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Four New Prenylated Isoflavonoids in *Tadehagi triquetrum*

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Investigation on the anthelminthic bioactive compounds of the ethanol extract of *Tadehagi triquetrum* resulted in the isolation of three new prenylated isoflavones, triquetrumones A (**1**), B (**2**), and C (**3**), and one new prenylated biisoflavanone, (*R*)-triquetrumone D (**4**), along with 16 known compounds, cyclokievitone (**5**), yukovanol (**6**), aromadendrin (**7**), kaempferol (**8**), astragalin (**9**), 2-*O*-methyl-L-chiro-inositol (**10**), galactitol (**11**), *p*-hydroxycinnamic acid (**12**), ursolic acid (**13**), betulinic acid (**14**), β-sitosterol (**15**), daucosterol (**16**), stigmasterol (**17**), stigmasta-5,22-dien-3-*O*-β-D-glucopyranoside (**18**), saccharose (**19**), and docosanoic acid (**20**). The structures of **1**−**4** were elucidated on the basis of spectroscopic and spectrometric methods. Compounds **1**−**3** displayed mild anthelminthic bioactivity, and compound **3** showed a significant binding ability to the estrogen receptor.

KEYWORDS: ***Tadehagi triquetrum*; Papilionaceae; coumaronochromone; triquetrumones A, B, C, D; anthelminthic**

INTRODUCTION

Herbivores, especially goats and rabbits, are commonly parasitized because grazing lands are frequently polluted by parasites. The control of parasites is primarily dependent upon continued applications of chemical drugs such as mebendozole and albendazole. Although effective, their repeated use has led to widespread development of resistance, undesirable effects on the environment and nontarget organisms, and especially human health concerns. For the majority of farmers, chemical drug treatment is not feasible and alternative treatments need to be evaluated. Plants may provide potential alternatives to currently used parasite control agents. Recently, herbal medicines possessing anthelminthic activity used as functional feeds have received growing attention, because the safety of herbal medicines has been partially verified in long-term use. Additionally, natural products in plants are often biodegradable to nontoxic products. These functional plants are therefore useful not only for the control of verminosis and the prevention of parasitization but also for lack of effects on human health, nontarget organisms, and the environment.

Tadehagi triquetrum (L.) Ohashi (Papilionaceae) is widely distributed in the southern area of Yunnan Province, People's Republic of China. It has been used as an anthelminthic, stomachic, antimicrobial, and antiinflammatory herbal medicine in traditional Chinese medicine (*1*) and also has been used as a nutrient and appetitive feedstuff. A previous study found some known flavonoids in *T. triquetrum* from Guangxi Province, but the bioactivity was not assayed (*2*, *3*). In the current investigation of the chemical constituents of the plant, we want to isolate the bioactive components and demonstrate that *T. triquetrum* can be used as an anthelminthic functional feed.

MATERIALS AND METHODS

General Experimental Procedures. One- and two-dimensional NMR experiments were performed on Bruker AM-400 and DRX-500 spectrometers with pyridine-d_5 or dimethyl sulfoxide (DMSO)-d_6 as the solvent and tetramethylsilane as the internal standard. MS spectra were taken on a VG Auto Spec-3000 (Micromass UK Ltd., Manchester) magnetic sector instrument. Optical rotations were measured on a SEPA-300 polarimeter. UV spectroscopic data were obtained on a UV-210A spectrometer (Shimadzu Co., Tokyo). IR spectra were recorded on a Bio-Rad FTS-135 spectrometer (California) with KBr pellets. High-performance liquid chromatography analysis used a Waters 996 instrument (Massachusetts). Column chromatography was performed on 200−300 mesh silica gel (Qingdao Marine Chemical Factory,

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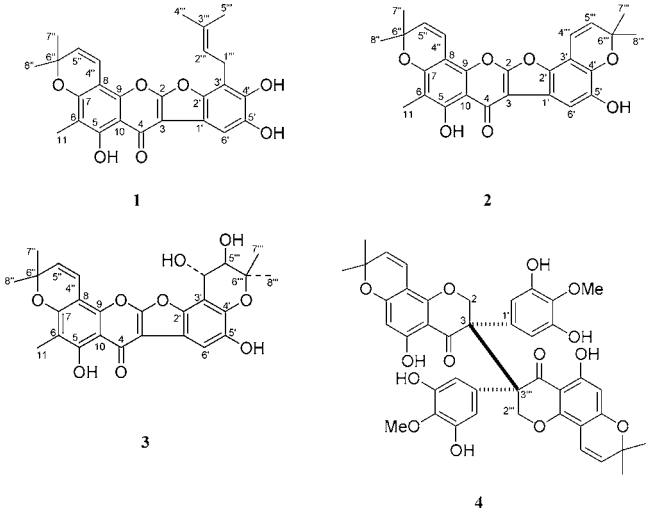

Figure 1. Structures of compounds **1**–**4** isolated from *T. triquetrum.*

People's Republic of China) and D101 resin (Tianjin Agricultural Chemical Co. Ltd., People's Republic of China). Fractions were monitored by thin-layer chromatography (TLC), with visualization under UV (254 or 365 nm) or by heating Si gel plates sprayed with 10% sulfuric acid in ethanol. MOPAC calculations were determined in the 3D Ultra 8.0 software (Cambridge Soft Co., Massachusetts). The small white mouse was a Kunming mouse (5 weeks old and ca. 20 g weight).

Plant Material. The whole plant of *T. triquetrum* was collected from Xishuangbanna, Yunnan Province, People's Republic of China, in June 2000. The identity of plant material was verified by Prof. Xi-Wen Li, and a voucher specimen (no. MYL-001, YCP) has been deposited at the Yunnan Academy of Forestry, Yunnan, People's Republic of China.

Bioassay. For activity against coccidian eggs, the same 13 coccidian egg samples (5 g) were prepared using feces of the rabbit, which was comprised of eggs from *Eimeria magna*, *Eimeria media*, *Eimeria stiedai*, *Eimeria perforans*, *Eimeria exigua*, and *Eimeria irresidua*. Ethanolic extracts (30, 20, and 10 mg) of the whole plant of *T. triquetrum* and compounds **1**, **2**, and **3** (10, 5, and 2.5 mg of each) (**Figure 1**) were dissolved in DMSO (10 mL), respectively, and then mixed into the coccidian egg samples, which were put in culture dishes and incubated at 35 °C. The total coccidian eggs in each culture dish were counted by the McMaster technique (*4*) at 0, 24, 48, and 144 h.

The binding responses of estrogen receptors (ER-α and ER-β) to the new compounds **1**−**4** were assayed as reported in the literature (*5*). The content of amino acid in whole plant of *T. triquetrum* was analyzed (mg/100 g) as follows: Asp (0.514), Thr (0.222), Ser (0.197), Glu (0.538), Gly (0.220), Ala (0.259), Cys (0.235), Val (0.302), Met (0.469), Ile (0.241), Leu (0.376), Tyr (0.140), Phe (0.246), Lys (0.331), His (0.130), Arg (0.225), and Pro (0.369).

Extraction and Isolation. The air-dried and ground whole plant of *T. triquetrum* (5 kg) was extracted with 95% EtOH (3 × 6 L) at 25 °C for 72 h. The combined ethanolic extracts were evaporated to dryness in vacuo and partitioned between water and EtOAc. The concentrated organic layer was dried to give 50 g of extract, which was adsorbed on D101 resin (2 kg) and eluted with a mixture of MeOH−H_2O (1:1, 3 × 5 L) followed by MeOH−H_2O (9:1, 3 × 5 L) to afford two fractions A and B. After evaporation of the solvent at reduced pressure, fraction B (11 g) was chromatographed on a silica gel (600 g) column, eluting with petroleum ether/$CHCl_3$ and $CHCl_3$/CH_3COCH_3 mixtures of increasing polarity. Fractions were combined based on their TLC pattern to give five pooled fractions B_1−B_5. Fraction B_1 was rechromatographed over a silica gel column (200 g), eluting with $CHCl_3$−CH_3COCH_3 (95:5) to yield compounds **2** (56 mg), **13** (66 mg), **14** (33 mg), **15** (52 mg), **17** (11 mg), and **20** (39 mg). Fraction B_2 was chromatographed on silica gel (200 g) eluting with $CHCl_3$−CH_3COCH_3 (90:10) to afford compounds **1** (30 mg), **4** (19 mg), **5** (21 mg), **6** (22 mg), **7** (32 mg), **8** (29 mg), and **9** (26 mg). Fraction B_3 was chromatographed on silica gel (200 g) eluting with $CHCl_3$−CH_3COCH_3 (80:20) to afford compounds **3** (15 mg), **16** (56 mg), and **18** (56 mg).

Table 1. ^{13}C and DEPT NMR Spectroscopic Data for **1**,[a] **2**,[a] **3**,[b] and **4**[b]

no.	1	2	3	4[c]
2	164.0 (C)	164.1 (C)	165.2 (C)	71.6 (CH_2)
3	112.7 (C)	113.6 (C)	115.3 (C)	47.7 (C)
4	178.4 (C)	178.6 (C)	179.9 (C)	199.3 (C)
5	158.9 (C)	158.9 (C)	160.6 (C)	165.1 (C)
6	108.2 (C)	108.4 (C)	109.9 (C)	98.0 (CH)
7	155.6 (C)	155.7 (C)	157.1 (C)	162.9 (C)
8	100.8 (C)	100.7 (C)	102.0 (C)	102.7 (C)
9	146.7 (C)	146.8 (C)	147.8 (C)	158.7 (C)
10	103.0 (C)	102.9 (C)	104.2 (C)	104.3 (C)
11	7.0 (CH_3)	6.9 (CH_3)	7.8 (CH_3)	
1′	97.6 (C)	97.3 (C)	98.7 (C)	114.5 (C)
2′	141.9 (C)	138.5 (C)	142.8 (C)	150.3 (C)
3′	111.9 (C)	106.9 (C)	112.6 (C)	137.5 (C)
4′	142.2 (C)	137.9 (C)	141.6 (C)	151.7 (C)
5′	143.9 (C)	144.2 (C)	146.6 (C)	108.6 (CH)
6′	103.1 (CH)	106.0 (CH)	107.1 (CH)	126.0 (CH)
4″	114.1 (CH)	113.9 (CH)	115.2 (CH)	116.4 (CH)
5″	128.6 (CH)	128.5 (CH)	129.1 (CH)	127.5 (CH)
6″	77.9 (C)	77.7 (C)	78.8 (C)	79.1 (C)
7″	27.7 (CH_3)	27.7 (CH_3)	28.4 (CH_3)	28.6 (CH_3)
8″	27.7 (CH_3)	27.7 (CH_3)	28.4 (CH_3)	28.6 (CH_3)
1‴	22.6 (CH_2)			
2‴	121.4 (CH)			
3‴	131.6 (C)			
4‴	17.7 (CH_3) (d)	114.4 (CH)	67.81 (CH)	
5‴	25.4 (CH_3) (d)	132.7 (CH)	76.7 (CH)	
6‴		76.3 (C)	80.7 (C)	
7‴		28.7 (CH_3)	21.0 (CH_3)	
8‴		28.7 (CH_3)	26.6 (CH_3)	
OMe				60.8 (CH_3)

[a] The δ values were recorded in DMSO-d_6, at 125 MHz (ppm). [b] The δ values were recorded in pyridine-d_5, at 125 MHz (ppm). [c] Compound **4** is symmetric, and only one set of NMR signals can be seen for the two monomer units. [d] Assignment may be interchanged.

Fraction A was combined with the water layer (69 g) and chromatographed on a silica gel column (600 g), eluting with $CHCl_3$/MeOH mixtures of increasing polarity to yield compounds **10** (586 mg), **11** (78 mg), **12** (52 mg), and **19** (598 mg).

Triquetrumone A (1). Yellow amorphous powder; $[\alpha]_D^{27}$ 0.0° (*c* 0.05, pyridine). UV (MeOH) λ_{max} (log ϵ): 214 (4.70), 250 (4.71), 256 (4.80), 262 (4.82), 280 (4.68), 360 (3.73) nm. IR (KBr) ν_{max}: 3431, 2926, 1647, 1610, 1450, 1376 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ 2.07 (3H, s, H-11), 7.19 (1H, s, H-6′), 6.73 (1H, d, J = 9.9 Hz, H-4″), 5.81 (1H, d, J = 9.9 Hz, H-5″), 1.44 (6H, s, H-7″, 8″), 3.47 (2H, d, J = 7.0 Hz, H-1‴), 5.27 (1H, t, J = 7.0 Hz, H-2‴), 1.77 (3H, s, H-4‴), 1.64 (3H, s, H-5‴), 13.33 (1H, br s, OH-5), 8.65 (1H, br s, OH-4′), 9.66 (1H, br s, OH-5′). ^{13}C NMR (DMSO-d_6, 125 MHz): see **Table 1**. Electron ionization mass spectrometry (EIMS) *m*/*z*: 448 $[M]^+$ (67), 433 (100), 419 (5), 389 (6), 377(21), 365 (6), 321 (2), 293 (1), 267 (1), 233 (2), 217 (12), 208 (7), 189 (9). High-resolution (HR)-EIMS *m*/*z*: M^+ calcd for $C_{26}H_{24}O_7$, 448.1522; found, 448.1512.

Triquetrumone B (2). Yellow amorphous powder; $[\alpha]_{27}^D$ 0.0° (*c* 0.19, pyridine). UV (MeOH) λ_{max} (log ϵ): 203 (4.07), 250 (4.31), 256 (4.41), 262 (4.40), 352 (3.10) nm. IR (KBr) ν_{max}: 3441, 2927, 1645, 1606, 1441, 1378 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ 2.03 (3H, s, H-11), 7.23 (1H, s, H-6′), 6.74 (1H, d, J = 9.6 Hz, H-4″), 5.82 (1H, d, J = 9.6 Hz, H-5″), 1.44 (6H, s, H-7″, 8″), 6.70 (1H, d, J = 9.6 Hz, H-4‴), 5.96 (1H, d, J = 9.6 Hz, H-5‴), 1.46 (6H, s, H-7‴, 8‴), 13.24 (1H, br s, OH-5), 8.96 (1H, br s, OH-5′). ^{13}C NMR (DMSO-d_6, 125 MHz): see **Table 1**. EIMS *m*/*z*: 446 $[M]^+$ (70), 431 (100), 401 (2), 377 (1), 345 (1), 303 (1), 267 (1), 233 (3), 216 (11), 208 (11), 188 (1). HR-EIMS *m*/*z*: M^+ calcd for $C_{26}H_{22}O_7$, 446.1365; found, 446.1361. ^1H NMR (pyridine-d_5, 500 MHz): δ 2.24 (3H, s, H-11), 7.95 (1H, s, H-6′), 6.84 (1H, d, J = 9.7 Hz, H-4″), 5.69 (1H, d, J = 9.7 Hz, H-5″), 1.38 (6H, s, H-7″, 8″), 6.79 (1H, d, J = 9.7 Hz, H-4‴), 5.82 (1H, d, J = 9.7 Hz, H-5‴), 1.46 (6H, s, H-7‴, 8‴). ^{13}C NMR (pyridine-d_5, 125 MHz): δ 164.8 (s, C-2), 115.2 (s, C-3), 179.5 (s, C-4), 160.4 (s, C-5), 109.6 (s, C-6), 156.8 (s, C-7), 101.6 (s, C-8), 147.6 (s, C-9),

104.0 (s, C-10), 7.5 (q, C-11), 98.5 (s, C-1′), 139.0 (s, C-2′), 107.8 (s, C-3′), 139.8 (s, C-4′), 146.0 (s, 5′), 107.9 (d, C-6′), 114.9 (d, C-4″), 128.6 (d, C-5″), 78.3 (s, C-6″), 27.5 (q, C-7″), 27.5 (q, C-8″), 115.5 (d, C-4‴), 132.8 (d, C-5‴), 77.2 (s, C-6‴), 28.2 (q, C-7‴), 28.2 (q, C-8‴).

Triquetrumone C (3). Yellow amorphous powder; $[\alpha]_{27}^{D}$ −14.3° (*c* 0.14, pyridine). UV (MeOH) λ_{max} (log ϵ): 263 (4.48), 295 (4.11), 353 (3.53) nm. IR (KBr) ν_{max}: 3426, 2927, 1645, 1615, 1456, 1366 cm^{-1}. ^{1}H NMR (pyridine-d_5, 500 MHz): δ 2.25 (3H, s, H-11), 8.10 (1H, s, H-6′), 6.70 (1H, d, J = 9.9 Hz, H-4″), 5.79 (1H, d, J = 9.9 Hz, H-5″), 1.32 (6H, s, H-7″, 8″), 5.68 (1H, d, J = 6.5 Hz, H-4‴), 4.45 (1H, d, J = 6.5 Hz, H-5‴), 1.76 and 1.51 (each 3H, s, H-7‴, 8‴). ^{13}C NMR (pyridine-d_5, 125 MHz): see **Table 1**. EIMS *m*/*z*: 480 [M]$^+$ (65), 465 (93), 447 (11), 431 (3), 409 (15), 393 (100), 380 (31), 365 (99), 351(5), 335 (4), 309 (2), 298 (12), 267 (3), 217 (13), 183 (9). HR-EIMS *m*/*z*: M$^+$ calcd for $C_{26}H_{24}O_9$, 480.1420; found, 480.1412.

(*R*)-Triquetrumone D (4). Yellow amorphous powder; $[\alpha]_{27}^{D}$ −24.8° (*c* 0.10, pyridine). UV (MeOH) λ_{max} (log ϵ): 205 (4.89), 224 (4.43), 269 (4.88), 294 (4.25), 306 (4.07) nm. IR (KBr) ν_{max}: 3433, 2928, 1641, 1590, 1471, 1375 cm^{-1}. ^{1}H NMR (pyridine-d_5, 500 MHz): δ 4.42 (1H, d, J = 10.9 Hz, H-2eq), 4.59 (1H, d, J = 10.9 Hz, H-2ax), 5.90 (1H, s, H-6), 6.38 (1H, d, J = 8.4 Hz, H-5′), 6.63 (1H, d, J = 8.4 Hz, H-6′), 6.48 (1H, d, J = 10.0 Hz, H-4″), 5.49 (1H, d, J = 10.0 Hz, H-5″), 1.36 (6H, s, H-7″, 8″), 3.76 (3H, s, OMe-3′), only ^{1}H NMR data of a monomer. ^{13}C NMR (pyridine-d_5, 125 MHz): see **Table 1**. FABMS *m*/*z*: 767 [M + 1]$^+$ (3), 384 (100), 245 (10), 191 (45). EIMS *m*/*z*: 384 (100), 369 (55), 351(19), 336 (3), 332 (1), 323 (1), 308 (1), 295 (1), 267 (1), 243 (1), 231 (3), 217 (23), 203 (76), 184 (3), 177 (9). HR-FABMS *m*/*z*: [M + H]$^+$ calcd for $C_{42}H_{39}O_{14}$, 767.2340; found, 767.2372.

RESULT AND DISCUSSION

The air-dried whole plant of *T. triquetrum* was extracted with ethanol and partitioned between EtOAc and water. The organic and aqueous layers were dried and repeatedly chromatographed on D101 resin and silica gel to afford compounds **1**−**20** in pure form.

Triquetrumone A (**1**) was obtained as a yellow amorphous powder. The molecular formula was determined as $C_{26}H_{24}O_7$ by HR-EIMS and ^{13}C NMR and DEPT experiments, which indicated 15 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl groups (3431 cm^{-1}) and a carbonyl group in conjugation with an aromatic ring and also hydrogen-bonded with a hydroxyl group (1647 cm^{-1}). The ^{1}H NMR spectrum provided signals of most of the functional groups, including an aromatic C-methyl group [δ_H 2.07 (3H, s)] (*6*), three phenolic hydroxyl groups [δ_H 13.33 (1H, br s), 9.66 (1H, br s), 8.65 (1H, br s)], a γ,γ-dimethylallyl group [δ_H 3.47 (2H, d, J = 7.0 Hz), 5.27 (1H, t, J = 7.0 Hz), 1.77 (3H, s), and 1.64 (3H, s)], a 2,2-dimethyldihydropyran ring [δ_H 6.73 (1H, d, J = 9.9 Hz), 5.81 (1H, d, J = 9.9 Hz) and 1.44 (6H, s)], and one aromatic proton at δ_H 7.19 (1H, s). In addition, the ^{13}C NMR spectrum of **1** (**Table 1**) showed signals for one carbonyl group [δ_C 178.4 (s)], one aromatic methine [δ_C 103.1 (d)], and 13 aromatic quaternary carbons appearing at δ_C 97.6−164.0, including seven oxygenated carbon signals at δ_C 141.9−164.0. The above NMR data indicated that **1** had one γ,γ-dimethylallyl group, a 2,2-dimethyldihydropyran ring, and a chromene ring, and considering the absorption bands in the IR [3431 and 1647 cm^{-1} (chelated OH and C═O)] and UV (250, 256, 262, 280, and 360 nm) spectra, the orange fluorescence under UV at 254 and 365 nm revealed that **1** had an isoflavone skeleton (*7*, *8*). On the basis of the analysis of its two-dimensional NMR spectra (HMQC and HMBC), **1** was deduced to be an isoflavone derivative. A coumaronochromone structure was proposed for **1** (*9*), because of the absence of a degree of unsaturation, a characteristic signal assignable for a proton at C-2 (ca. δ_H 8.1) and a carbon at C-2 (ca. δ_C 153) of the isoflavone nucleus in the ^{1}H and ^{13}C NMR spectra (*10*, *11*). In the HMBC spectrum (**Figure 2**), the proton of the hydrogen-bonded hydroxyl group at δ_H 13.33 correlated with C-5 (δ_C 158.9), C-6 (δ_C 108.2), and C-10 (δ_C 103.0). One *cis*-olefinic proton at δ_H 5.81 (H-5″) coupled with C-8 (δ_C 100.8) and another at δ_H 6.73 (H-4″) showed cross-peaks with C-7 (δ_C 155.6) and C-9 (δ_C 146.7). An aromatic methyl group at δ_H 2.07 (CH_3-11) correlated with C-6 (δ_C 108.2), C-5 (δ_C 158.9), and C-7 (δ_C 155.6). These results indicated the presence of a 5-hydroxy-6-methyl-benzoyl moiety with a 2,2-dimethyl-dihydropyran ring. The following long-range cross-peaks (^2J and ^3J) were observed for the B ring: a singlet at δ_H 7.19 (H-6′) with C-3 (δ_C 112.7), C-2′ (δ_C 141.9), and C-4′ (δ_C 142.2); a methylene doublet at δ_H 3.47 (H_2-1‴) with C-2′ (δ_C 141.9), C-3′ (δ_C 111.9), and C-4′ (δ_C 142.2), which indicated the attachment of the γ,γ-dimethylallyl group at C-3′ and the nonhydrogen-bonded hydroxyl groups at C-4′ and C-5′ on the aromatic B ring, respectively. Moreover, the retro-Diels−Alder cleavage (**Figure 3**) in the EIMS indicated that the above structural elucidation of **1** was reasonable (*12*). Thus, the structure of **1** was established as 5,4′,5′-trihydroxy-6-methyl-3′-(3,3-dimethylallyl)-[6″,6″-dimethylpyrano(2″,3″:7,8)] coumaronochromone, a new natural product named triquetrumone A.

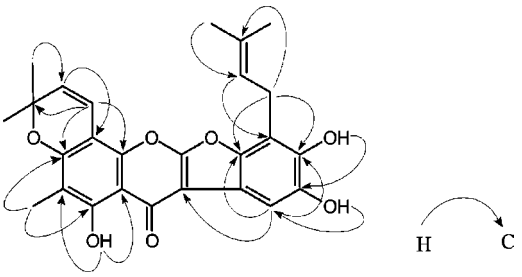

Figure 2. Selected HMBC correlations of **1**.

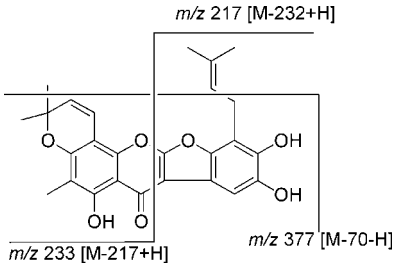

Figure 3. Selected EIMS fragments.

Compound **2** was obtained as a yellow amorphous powder and showed an orange fluorescence under UV at 365 nm. The HR-EIMS of **2** indicated the molecular formula $C_{26}H_{22}O_7$, which was supported by its ^{13}C NMR and DEPT spectra. The UV (λ_{max} 250, 256, 262, and 352 nm), IR (ν_{max} 3441, 1645, and 1378 cm^{-1}), and NMR spectroscopic data of **2** also suggested a coumaronochromone structure, which was very similar to **1**, except that **2** was two mass units less than **1**. Careful comparison of the NMR data of these two compounds revealed that the only difference between **1** and **2** was the existence of another 2,2-dimethyldihydropyran ring on ring B of **2** instead of the γ,γ-dimethylallyl group in **1**. Thus, the structure of **2** was established as 5,5′-dihydroxy-6-methyl[6″,6″-dimethylpyrano-(2″,3″:7,8)]-[6‴,6‴-dimethylpyrano (2‴,3‴:4′,3′)] coumarono-chromone, named as triquetrumone B.

Compound **3** was also obtained as a yellow amorphous powder. The molecular formula of $C_{26}H_{24}O_9$ established by HR-EIMS was 34 mass units (2 × OH) higher than in **2**. In the

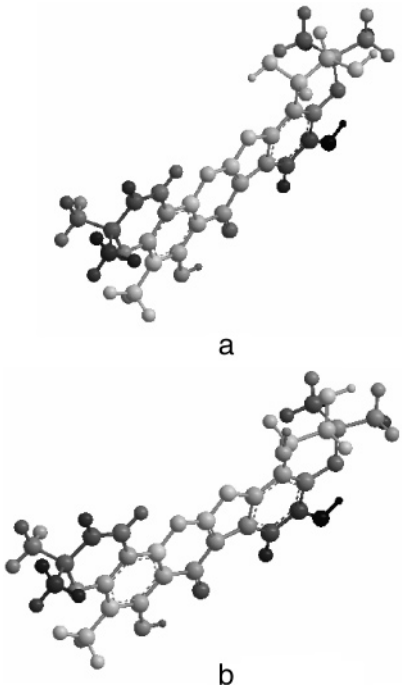

Figure 4. Two isoforms of compound **3** in minimization energy.

^{13}C NMR spectrum of **2**, the double bond signals for C-4‴ (δ_C 114.4) and C-5‴ (δ_C 132.7) were replaced in **3** by signals at δ_C 67.8 (C-4‴) and 76.7 (C-5‴), clearly indicating the location of the two hydroxyl groups at C-4‴ and C-5‴. The vicinal diol groups were trans according to the coupling constant (J = 6.5 Hz) between H-4‴ and H-5‴, which was obviously different from that (J = 3.5 Hz) of 4,5-dihydro-5′α-hydroxy-4′α -methoxy-6a,12a-dehydro-α-toxicarol (*13*), and no nuclear Overhauser effect correlation was observed between these two protons (*13*−*15*). In the trans form, there are still two possible isoforms for these two hydroxyl groups. Using the MOPAC procedure, the three-dimensional molecular configurations of the two isomers of **3** (**Figure 4**) showed that only the 3a (4‴*S*,5‴*R*) configuration was reasonable. In the 3b (4‴*R*,5‴*S*) configuration, a γ-gauche steric compression effect between OH-5‴ and CH_3-7‴ was almost the same as that between OH-5‴ and CH_3-8‴, so that the chemical shifts of C-7‴ and C-8‴ should be almost equal also. In fact, as compared with C-8‴ (δ_C 26.6), the chemical shift of C-7‴ (δ_C 21.0) shifted upfield 5.6 ppm, which definitely indicated that the orientation of OH-5‴ was the same as that of C-7‴ (*16*). Therefore, compound **3** was established as 5,5′-dihydroxy-6-methyl[6″,6″-dimethylpyrano-(2″,3″:7,8)]-[2H-4‴(*S*),5‴(*R*)-dihydroxy-6‴,6‴-dimethylpyrano-(2‴,3‴:4′,3′)] coumaronochromone, named triquetrumone C.

Compound **4** was obtained as a yellow amorphous powder. The HR-FABMS of **4** clearly showed the $[M + 1]^+$ in agreement with the molecular formula $C_{42}H_{38}O_{14}$. According to its EIMS and NMR spectra, an isoflavanone, which was structurally similar to 2′,3′,4′,5,7-pentahydroxyisoflavanone and orientanol E (*17*), could be established. However, in comparison to the NMR data of compound **4** with those of orientanol E, a distinct difference was in the C-3 position, which appeared as a quaternary carbon (δ_C 47.7, s) in **4** instead of a methine (δ_C 47.3, d) in orientanol E. In addition, H_2-2 of **4** exhibited AB doublets (δ_H 4.42, 4.59, d, J = 10.9 Hz) instead of dd (δ_H 4.50, 4.61, dd, J = 11.0, 5.1 Hz) peaks in orientanol E. These features revealed that compound **4** was an isoflavanone dimer possessing a C-3/C-3‴ linkage. An *R* configuration of both chiral centers (C-3 and C-3‴) was tentatively assigned on the basis of the comparison of the optical rotation ($[\alpha]_D = -24.8°$) of **4** with that of (*R*)-saclenone (*18*). In conclusion, the structure of compound **4** was established as 5,2′,4′,5‴,2⁗,4⁗-hexahydroxy-3′,3⁗-dimethoxy[6″,6″-dimethylpyrano(2″,3″:7,8)]-[6⁗,6⁗-dimethylpyrano(2⁗,3⁗:7‴,8‴)]-3,3‴-biisoflavanone and was named (*R*)-triquetrumone D. To the best of our knowledge, this is the first time a bi-isoflavanone possessing a C-3/C-3‴ linkage has been found.

Table 2. Bioactivity of Ethanol Extract of *T. triquetrum* and Triquetrumone A, B, and C to Rabbit Coccidian Eggs

compounds	amount	0 h	24 h	48 h	144 h
triquetrumone A	10.0 mg	43200/5 g	25500/5 g	24310/5 g	20020/5 g
	5.0 mg	43200/5 g	28450/5 g	27210/5 g	20100/5 g
	2.5 mg	43200/5 g	30900/5 g	28200/5 g	20920/5 g
triquetrumone B	10.0 mg	43200/5 g	23350/5 g	21260/5 g	17520/5 g
	5.0 mg	43200/5 g	24400/5 g	21350/5 g	20020/5 g
	2.5 mg	43200/5 g	25550/5 g	23010/5 g	20070/5 g
triquetrumoneC	10.0 mg	43200/5 g	17770/5 g	17730/5 g	17720/5 g
	5.0 mg	43200/5 g	19650/5 g	19630/5 g	18720/5 g
	2.5 mg	43200/5 g	19800/5 g	19780/5 g	18700/5 g
ethanolic extract	30.0 mg	43200/5 g	17750/5 g	16790/5 g	16100/5 g
	20.0 mg	43200/5 g	27800/5 g	26670/5 g	25600/5 g
	10.0 mg	43200/5 g	33560/5 g	31100/5 g	29100/5 g
DMSO	10.0 mL	43200/5 g	35500/5 g	34500/5 g	31050/5 g

Seven known compounds were identified as cyclokievitone (**5**) (*19*), yukovanol (**6**) (*20*), aromadendrin (**7**) (*21*), kaempferol (**8**) (*22*), astragalin (**9**) (*23*), 2-*O*-methyl-L-chiro-inositol (**10**) (*24*), and galactitol (**11**) (*25*), respectively, by comparison of their spectroscopic data (^{1}H, ^{13}C NMR and MS) with those reported in the literature. The structures of the other nine known compounds were established as *p*-hydroxycinnamic acid (**12**), ursolic acid (**13**), betulinic acid (**14**), β-sitosterol (**15**), daucosterol (**16**), stigmasterol (**17**), stigmasta-5,22-dien-3-*O*-β-D-glucopyranoside (**18**), saccharose (**19**), and docosanoic acid (**20**) according to their spectroscopic data (^{1}H, ^{13}C NMR and MS), and their R_f values were then compared with those of the standard samples on TLC.

In comparison with the control sample, the ethanolic extract and compounds **1**−**3** displayed an incipient tendency to reduce the coccidian eggs from a rabbit (**Table 2**), but they could not destroy these eggs completely. In animal clinic bioassays, the ethanolic extract of the whole plant of *T. triquetrum* also showed mild anthelminthic activity to the parasites living in sheep and rabbits (*26*, *27*). According to a report from a human clinic (*28*), the aqueous decoction of the whole plant of *T. triquetrum* showed ca. 50% healing rate to the helminthiasis. All of these bioassay results were similar and suggested that *T. triquetrum* possessed mild anthelminthic bioactivity.

In a feeding experiment, the ethanol extract of *T. triquetrum* was given to small white mice (the Kunming mouse). *T. triquetrum* did not show any obvious poisonous effects to the mice at 2.5 g/kg. When the dosage was more than 5.0 g/kg, the mice appeared to be cachexia (*29*). The analysis of the amino acid implied that the content of amino acid in *T. triquetrum* was similar to other Chinese leguminous feeds (*30*). These two results suggested that a nutrient feed usage of *T. triquetrum* was reasonable.

Isoflavonoids are a major group of phytoestrogens possessing a wide variety of biological activity (*31*). The binding response of estrogen receptors (ER-α and ER-β) to the new compounds **1**−**4** was determined as reported in the literature (*5*). Only compound **3** showed a significant binding ability. The preliminary test showed its binding ability to ER-α as 54% at a concentration of 6.24 μg/mL and to ER-β as 51% at a

concentration of 0.78 μg/mL. Compound **1** showed a 55% binding ability to ER-α at 200 μg/mL, and compound **4** showed a 38% binding ability to ER-α at 200 μg/mL. Compound **2** was not active at 200 μg/mL to ER-α and ER-β.

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