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FOUR CHROMONES FROM *ERIOSEMA TUBEROSUM*

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Key Word Index–*Eriosema tuberosum*; Leguminosae; roots; chromones; antifungal activity.

Abstract—Four new prenylated chromones have been isolated from a dichloromethane extract of the roots of *Eriosema tuberosum*. Their structures were elucidated by spectroscopic methods. Eriosematin A exhibited antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* in TLC bioautographic assays.

INTRODUCTION

In the course of our studies on the secondary metabolites of the Chinese plant *Eriosema tuberosum*, nine phenolic compounds were described in a previous paper [1]. Continuation of this work on the antifungal metabolites of this species has led to the characterization of a further four new prenylated chromones from a dichloromethane extract of the roots. The structural elucidation and antifungal activities of these compounds are reported.

RESULTS AND DISCUSSION

Compound **1**, obtained as yellow powder, had a molecular formula of $C_{19}H_{20}O_4$ based on TSP- and EI-mass spectra and ^{13}C NMR spectral data. The ^1H NMR spectrum revealed clearly the presence of an *iso*prenyl group [δ1.67, 1.79 (2 × Me), 3.32 (1 × CH_2) and 5.21 (1 × CH)] and a chelated hydroxyl group (δ12.86). The remaining proton signals were assigned to a dimethylchromene ring [(δ1.45 (2 × Me), 6.65 and 5.57 (J = 10 Hz)] and an α,β-unsaturated ketone moiety [δ7.74 and 6.18 (J = 6 Hz)]. These findings were quite similar to the ^1H NMR data (Table 1) or eriosematin [1]. However, careful comparison of the chemical shift of the chelated proton in **1** and eriosematin (δ12.86 for **1** and 12.78 for eriosematin) suggested that the dimethylchromene ring was fused at the C-7 and C-8 positions on the A ring of **1** instead of C-6 and C-7 in eriosematin [2,3]. The ^{13}C NMR data (Table 2) of **1** showed that it has an angular fusion pattern of the dimethylchromene ring on the A ring because the signals of C-5, C-6, C-8 and C-9 were different from those of eriosematin. This angular fusion pattern of **1** was finally confirmed by the long-range coupling observed in a FLOCK experiment (Fig. 1) [4]. Thus, compound **1** was characterized as 5-hydroxy-6-γ,γ-dimethylallyl-6′,6′-dimethyl-pyrano (2′, 3′: 7, 8) chromone, named iso-eriosematin.

Compound **2**, obtained as yellow powder, possessed a molecular formula of $C_{14}H_{14}O_4$, as deduced from the EI-mass spectrum and analysis of ^{13}C NMR spectral data (Table 2). In the ^1H NMR spectrum of **2**, a set of signals characteristic of an isoprenyl group [δ1.73, 1.81 (2 × Me), 3.45 (1 × CH_2) and 5.21 (1 × CH)] was unambiguously assigned. Moreover, the presence of two olefinic protons resonating at δ7.80 (J = 6 Hz) and 6.22 (J = 6 Hz) and a chelated hydroxyl group (δ12.51) suggested that **2** was a chromone. However, the absence of the proton signals for a dimethylchromene ring, which were replaced by one proton at δ6.32, indicated that **2** was not completely substituted on the A ring. This assumption was subsequently confirmed by the ^{13}C NMR spectrum and DEPT of **2**, which provided a total of 14 resonance lines consisting of two methyl, one methylene, four methine and seven quaternary carbons. Comparison of these data, especially the signal resonating at δ99.8, with those of heteroflavanones B and C, leachianones F and G, 2-hydroxymethlalloptaeroxylins 1 and 2 [3, 5–7] revealed that C-5 and C-7 were substituted by a hydroxyl group, with C-8 prenylated. Thus, the structure of compound **2** was established as 5,7-dihydroxy-8-γ,γ-dimethylallyl chromone, named eriosematin A.

The molecular formula of compound **3** was found to be $C_{19}H_{24}O_5$, by EI-mass ([M]$^+$ at m/z 332) and ^{13}C NMR spectral data (Table 2). The ^1H NMR spectrum showed some common signals similar to those of compound **2**. Moreover, the occurrence of a six-proton singlet at δ1.29 and a pair of two-proton triplets (J = 6 Hz) at δ1.78 and δ2.77 indicated the presence of a 3-hydroxy-3,3-dimethylbutyl group. The EI-mass spectrum revealed

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Table 1. ^{1}H NMR data of compounds **1**–**4** and eriosematin (200 MHz)

H	1	Eriosematin	2	3	4
2	7.74 (*d*, *J* = 6)	7.75 (*d*, *J* = 6)	7.80 (*d*, *J* = 6)	7.77 (*d*, *J* = 6)	7.77 (*d*, *J* = 6)
3	6.18 (*d*, *J* = 6)	6.16 (*d*, *J* = 6)	6.22 (*d*, *J* = 6)	6.18 (*d*, *J* = 6)	6.20 (*d*, *J* = 6)
5-OH	12.86 (*s*)	12.78 (*s*)	12.51 (*s*)	12.80 (*s*)	12.90 (*s*)
6			6.32 (*s*)		
7-OH			6.54 (*br s*)	8.30 (*br s*)	
Chromene ring					
4′	6.65 (*d*, *J* = 10)	6.69 (*d*, *J* = 10)			
5′	5.57 (*t*, *J* = 10)	5.59 (*d*, *J* = 10)			
6′	1.45 (*s*)	1.42 (*s*)			
6′	1.45 (*s*)	1.42 (*s*)			
Isoprenyl					
1′			3.45 (*d*, *J* = 8)	3.44 (*d*, *J* = 6)	3.39 (*d*, *J* = 6)
2′			5.21 (*t*, *J* = 8)	5.20 (*t*, *J* = 6)	5.20 (*t*, *J* = 6)
4′			1.73 (*s*)	1.68 (*s*)	1.82 (*s*)
5′			1.81 (*s*)	1.69 (*s*)	1.80 (*s*)
1″	3.32 (*d*, *J* = 6)	3.34 (*d*, *J* = 7.4)		2.77 (*t*, *J* = 6)	3.41 (*d*, *J* = 6)
2″	5.21 (*t*, *J* = 6)	5.13 (*t*, *J* = 7.4)		1.78 (*t*, *J* = 6)	4.94 (*d*, *J* = 6)
4″	1.79 (*d*, *J* = 2)	1.62 (*s*)		1.29 (*s*)	1.75 (*s*)
5″	1.67 (*d*, *J* = 2)	1.67 (*s*)		1.29 (*s*)	1.72 (*s*)

Eriosematin recorded under same conditions as compounds **1**–**4** [1].

Table 2. ^{13}C NMR data of compounds **1**–**3** and eriosematin (50 MHz, measured in $CDCl_3$)

C	1	Eriosematin	2	3
2	155	155.3	155.6	155.3
3	111.2	110.9	111	110.7
4	182	182.3	182.4	182.4
5	158.9	154.8	160.2	153.4
6	112.9	106.3	99.8	112.8
7	157.2	156.9	155.2	156.9
8	100.9	107.6	106.7	106.6
9	150.6	154.7	161	159.5
10	106.4	105.4	105.6	105.9
Chromene ring				
4′	127.6	128.1		
5′	114.9	115.7		
3′	77.8	78.1		
6′	28.1	28.2		
6′	28.1	28.2		
Isoprenyl				
1′			21.7	21.9
2′			121	121.9
3′			135	133
4′			17.9	17.8
5′			25.8	25.7
1″	21.2	21.9		16.5
2″	121.8	121.8		41.2
3″	131.7	131.5		72.2
4″	17.9	17.4		29.6
5″	25.8	25.7		29.6

Eriosematin recorded under the same conditions as compounds **1**–**3** [1].

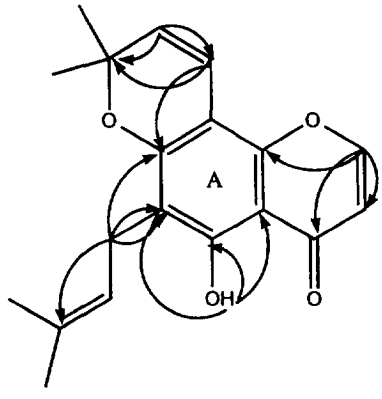
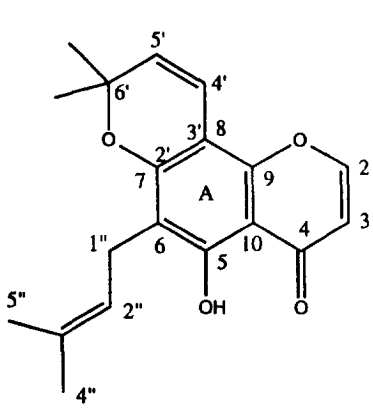
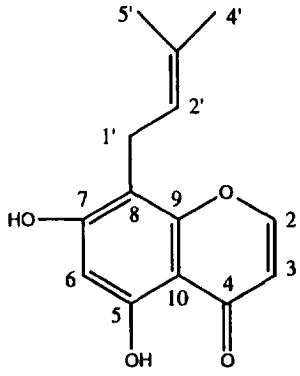


Fig. 1. Long-range couplings between protons and carbons deduced from the FLOCK NMR spectrum of compound **1**.

that the $[M]^+$ was fragmented through a process of water loss from the parent ion followed by successive eliminations of two γ,γ-dimethylallyl groups. Loss of 55 (C_4H_8) in the cleavage pathway A (Fig. 2) suggested that the C-6 position was substituted by one γ,γ-dimethylallyl group and the cleavage pathway B indicated that the C-8 position was connected with another γ,γ-dimethylallyl group [8,9]. After comparing the ^{1}H and ^{13}C NMR spectral data of **3** with those of analogues [10,11], the presence of a 3-hydroxy-3,3-dimethylbutyl group could be ascertained. The long-range couplings observed in a FLOCK experiment (Fig. 3) finally confirmed that the C-6 position was connected with the 3-hydroxy-3,3-dimethylbutyl group. Therefore, the structure of **3** was



1



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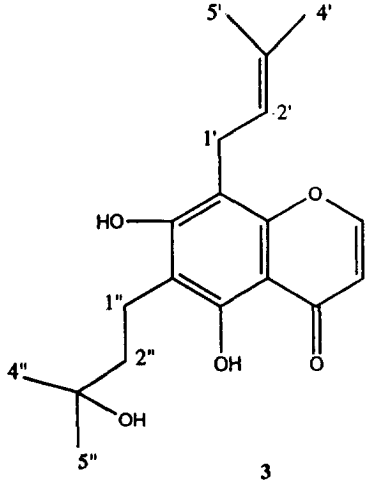

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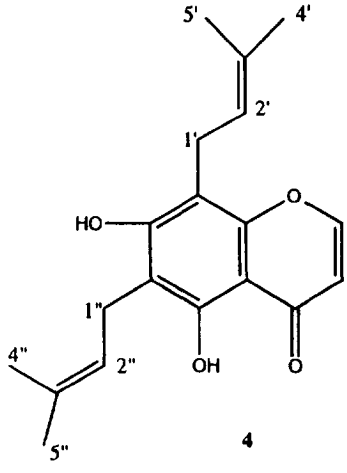

4

established as 5,7-dihydroxy-6-(3-hydroxy-3,3-dimethylbutyl)-8-γ,γ-dimethylallyl chromone, named eriosematin B.

Compound **4** had a molecular formula $C_{19}H_{22}O_4$, as deduced from the EI-mass spectrum ($[M]^+$ at m/z 314, 18 amu lower than that of compound **3**) and ^1H NMR spectral data (Table 1). The ^1H NMR spectrum demonstrated the presence of two γ,γ-dimethylallyl groups and a chelated hydroxyl group. The EI-mass data were similar to those of compound **3**, but the fragment produced by loss of water from the $[M]^+$ could not be seen. These findings could only be explained by assuming that compound **4** was a dehydrated derivative of compound **3**. Therefore, the structure of **4** was characterized as 5,7-dihydroxy-6-γ,γ-dimethylallyl-8-γ,γ-dimethylallyl chromone, named eriosematin C.

Despite their rather simple structures, the four chromones isolated from the lipophilic root extract of *E. tuberosum* are new natural compounds. All four chromones were evaluated for their antifungal activities against *Cladosporium cucumerinum* and *Candida albicans* according to established methods [12, 13]. Compared with miconazole (activity against *C. albicans* is 0.01 μg) and propiconazole (activity against *C. cucumerium* is 0.001 μg) the minimal amount of compound **2** needed to inhibit fungal growth on TLC plates was 2.5 μg for both fungi; the other three compounds were not active. It appears that prenylation at the C-6 position reduces antifungal activities significantly.

EXPERIMENTAL

Details of instruments and chromatographic conditions used in this work were essentially the same as described in ref. [1].

Plant material. Roots of *E. tuberosum* (1.67 kg) were collected in June 1992 in Fu Ming County, Yunnan Province, P. R. China. A voucher specimen is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Science.

Extraction and isolation. Powdered roots were extracted at room temp. successively with CH_2Cl_2 and MeOH.

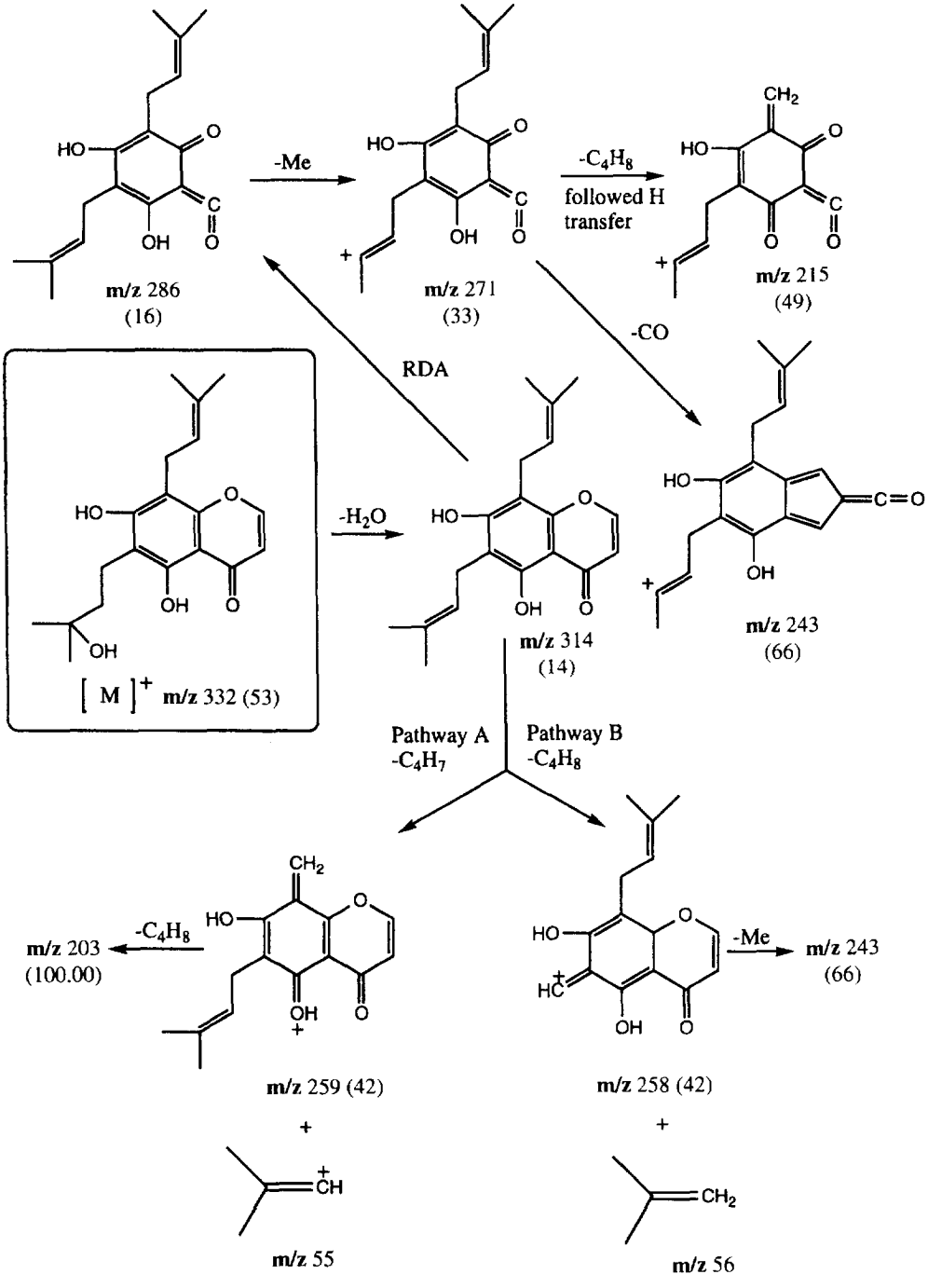

Fig. 2. Mass spectral fragmentations of compound **3** showing different possible cleavage pathways.

The CH_2Cl_2 extract (120 g) was submitted to CC on silica gel (40–63 μm, 2000 g) using step-gradient elution (*n*-hexane–EtOAc, 9:1 to 0:100); 15 frs (A–O) were collected. Fr. H (4 g) was chromatographed by MPLC on a Diol column (*n*-hexane–EtOAc, 4:1) yielding 5 frs. Fr. 3 was submitted to semi-prep. HPLC on RP-18 (MeOH–H_2O, 7:3) to give compounds **2** (15 mg) and **3** (6.6 mg). Fr. G (2 g) was submitted to CC on silica gel eluted with *n*-hexane–EtOAc (2:1) giving three frs a–c. Fr. a was chromatographed by semi-prep. HPLC on RP-18 (MeOH–H_2O, 7:3) to give compounds **1** (14 mg) and **4** (1 mg).

Compound **1** [*5-hydroxy-6-γ,γ-dimethylallyl-6′,6′-dimethyl -pyrano* (2′, 3′: 7, 8) *chromone, iso-eriosematin*]. Yellow powder, mp 97–101°. TLC (silica gel, *n*-hexane–EtOAc, 2:1): R_f 0.55. UV: λ_{max} nm (MeOH) (log ε): 265 (4.14), 335 (3.98); + NaOMe: 272, 311; +$AlCl_3$/HCl: 278, 327; +NaOAc/$B(OH)_3$: 265, 299. IR ν_{max} (KBr) cm^{-1}: 3450,

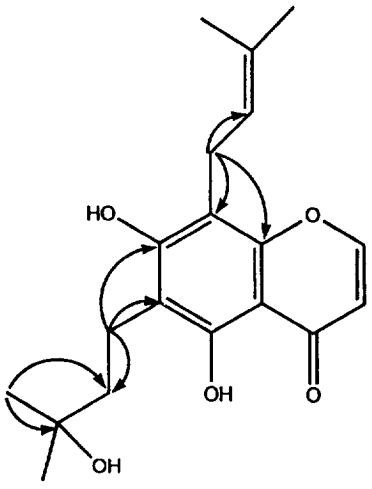

Fig. 3. Long-range couplings between protons and carbons observed in the FLOCK NMR spectrum of compound **3**.

2950, 1650, 1570, 1420, 1280, 1126, 850. TSPMS (pos. mod.) *m/z* (rel. int.): 313 $[M + H]^+$ (100); El-MS *m/z* (rel. int.): 312 $[M]^+$ (52.32): 297 (100), 269 (27.28), 257 (34.23), 241 (23.31), 215 (9.82). ^1H, ^{13}C NMR: Tables 1 and 2.

Compound **2** [*5,7-dihydroxy-8-γ,γ-dimethylallyl chromone, eriosematin A*]. Yellow powder, mp 64–68°. TLC (silica gel, *n*-hexane–EtOAc 2:1): R_f 0.54. UV: λ_{max} nm (MeOH) (log ε): 258 (4.16), 302 (4.10); +NaOMe: 272, 335; + $AlCl_3$/HCl: 268, 311; + NaOAc/$B(OH)_3$: 298, 306. IR ν_{max} (KBr) cm^{-1}: 3500 2950, 1670, 1625, 1415, 1300, 1205, 1110, 850. DCl-MS (NH_3, pos.) *m/z* (rel. int.): 246 $[M]^+$ (100); El-MS *m/z* (rel. int.):246 $[M]^+$ (84.93), 231 (100), 191 (57.22), 178 (29.93). ^1H and ^{13}C NMR: Tables 1 and 2.

Compound **3** [*5,7-dihydroxy-6-(3-hydroxy-3,3-dimethylbutyl)-8-γ,γ-dimethylallyl chromone, eriosematin B*]. Powder, mp 71–74° TLC (silica gel, *n*-hexane: EtOAc 2:1): R_f 0.38. UV: λ_{max} nm (MeOH) (log ε): 264 (4.02), 296 (3.64); +NaOMe: 271, 339; + $AlCl_3$/HCl: 273, 315; + NaOAc/$B(OH)_3$; 263, 335. IR ν_{max} (KBr) cm^{-1}: 3450, 2980, 1650, 1580, 1420, 1380, 1260, 1150, 1184, 850. El-MS *m/z* (rel. int.): 332 $[M]^+$ (52.82), 314 (14.06), 286 (15.45), 271 (32.54), 259 (42.11), 258 (42.11), 215 (48.81), 243 (66.37), 203 (100). ^1H and ^{13}C NMR: Tables 1 and 2.

Compound **4** [*5,7-dihydroxy-6-γ,γ-dimethylallyl-8-γ,γ-dimethylallyl chromone, eriosematin C*]. W Powder, mp 140–142°. TLC (silica gel, *n*-hexane–EtOAc, 2:1): R_f 0.54. UV: λ_{max} nm (MeOH) (log ε): 261 (4.01), 307 (3.99); + NaOMe: 265, 328; + $AlCl_3$/HCl: 265, 310; +NaOAc/$B(OH)_3$: 264, 311. IR ν_{max} (KBr) cm^{-1}: 3500 2980, 1655, 1570, 1450, 1380, 850. El-MS *m/z* (rel. int.): 314 $[M]^+$ (32.88), 286 (15.45), 271 (29.46), 259 (25.84), 258 (17.11), 215 (49.08), 243 (43.06), 203 (97.50), ^1H NMR: Tables 1.

Bioassays. Bioautography with *Cladosporium cucumerinum* and *Candida albicans* for evaluating antifungal activity of compounds **1**–**4** was performed by the methods described in refs [12, 13].

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