient Agar (NA, Conda) and Sabouraud Dextrose Agar (SDA, Conda), were used for culturing bacteria and fungi

Table 1
NMR data of Compound **1** recorded in $CDCl_3$ (δ in ppm, *J* in Hz).

No.	^{1}H NMR[a]	^{13}C NMR[b]	HMBC
1	4.54 (d, 8.4)	78.5 (d)	6.17, 3.97,
2	3.97 (d, 8.4)	88.7 (d)	6.92, 6.17, 4.54, 3.25
1′		134.8 (s)	4.54, 3.97
2′	6.17 (s)	103.9 (d)	6.17, 4.54
3′		152.4 (s)	6.17, 3.62
4′		136.9 (s)	6.17, 3.73
5′		152.4 (s)	6.17, 3.62
6′	6.17 (s)	103.9 (d)	6.17, 4.54
1″		129.4 (s)	6.75, 4.54
2″	6.92 (d, 8.4)	128.9 (d)	6.92, 3.97
3″	6.75 (d, 8.4)	113.4 (d)	6.92, 6.75
4″		159.3 (s)	6.92, 6.75, 3.71
5″	6.75 (d, 8.4)	113.4 (d)	6.92, 6.75
6″	6.92 (d, 8.4)	128.9 (d)	6.92, 3.97
2-OCH_3	3.25 (s)	56.6 (q)	3.97
3′-OCH_3	3.62 (s)	55.7 (q)	
4′-OCH_3	3.73 (s)	60.7 (q)	
5′-OCH_3	3.62 (s)	55.7 (q)	
4″-OCH_3	3.71 (s)	55.1 (q)	

[a] Measured at 400 MHz.
[b] Measured at 100 MHz.

Table 2
^{1}H NMR data of compounds 2–4 (δ in ppm, J in Hz).

No	2[a]	3[b]	4[c]
2	7.80 (s)	8.15 (1H, s)	8.20 (1H, s)
3			
4			
4a			
5			
6			6.29 (d, 2.4)
7			
8	6.66 (s)	6.66 (s)	6.42 (d, 2.4)
8a			
1′			
2′	7.33 (d, 8.0)	7.06 (s)	7.54 (d, 8.4)
3′	6.83 (d, 8.0)		6.99 (d, 8.4)
4′			
5′	6.83 (d, 8.0)	6.93 (d, 8.0)	6.99 (d, 8.4)
6′	7.33 (d, 8.0)	6.96 (d, 8.0)	7.54 (d, 8.4)
7′	3.42 (d, 6.7)	3.26 (d, 6.7)	4.60 (d, 6.6)
8′	5.18 (t, 6.4)	5.12 (t)	5.49 (t, 6.6)
9′			
10′	1.67 (s)	1.60 (s)	1.78 (s)
11′	1.79 (s)	1.71 (s)	1.76 (s)
5-OCH_3	3.87 (s)	3.69 (s)	
7-OCH_3	3.92 (s)		
OCH_2O		6.01 (s)	
5-OH			13.02 (s)
7-OH			9.79 (s)

[a] Measured at 400 MHz in $CDCl_3$.
[b] Measured at 400 MHz in DMSO-d_6.
[c] Measured at 600 MHz in acetone-d_6.

respectively, while Mueller Hinton Broth (MHB, Conda), and Sabouraud Dextrose Broth (SDB, Conda) were used for the determination of minimum inhibitory concentrations (MIC).

Preparation of microbial inocula: The inocula of bacteria and yeasts were prepared from 24 h and 48 h old agar cultures respectively. The absorbance was read at 600 and 530 nm respectively and adjusted with sterile physiological solution to an optical density of 0.10 (0.5 McFarland standards). These solutions corresponded to about 10^8 colony-forming units (CFU) per milliliter and 2.5×10^5 cells/ml for bacteria [9] and yeasts [10] respectively. From the prepared bacterial solutions, other dilutions with sterile physiological solution were prepared to give a final concentration of 10^6 CFU/ml for bacteria [11]. Conidia suspensions of dermatophyte species were prepared from 10 day old cultures. The number of conidia was determined using a spectrophotometer and adjusted with sterile saline (NaCl) solution (0.9%) to an absorbance of 0.600 at 450 nm (Jenway 6105 UV/Vis spectrophotometer-50 Hz/60 Hz) corresponding to a final concentration of about 4×10^3 spores/ml [12].

Determination of minimum inhibitory concentration (MIC): The MICs of the isolated compounds were determined by the broth microdilution method in 96-well micro-titre plates as described by Tchakam et al. [13]. The 96-well plates were prepared by dispensing into each well 100 μl of Mueller Hinton Broth for bacteria and Sabouraud Dextrose broth for fungi. The test substances were initially prepared in 10% DMSO in broth medium at 400 μg/ml for compounds or 50 μg/ml for the reference antibiotics. A volume of 100 μl of each test sample was added into the first wells of the micro-titre plate (whose wells were previously loaded with 100 μl of broth medium). Serial two-fold dilutions of the test samples were made and 100 μl of the inocula prepared above was added into respective wells [9]. This gave final concentration ranges of 100 to 0.781 μg/ml for the compounds and 12.5 to 0.097 μg/ml for

Table 3
^{13}C NMR and HMBC data of compounds 2–4 (δ in ppm).

No	2 in $CDCl_3$		3 in DMSO-d_6		4 in acetone-d_6	
	δ_C[a]	HMBC	δ_C[a]	HMBC	δ_C[b]	HMBC
2	150.5 (d)		151.4 (d)		154.5 (d)	
3	126.0 (s)	7.80, 7.33	124.1 (s)	8.15, 7.06, 6.96	123.8 (s)	8.20, 6.99
4	175.4 (s)	7.80, 6.66	173.5 (s)	8.15, 6.66	181.6 (s)	13.02, 8.20, 7.54, 6.42, 6.29
4a	112.9 (s)	6.66	111.2 (s)	8.16, 6.66	106.1 (s)	13.02, 6.42, 6.29
5	158.0 (s)	3.87, 3.42	158.0 (s)	3.69, 3.26	163.9 (s)	13.02, 6.29
6	122.3 (s)[c]	6.66, 3.42	120.4 (s)	6.66, 3.26	99.9 (d)	13.02, 6.42
7	162.2 (s)	6.66, 3.92, 3.42	160.7 (s)	6.66, 3.26	165.1 (s)	6.42
8	95.1 (d)	3.92	98.3 (d)		94.5 (d)	6.29
8a	157.8 (s)	7.80, 6.66	156.8 (s)	8.15, 6.66	159.0 (s)	8.20, 6.42
1′	123.8 (s)	7.80, 6.83	126.0 (s)	8.15, 6.93	124.0 (s)	8.20, 6.99
2′	130.4 (d)	7.33, 7.80	109.7 (d)	6.96	131.1 (d)	7.54, 6.99
3′	115.6 (d)	6.83	146.9 (s)[d]	7.06, 6.96, 6.93, 6.01	115.2 (d)	7.54, 6.99
4′	155.9 (s)	7.33, 6.83	146.8 (s)[d]	7.06, 6.96, 6.93, 6.01	159.9 (s)	7.54, 6.99, 4.60
5′	115.6 (d)	6.83	108.0 (d)	7.06	115.2 (d)	7.54, 6.99
6′	130.4 (d)	7.33, 7.80	122.5 (d)	7.06	131.1 (d)	7.54, 6.99
7′	22.3 (t)	5.18	22.0 (t)	6.66, 5.12	65.4 (t)	5.49
8′	122.4 (d)[c]	3.42, 1.79, 1.67	122.8 d)	3.26, 1.71, 1.60	121.1 (d)	4.60, 1.78, 1.76
9′	131.8 (s)	3.42, 1.79, 1.67	130.6 (s)	3.26, 1.71, 1.60	137.9 (s)	4.60, 1.78, 1.76
10′	25.7 (q)	5.18, 1.79	25.5 (q)	5.12, 3.26, 1.71	25.8 (q)	5.49, 1.76
11′	17.8 (q)	5.18, 1.67	17.7 (q)	5.12, 1.60	18.2 (q)	5.49, 1.78
5-OCH_3	62.3 (q)		61.2 (q)			
7-OCH_3	55.9 (q)					
OCH_2O			101.0 (CH_2)			

[a] Measured at 100 MHz.
[b] Measured at 150 MHz.
[c] Interchangeable.
[d] Interchangeable.

reference substances. The plates were sealed with parafilm, then agitated with a plate shaker to mix their contents and incubated at 35 °C for 24 h for bacteria, 48 h for yeast and at 28 °C for 5 days for dermatophytes.

For bacteria, MICs were determined upon addition of 50 μl (0.2 mg/ml) p-iodonitrotetrazolium chloride (INT, Sigma-Aldrich). Viable bacteria reduced the yellow dye to a pink colour. For yeasts and dermatophytes, MICs were determined by visualizing the turbidity of the wells. The MIC corresponded to the lowest well concentration where no colour/turbidity change was observed, indicating no growth of microorganism. All tests were performed in triplicates. Gentamycin for bacteria, nystatin for yeast and griseofulvin for dermatophytes were used as positives controls.

3. Results and discussion

Compound **1**, was obtained as gray oil, [α]17 D −4.2 (*c* 0.26, MeOH). The molecular formula, $C_{19}H_{24}O_6$, with eight degrees of unsaturation, was deduced by high-resolution (HR) EI-MS (*m/z* 348.1582, $[M]^+$), which was compatible with the results of ESI-MS (*m/z* 371, $[M + Na]^+$) and NMR data analysis. The UV spectrum exhibited maxima at λ_{max} (MeOH) 274 nm, 204 nm (log ε 3.02, 4.25). The IR spectrum suggested the presence of hydroxyl group (3455 cm^{-1}) and aromatic ring (1592, 1511 cm^{-1}). The ^1H NMR spectrum (Table 1) of **1** contained four sets of signals, including one set of ortho-coupled protons assignable to an AA′BB′-type phenyl group [δ_H: 6.92, 6.75 (2H each, d, $J = 8.4$ Hz)], a set of protons assignable to 1,3,4,5-tetrasubstituted phenyl group (δ_H: 6.17, 2H, s), two aliphatic protons [δ_H: 4.54, 3.97 (1H each, d, $J = 8.4$ Hz)], and four methoxyl groups. The ^{13}C NMR spectrum (Table 1) exhibited 19 signals. Comparing its ^1H NMR and ^{13}C NMR spectrum with those of compound **5** [14], the significant difference was observed at benzylic signals; the benzylic double bond of **5** was saturated and oxygenated. The hydroxyl positioned at C-1 and the methoxyl positioned at C-2 were disclosed by the HSQC and HMBC correlations (Fig. 1 and Table 1). HMBC correlations were observed from the protons at δ_H 6.17 (H-2′ and H-6′) to the carbon at δ_C 78.5 (d, C-1), from the protons at δ_H 3.25 (3H, s) to the carbon at δ_C 88.7 (d, C-2), from the protons at δ_H 6.92 (H-2″ and H-6″) to the carbons at δ_C 159.3 (s, C-4″) and 88.7 (d, C-2). Based on the coupling constants of the two aliphatic protons ($J = 8.4$ Hz), the hydroxyl and methoxyl should be in the *threo* form [15,16]. Thus, the structure of **1** was elucidated as 1-(3′,4′,5′-trimethoxyphenyl)-2-methoxy-2-(4″-methoxyphenyl)-ethane-1-ol.

Compound **2**, was obtained as white amorphous powder. The molecular formula, $C_{22}H_{22}O_5$, with 12 degrees of unsaturation, was deduced by high-resolution (HR) EI-MS (*m/z* 366.1440, $[M]^+$), which was compatible with the results of ESI-MS (*m/z* 389, $[M + Na]^+$) and NMR data analysis. The UV spectrum exhibited maxima at λ_{max} (MeOH) 260 nm, 203 nm (log ε 4.15 and 4.19). The IR spectrum suggested the presence of hydroxyl group (3415 cm^{-1}) and aromatic ring (1629, 1610 cm^{-1}). The ^1H NMR spectrum (Table 2) displayed two sets of signals at δ_H 7.33 and 6.83 (each 2H, d, $J = 8.0$ Hz) ascribed to a *p*-substituted phenyl, one aromatic and one olefinic proton singles at δ_H 6.66 and 7.80 (each 1H, s), two methoxyl groups at δ_H 3.92 and 3.87 (each 3H, s). The ^1H NMR spectrum also exhibited a 3-methyl-2-butenyl group [δ_H 3.42 (2H, d, $J = 6.7$ Hz), 5.18 (1H, t, $J = 6.4$ Hz), 1.67 and 1.79 (each 3H, s)]. The ^{13}C NMR spectrum (Table 3) showed 22 carbon signals corresponding to a prenylated isoflavone derivative with two methoxyl groups. One methoxyl group (δ_H 3.92, 3H, s) positioned at C-7, was confirmed by the HMBC correlations (Fig. 1 and Table 3) from the protons at δ_H 3.92 (3H, s) to the carbon at δ_C 162.2 (s, C-7), from the proton at δ_H 6.66 (H-8) to the carbons at δ_C 175.4 (s, C-4), 162.2 (s, C-7), 157.8 (s, C-8a), and 112.9 (s, C-4a). The other methoxyl group (δ_H 3.87, 3H, s) was positioned at C-5 by the HMBC correlations from the protons at δ_H 3.87 and the protons at δ_H 3.42 (H-7′) to the carbon at δ_C 158.0 (s, C-5). Therefore, the structure of **2** was determined as 4′-hydroxy-5,7-dimethoxy-6-(3- methyl-2-butenyl)-isoflavone.

Compound **3**, was obtained as white amorphous powder. The molecular formula, $C_{22}H_{20}O_6$, with 13 degrees of

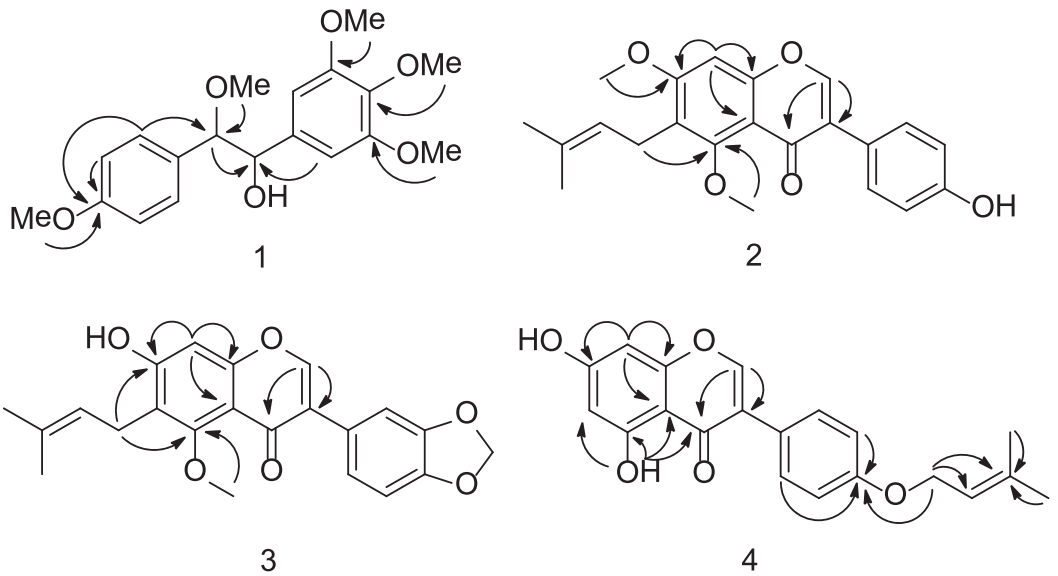
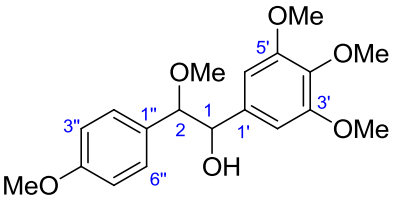
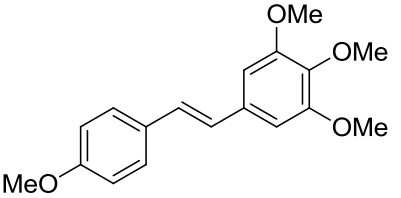


Fig. 1. The selected key HMBC correlations of **1**–**4**.

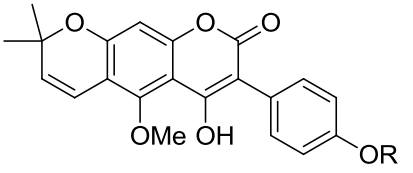
unsaturation, was deduced by high-resolution (HR) EI-MS (*m/z* 380.1269, $[M]^+$), which was compatible with the results of ESI-MS (*m/z* 403, $[M + Na]^+$) and NMR data analysis. The UV spectrum exhibited maxima at λ_{max} (MeOH) 293 nm, 258 nm, 204 (log ε 3.77, 3.93 and 4.13). The IR spectrum suggested the presence of hydroxyl group (3222 cm^{-1}) and aromatic ring (1632, 1582 cm^{-1}). Comparison of its ^1H NMR spectrum (Table 2) with that of compound **11** [17] showed that, for compound **3**, the signal of 5-OH or 7-OH in compound **11** was replaced by the signal of a methoxyl group (δ_H: 3.69, 3H, s) and it was confirmed in the spectrum by the corresponding signals (δ_C: 61.6). It assigned the 7-OH and 5-OMe from the HMBC correlations from the protons at δ_H 6.66 (H-8) to the carbons at δ_C 173.5 (s, C-4), 111.2 (s, C-4a), 120.4 (s, C-6), 160.7 (s, C-7) and



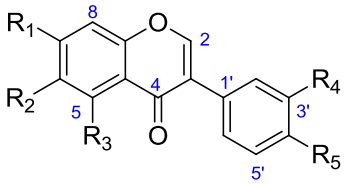
1

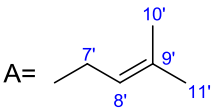


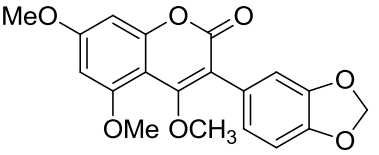
5



6 R=OMe
7 R=A

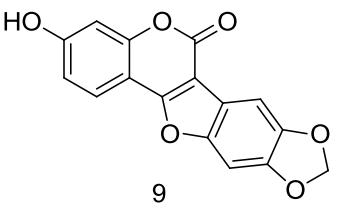




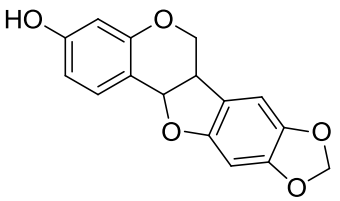


8

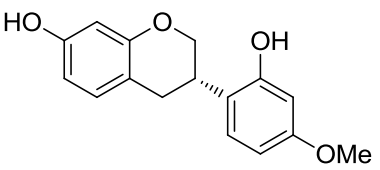
2 R_1=R_3=OMe, R_2=A, R_4=H, R_5=OH
3 R_1=OH, R_2=A, R_3=OMe, R_4=R_5=-OCH2O-
4 R_1=R_3=OH, R_2=R_4=H, R_5=-OA
11 R_1=R_3=OH, R_2=A, R_4=R_5=-OCH2O-
12 R_1=OH, R_2=OMe, R_3=H, R_4=R_5=-OCH2O-
13 R_1=OMe, R_2=R_3=R_4=H, R_5=OH
14 R_1=R_5=OH, R_2=R_3=R_4=H



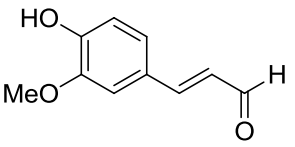
9



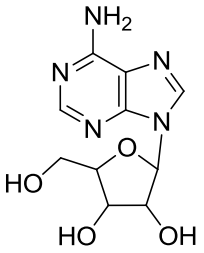
10



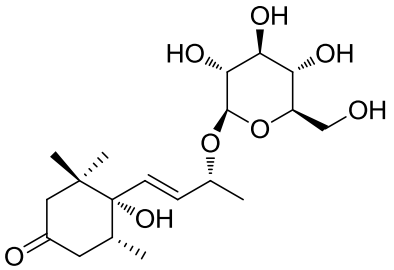
15



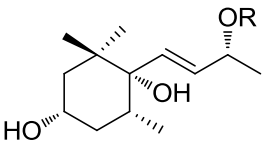
16



17



18



19 R=β -D-glu
20 R=OH

Fig. 2. The structure of compounds **1**–**20**.

156.8 (s, C-8a), and from the protons at δ_H 3.69 (3H, s) to the carbon at δ_C 158.0 (s, C-5), as well as from the protons at δ_H 3.26 (H-7′) to the carbon at δ_C 158.0 (s, C-5), 120.4 (s, C-6), and 160.7 (s, C-7) (Fig. 1 and Table 3). Thus, the structure of **3** was elucidated as 7-hydroxy-5-methoxy-6-(3-methyl-2-butenyl)-3′,4′-(methylenedioxy)-isoflavone, and named as derrubone 5-methyl ether.

Compound **4**, was obtained as white amorphous powder. The molecular formula, $C_{20}H_{18}O_5$, with 12 degrees of unsaturation, was deduced by ESI-MS (*m/z* 338, $[M + H]^+$; *m/z* 361, $[M + Na]^+$) and NMR data analysis. The UV spectrum exhibited maxima at λ_{max} (MeOH) 264 nm, 202 nm (log ε 4.33, 4.32). The IR spectrum suggested the presence of hydroxyl group (3423 cm^{-1}) and aromatic ring (1655, 1572 cm^{-1}). The ^1H NMR spectrum (Table 2) showed two phenolic hydroxyl groups at δ_H 13.02, 9.79 (each 1H, s), an aromatic or olefinic proton at δ_H 8.20 (1H, s), two sets of signals at δ_H 7.54 and 6.99 (each 2H, d, $J = 8.4$ Hz) ascribed to a *p*-substituted phenyl, two *meta*-coupled aromatic protons at δ_H 6.29 and 6.42 (each 1H, d, $J = 2.4$ Hz), a *O*-(3-methyl-2-butenyl) group [δ_H 4.60 (2H, d, $J = 6.6$ Hz), 5.49 (1H, t, $J = 6.6$ Hz), 1.78 and 1.76 (each 3H, s)]. The ^{13}C NMR spectrum (Table 3) showed 22 carbon signals. Comparison of its ^1H NMR and ^{13}C NMR spectrum with those of genistein [18] showed that, for compound **4** the signal of one phenolic hydroxyl group of the later compound was replaced by an *O*-(3-methyl-2-butenyl) group. The *O*-(3-methyl-2-butenyl) group in compound **4** positioned at C-4′, was confirmed by the HMBC correlations from the protons at δ_H 7.54 (H-2′ and H-6′), 6.99 (H-3′ and H-5′) and δ_H 4.60 (H-7′) to the carbon at δ_C 159.9 (s, C-4′). The 5-OH and 7-OH were further supported by the HMBC correlations (Fig. 1 and Table 3) from the proton at δ_H 13.02 (5-OH) to the carbons at δ_C 181.6 (s, C-4), 163.9 (s, C-5), 99.9 (d, C-6), from the proton at δ_H 6.42 (1H, d, $J = 2.4$ Hz) to the carbons at δ_C 181.6 (s, C-4), 106.1 (s, C-4a), 99.9 (d, C-6), 165.1 (s, C-7), 159.0 (s, C-8a) respectively. In a previous phytochemistry investigation, compound **4** had been synthesized [19]; however NMR spectral data were not available. In the present study, we carried out detailed NMR data analysis and confirmed its structure as 5,7-dihydroxy-3-[4′-*O*-(3-methyl-2-butenyl)-phenyl]-isoflavone.

The known compounds were 3,4,4′,5-tetramethoxystilbene (**5**) [14], robustic acid (**6**) [20,21], 4-hydroxy-3-[4′-*O*-(3,3-dimethylallyl)phenyl]-5-methoxy-2″,2″-dimethylpyrano-(5″,6″:6,7)-coumarin (**7**) [22], derrusnin (**8**) [23], medicagol (**9**) [24,25], maackiain (**10**) [26], derrubone (**11**) [17], 6-methoxy-7-hydroxy-3′,4′-methyl-ene-dioxyisoflavone (**12**) [27], formononetin (**13**) [28], daidzein (**14**) [29], (3*S*)-vestitol (**15**) [30], coniferaldehyde (**16**) [31], β-adenosine (**17**) [32,33], alangioside A (**18**) [34], ampelopsisionoside (**19**) [32], and (3*S*,5*R*,6*S*,7*E*,9*R*)-3,6-dihydroxy-5,6-di-hydro-β-ionol (**20**) [35] (Fig. 2), which were identified comparison of their physical and spectroscopic data with those reported in the literature.

The new compounds **1**–**3** were evaluated for their antimicrobial activities against three bacterial, three yeasts and three dermatophytes strains, and the MIC values were summarized in Table 4. The results showed that bacteria were more resistant to these compounds compared to fungi, with *Providencia stuartii* being the most susceptible strain. Compound **2** exhibited inhibitory activities against most of the tested microbial strains, in which the best activities showed on *Candida guilliermondii*, *C. albicans*, and *M. gypseum* with the minimal inhibitory concentration (MIC) values of 12.5 μg/ml. Compound **3** showed inhibitory activities against six tested microbial strains in which the lowest MIC value of 25 μg/ml on four of the tested trains. It is worth noting that the antifungal activity of compounds **2** and **3** on *T. mentagrophytes* (with MIC value of 25 μg/ml) and *M. gypseum* (with MIC values of 12.5 μg/ml and 25 μg/ml, respectively) was close to that of the reference drug, as well as compound **2** on *C. albicans*. Compound **1** showed better inhibitory activity against the tested yeast strains compared to bacterial and dermatophyte strains. Thus these compounds showed varied activities depending on the type and species of the microorganism. In the previously antimicrobial activities investigation, isoflavone erythrinin B isolated from *Erythrina porppigiana* had been reported to possess antibacterial activity against methicillin-resistant *S. aureus* with MIC value of 6.25 μg/ml [36]. In our study, the new isoflavones compound **2** and **3** have antibacterial activity against *S. aureus* with a MIC value more than 100 μg/ml. Comparing erythrinin B with compounds **2** and **3**, it was observed that more than one hydroxyl groups in erythrinin B were replaced by methoxyl groups. So we infer that the hydroxyl groups in this type compounds may contribute to the antibacterial activity.

In conclusion, twenty compounds were isolated from 80% methanol extract of the *D. eriocarpa* stem for the first time,

Table 4
Anti-microorganism activities of new compounds 1-3RD: Reference drug (Gentamycin for bacteria, Nystatin for yeasts and Griseofulvin for dermatophytes).

Microorganism	MIC μg/ml			
	Comp.1	Comp.2	Comp.3	RD
Bacteria				
Staphylococcus aureus	>100.000	>100.000	>100.000	0.195
Providencia stuartii	100.000	100.000	25.000	0.195
Enterococcus faecalis	100.000	>100.000	>100.000	1.963
Yeast				
Cryptococcus neoformans	100.000	50.000	>100.000	6.250
Candida guilliermondii	50.000	12.500	25.000	3.125
C. albicans	50.000	12.500	50.000	6.250
Dermatophyte				
Trichophyton mentagrophytes	>100.000	25.000	25.000	12.500
T. terrestre	100.000	100.000	50.000	12.500
Microsporium gypseum	100.000	12.500	25.000	3.125

and three of them are new (**1**, **2** and **3**), together with a new natural compound (**4**). The compound types reported here include resveratrol analogues, prenylisoflavones, coumarins and ionoe derivatives. In the antimicrobial bioassay, compound **2** showed the best inhibitory activities against three of the tested microbial strains. The above mentioned information makes the present study a starting and vital aspect for systemic investigation of the ethnomedicinal plant.

Conflict of interest

The authors declare no conflict of interest statement.

Acknowledgments

This work was sponsored by grants from the Natural Science Foundation of the Yunnan Province (2013FB065), the 45th Scientific Research Foundation for the Returned Overseas Chinese Scholars from State Education Ministry and the National Science and Technology Support Program of China (2013BAI11B02).

References

[1] Guangxi Institute of Chinese Medicine, Pharmaceutical Science. Medicinal plants directory of Guangxi. Guangxi: Guangxi People's Publishing House; 1986 232.
[2] Editorial Committee of the Administration Bureau of Traditional Chinese Medicine. Chinese Matrria Medica (Zhonghua Bencao), 4. Shanghai: Shanghai Press of Science and Technology; 1998 442.
[3] Yang DA, Yu SM, Huang LY, Chen SF, Guo LC. Toxicologic study on water soluble extract and ethanol soluble extract of *Derris eriocarpa* How in Zhuang medicines. Lishizhen Med Mater Med Res 2009;20(7):1586–7.
[4] Guo LC, Yang DA, Yu SM, Huang LY, Chen SF. Pharmacological effects of Tugancao. Lishizhen Med Mater Med Res 2010;21(1):154–5.
[5] Yang DA, Guo LC, Yu SM, Huang L, Yang Y, Chen SF, et al. Anti-inflammatory and analgesic effects of Tugancao. J Med Pharm Chin Minor 2009;15(11):54–5.
[6] Chen SF, Yu SM, Huang LY, Guo LC, Yang DA. Cough-relieving and sputum-removing effect of Tugancao. Lishizhen Med Mater Med Res 2009;20(11):2691–2.
[7] Yang DA, Guo LC, Yu SM, Huang LY, Chen SF. Anti-inflammatory and analgesic effects of Tugancao butanol extract. Acta Chin Med Pharm 2011;39(2):36–7.
[8] Tian H, Cui J, Jiang CY, Yang ZL. Preliminary study on chemical components of *Derris eriocarpa* How. J Liaoning Univ TCM 2013;15(2):66–7.
[9] Tereschuk ML, Riere MVQ, Castro GR, Abdala LR. Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. J Ethnopharmacol 1997;56:227–32.
[10] Clinical and Laboratory Standards Institute (formerly NCCLS, National Committee for Clinical and Laboratory Standards). Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard M27-A2 and filamentous fungi M38-A, vol. 22(15). Wayne, PA: National Committee for Clinical and Laboratory Standards; 2002 1.
[11] Tamokou JD, Simo MDJ, Lunga PK, Tene M, Tane P, Kuiate JR. Antioxidant and antimicrobial activities of ethylacetate extract, fractions and compounds from stem bark of *Albizia adianthifolia* (Mimosoideae). BMC Complement Altern Med 2012;12:99.
[12] Venugopal PV. In vitro susceptibility of dermatophytes to imidazoles. Indian J Dermatol 1992;37:35–41.
[13] Tchakam PD, Lunga PK, Kowa TK, Lonfouo AH N, Wabo HK, Tapondjou LA, et al. Antimicrobial and antioxidant activities of the extracts and compounds from the leaves of *Psorospermum aurantiacum* Engl. and *Hypericum lanceolatum* Lam. BMC Complement Altern Med 2012;12:136.
[14] Marek M, Norbert H, Thomas E, Karin P, Gerhard E, Philipp S, et al. Resveratrol analogues as selective cyclooxygenase-2 inhibitors: synthesis and structure–activity relationship. Bioorg Med Chem 2004;12(21):5571–8.
[15] Xiao K, Xuan LJ, Xu YM, Bai DL, Zhong DX. Constituents from *Polygonum cuspidatum*. Chem Pharm Bull 2002;50(5):605–8.
[16] Imuta M, Ziffer H. Microbial reduction of a series of substituted benzils. Optical properties and nuclear magnetic resonance spectra of products. J Org Chem 1978;43(17):3319.
[17] Hastings JM, Hadden MK, Blagg BSJ. Synthesis and evaluation of eerrubone and select analogues. J Org Chem 2008;73(2):369–73.
[18] Anyanwutaku IO, Zirbes E, Rosazza JPN. Isoflavonoids from streptomycetes: origins of genistenin, 8-chlorogenistein, and 6,8-dichorogenistein. J Nat Prod 1992;55(10):1498–504.
[19] Ozaki T, Mishima S, Nishiyama M, Kuzuyama T. NovQ is a prenyltransferase capable of catalyzing the addition of a dimethylallyl group to both phenylpropanoids and flavonoids. J Antibiot (Tokyo) 2009;62(7):385–92.
[20] Khalid SA, Waterman PG. Thonningine-A and thonningine-B: two 3-phenylcoumarins from the seeds of *Millettia thonningii*. Phytochemistry 1983;22(4):1001–3.
[21] Olivares EM, Lwande W, Monache FD, Bettolo GBM. A pyrano-isoflavone from seeds of *Milletia thonningii*. Phytochemistry 1982;21(7):1763–5.
[22] Magalhaes AF, Tozzi AMGA, Magalhaes EG, Souza-Neta LC. New prenylated metabolites of *Deguelia longeracemosa* and evaluation of their antimicrobial potential. Planta Med 2006;72(4):358–63.
[23] East AJ, Ollis WD, Wheeler RE. Natural occurrence of 3-aryl-4-hydroxycoumarins. I. Phytochemical examination of *Derris robusta*. J Chem Soc C 1969;3:365–74.
[24] Tang YP, Hu J, Wang JH, Lou FC. A new coumaronochromone from *Sophora Japonica*. J Asian Nat Prod Res 2002;4(1):1–5.
[25] Takai M, Yamaguchi H, Saiton T, Shibata S. Chemical studies on the oriental plan drugs. XXXV. The chemical constituents of the heartwood of *Maackia amurensis* var. buergeri. Chem Pharm Bull 1972;20(11):2488–90.
[26] Bedir E, Calis a I, Aquino R, Piacente S, Pizza C. Trojanoside H: a cycloartane-type glycoside from the aerial parts of *Astragalus trojanus*. Phytochemistry 1999;51:1017–20.
[27] Rao EV, Murthy MSR, Ward RS. Nine isoflavones from *Tephrosia maxima*. Phytochemistry 1984;23(7):1493–501.
[28] Chang YC, Nair MG, Santell RC, Helferich WG. Microwave-mediated synthesis of anticarcinogenic isoflavones from soybeans. J Agric Food Chem 1994;42:1869–71.
[29] Chi JF, Zhang GG, LI P, Kou LP. Chemical constituents of *Radix puerariae* from Anhui Province. Chin J Med Chem 2007(1):47–9.
[30] Piccinelli AL, Fernandez MC, Cuesta-Rubio O, Ndez IMR, Simone FD, Rastrelli L. Isoflavonoids isolated from *Cuban propolis*. J Agric Food Chem 2005;53:9010–6.
[31] Sy LK, Brown GD. Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*. Phytochemistry 1999;50:781–5.
[32] Ciuffreda P, Casati S, Manzocchi A. Spectral assignments and reference data: complete ^{1}H and ^{13}C NMR spectral assignment of alpha- and beta-adenosine, 2′-deoxyadenosine and their acetate derivatives. Magn Reson Chem 2007;45(9):781–4.
[33] Domondona DL, He WD, Kimp ND, Hofte M, Poppea J. β-Adenosine, a bioactive compound in grass chaff stimulating mushroom production. Phytochemistry 2004;65(2):181–7.
[34] Marino SD, Borbone N, Zollo F, Ianaro A, Meglio PD, Iorizzi M. Megastigmane and phenolic components from *Laurus nobilis*. J Agric Food Chem 2004;52:7525–31.
[35] Pérez C, Trujillo J, Almonacid LN, Trujillo J, Navarro E, Alonso SJ. Absolute structures of two new C_{13}-norisopernoids from *Apollonias barbujana*. J Nat Prod 1996;59(4):69–72.
[36] Sato M, Tanaka H, Tani N, Nagayama M, Yamaguchi R. Different antibacterial actions of isoflavones isolated from *Erythrina poeppigiana* against methicillin-resistant *Saphylococcus aureus*. Lett Appl Microbiol 2006;43:243–8.