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FURTHER CHROMONES FROM *ERIOSEMA TUBEROSUM*

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

Abstract—Two new prenylated chromones, eriosematins D and E, have been isolated from a dichloromethane extract of the roots of *Eriosema tuberosum*. Their structures were elucidated by spectroscopic methods, including 2D NMR heteronuclear correlation spectra. In solution, both compounds were shown to be in equilibrium at room temperature; stable derivatives were obtained after acetylation. Both chromones exhibited antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* in TLC bioautographic assays. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

As part of our search for new antifungal lead compounds from plants, we have investigated the Chinese medicinal plant, *Eriosema tuberosum*. In previous papers [1, 2], we described the isolation and characterization of a series of flavanones, isoflavones and chromones possessing antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* in TLC bioautographic assays [3–5]. The present work deals with the isolation and structural elucidation of two additional new antifungal chromones (**1** and **2**), together with a known compound, lupinifolin (**3**). In addition, the dehydration products eriosematin (**4**), and isoeriosematin (**5**), as well as their acetylated derivatives (**6** and **7**), were obtained by acetylation of a mixture of **1** and **2**.

RESULTS AND DISCUSSION

A dichloromethane root extract of *E. tuberosum* was separated into 15 fractions by chromatography on silica gel as previously described [1]. Further chromatography of fraction H by MPLC followed by preparative HPLC on Diol material afforded pure compound **1** and **2** containing a small quantity of **1**. When the collected eluate was left standing, the percentage of **1** in **2** gradually increased. This information suggested that **1** and **2** were in equilibrium at room temperature. The relative stability of **1** (>95% purity in $CDCl_3$ for 2 hr) allowed the measurement of the ^1H and ^{13}C NMR spectra of the pure compound, while the NMR spectra of **2** could only be recorded for the mixture containing *ca* 80% of **2** and 20% of **1**.

Compound **1** was obtained as a yellow powder. The EI mass spectrum exhibited a $[M]^+$ at *m/z* 330. The molecular formula was deduced as $C_{19}H_{22}O_5$ from ^1H and ^{13}C NMR data. The ^1H NMR spectrum of **1** revealed the presence of a chelated hydroxyl group, a dimethylallyl substituent and a dimethylchromene ring system. Comparison with the NMR data for previously reported compounds [1, 2] indicated that **1** was a chromone derivative. However, the presence of three typical one-proton double doublets which were attributed to H-2 and H-3 (2H), together with the ^{13}C NMR signals at δ 94.3 (C-2) and 42.6 (C-3), revealed that the C-2, C-3 bond was saturated. Moreover, the resonance of C-2, (δ 94.3) indicated the presence of a hydroxyl group at this position. The EI mass spectrum of **1** exhibited the typical cleavage of chromones [1]. The fragment ion peak at *m/z* 286 (10%), due to RDA-cleavage, confirmed that the B ring was bearing a hydroxyl group. The chelated hydroxyl proton resonating at δ 12.23 and the olefinic proton H-4″ observed at δ 6.53 (both appearing at higher fields than those of eriosematin [1]) indicated the angular fusion pattern of the rings. This was confirmed by long-range couplings between H-4″ and C-8 and C-9 (Fig. 1) in a FLOCK experiment [6]. Therefore, the structure of **1**, named eriosematin D, was established as 2,5-dihydroxy-6-γ,γ-dimethylallyl - 6″,6″ - dimethyl - pyrano(2″,3″:7,8)chromone.

Compound **2**, was obtained as a yellow powder containing *ca* 20% of **1**. In the EI mass spectrum, a $[M]^+$ was observed at *m/z* 330. Compound **2** was found to be an isomer of **1** from its ^1H and ^{13}C NMR data. The ^1H NMR spectrum of **2** was quite similar to

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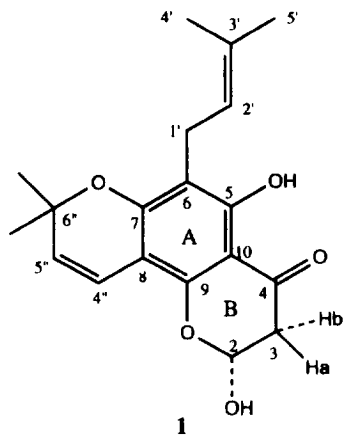
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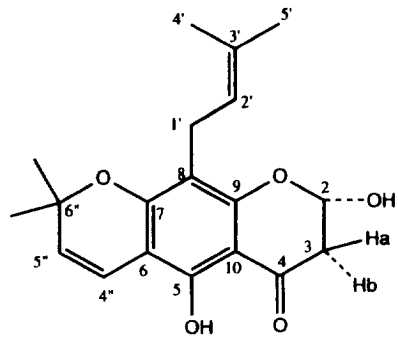
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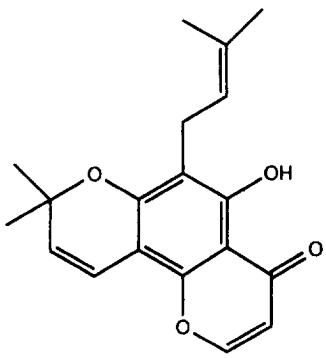
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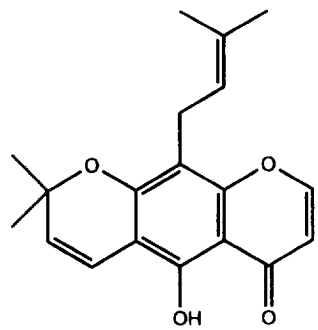
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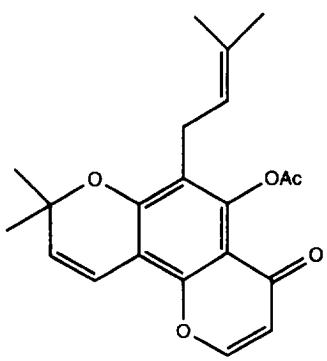
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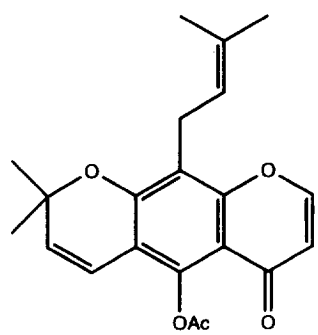
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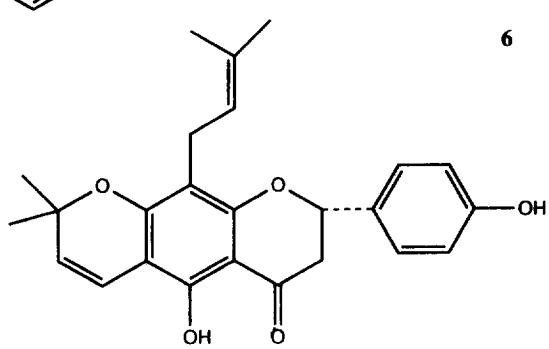
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that of **1**. Differences were observed for the signals appearing at δ 12.14 (chelated hydroxyl proton) and 6.59 (H-4″), suggesting the linear fusion pattern of the rings [1]. This was definitely established from a long-range coupling observed between H-1′ and C-9 in a FLOCK experiment (Fig. 1). Thus, the structure of **2**, named eriosematin E, was deduced as 2,5-dihydroxy-8-γ,γ-dimethylallyl-6″,6″-dimethyl-pyrano(2″,3″:7,6) chromone.

In order to stop the interconversion of **1** and **2**, 200 mg of fraction 4 containing compounds **1** and **2** were acetylated with pyridine–acetic anhydride (1:1). After acetylation, four compounds (**4–7**) were obtained from the reaction mixture. Compounds **4** and **5** were identified as eriosematin (**4**) and isoeriosematin (**5**) [1, 2], the dehydrated derivatives of **2** and **1**, respectively. Compounds **6** and **7** were shown to be the acetylated products of **4** and **5**, respectively, according to their ^{1}H, ^{13}C NMR and EI mass spectral data. There was no acetylated product which came directly from **1** or **2**. This indicated that, under the conditions of the acetylation, **1** and **2** transferred into dehydrated derivatives before the hydroxyl group at C-2 was acetylated.

Compound **3** was obtained after filtration of fraction 2 over Sephadex LH-20 in methanol. It was identified as lupinifolin by comparison of its ^{1}H, ^{13}C NMR and EI mass spectral data with literature values [7–9]. Lupinifolin was previously isolated from *Tephrosia lupinifolia* [7], *Mundulea sericea* [8] and *Lonchocarpus minimiflorus* [9].

Following the procedure described previously [3–5], **1–3**, **6** and **7** were subjected to antifungal testing against *C. cucumerinum* and *C. albicans*. The minimum amounts of **1–3** required to inhibit growth of *C. cucumerinum* on TLC plates were 0.5 (**1** and **2**) and 10 μg (**3**), respectively. On the other hand, inhibition of *C. albicans* needed as much as 30 μg of **1–3**. Acetylated derivatives **6** and **7** were inactive against both fungi. For comparison, propiconazole was active at 0.1 μg against *C. cucumerinum*, while miconazole inhibited growth of *C. albicans* at 0.001 μg.

To our knowledge, **1** and **2** are the first examples of natural chromones which exist in equilibrium in solution. This phenomenon results from a Wessely–Moser rearrangement, involving opening of the pyrone ring followed by ring closure with either of the two phenolic hydroxyl groups *ortho* to the carbonyl function. A mixture of the two isomers is thus produced [10].

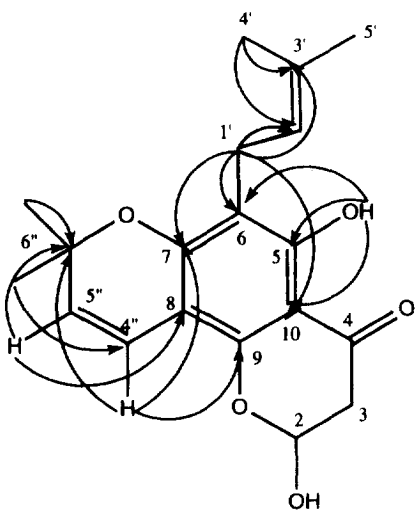
The isolation and structural determination of further antifungal constituents of *E. tuberosum* is in progress.

EXPERIMENTAL

General. Mps: uncorr. For open CC, silical gel (40–63 μm, Merck) was used. TLC was carried out on silica gel precoated aluminium sheets and HPTLC Diol glass plates (Merck). Prep. HPLC was performed on a Lichrosorb Diol column (7 μm, 250 × 16 mm i.d., Knaver). ^{1}H and ^{13}C NMR were recorded at 200 and 50 MHz, respectively, with TMS as int. standard. EI-MS were obtained at 70 eV on a triple-stage quadrupole instrument.

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

Extraction and isolation. Powdered roots were extracted at room temp. successively with CH_2Cl_2 and MeOH. The CH_2Cl_2 extract (120 g) was submitted to CC on silica gel (40–63 μm, 2 kg) using step-gradient



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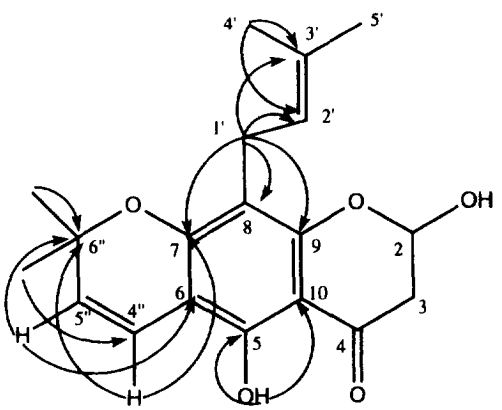

2

Fig. 1. Long-range couplings observed in FLOCK NMR spectra of compounds **1** and **2**. Atom numbering as used in the literature.

elution (petrol–EtOAc, 9:1–0:1); 15 frs (A–O) were collected. Fr. H (4 g) was subjected to MPLC on a Diol column (petrol–EtOAc, 4:1) to afford 5 subfrs (frs 1–5). Compounds **1** (40 mg) and **2** containing 20% **1** (80 mg) were isolated from fr. 4 by prep. HPLC on a Diol column (petrol–EtOAc, 6:1). Compound **3** (200 mg) was obtained from fr. 2 after purification over Sephadex LH-20 with MeOH.

Acetylation of fr. 4. A 200 mg portion of fr. 4 was kept in pyridine–Ac_2O (1:1, 8 ml) at room temp. for 12 hr. After usual work-up, **4** (6.9 mg), **5** (35 mg), **6** (40 mg) and **7** (60 mg) were isolated from the crude mixt. by semi-prep. HPLC on a Diol column (petrol–EtOAc, 17:13; 10 ml min^{-1}).

Eriosematin D (**1**) {2,3-*dihydro*-2,5-*dihydroxy*-8,8-*dimethyl*-6-(3-*methylbut*-2-*enyl*)-4*H*,8*H*-*pyrano*[2,3-f][1]*benzopyran*-4-*one*}. Yellow powder, mp 110–112°. $[\alpha]_D^{25}$ −0.02° (MeOH, *c* 0.06). TLC (Diol, petrol–EtOAc, 2:1) R_f 0.49, TLC (silica gel, petrol–EtOAc, 2:1) R_f 0.43. UV λ_{max}^{MeOH} nm (log ε): 265 (3.8), 315 (3.2), 365 (2.4). IR ν_{max}^{KBr} cm^{-1}: 3250, 2900, 1600, 1480, 1350, 1280, 980, 910, 880. EI MS *m/z* (rel. int): 330 (63), 315 (100), 286 (10), 271 (16), 259 (13), 243 (11), 215 (46). ^1H NMR (200 MHz, $CDCl_3$): δ 5.78 (1H, *dd*, *J* = 8.0, 4.0 Hz, H-2), 2.98 (1H, *dd*, *J* = 16.0, 4.0 Hz, H-3a), 2.78 (1H, *dd*, *J* = 16.0, 8.0 Hz, H-3b), 12.23 (1H, *s*, C-5-OH), 6.53 (1H, *d*, *J* = 10.0 Hz, H-4″), 5.49 (1H, *d*, *J* = 10.0 Hz, H-5″), 1.43 (6H, *s*, 6″-Me × 2), 3.22 (2H, *d*, *J* = 8.0 Hz, H-1′), 5.18 (1H, *t*, *J* = 8.0 Hz, H-2′), 1.66 (3H, *br s*, H-4′), 1.77 (3H, *br s* H-5′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 94.5 (C-2), 42.5 (C-3), 193.9 (C-4), 160.8 (C-5), 110.1 (C-6), 159.8 (C-7), 102.0 (C-8), 151.2 (C-9), 102.0 (C-10), 126.5 (C-4″), 115.6 (C-5″), 77.9 (C-6″), 28.3 (C-6″ × 2 Me), 20.8 (C-1′), 122.2 (C-2′), 131.3 (C-3′), 17.8 (C-4′), 25.8 (C-5′).

Eriosematin E (**2**) {2,3-*dihydro*-2,5-*dihydroxy*-8,8-*dimethyl*-10-(3-*methylbut*-2-*enyl*)-4*H*,8*H*-*pyrano*[2,3-g[1]*benzopyran*-4-*one*}. Yellow powder, mp 108–109°. $[\alpha]_D^{25}$ −0.05° (MeOH, *c* 0.03). TLC (Diol, petrol–EtOAc, 2:1) R_f 0.37, TLC (silica gel, petrol–EtOAc, 2:1) R_f 0.43. UV λ_{max}^{MeOH} nm (log ε): 265 (3.8), 315 (3.2), 365 (2.4). IR ν_{max}^{KBr} cm^{-1}: 3220, 2890, 1600, 1500, 1400, 1350, 1290, 1250, 1100, 980, 910. EI MS *m/z* (rel. int): 330 (63), 315 (100), 286 (10), 271 (16), 259 (13), 243 (11), 215 (46). ^1H NMR (200 MHz, $CDCl_3$): δ 5.77 (1H, *dd*, *J* = 8.0, 6.0 Hz, H-2), 2.97 (1H, *dd*, *J* = 17.0, 6.0 Hz, H-3a), 2.77 (1H, *dd*, *J* = 17.0, 8.0 Hz, H-3b), 12.14 (1H, *s*, C-5-OH), 6.59 (1H, *d*, *J* = 10.0 Hz, H-4″), 5.47 (1H, *d*, *J* = 10.0 Hz, H-5″), 1.42 (6H, *s*, 6″-Me × 2), 3.18 (2H, *d*, *J* = 8.0 Hz, H-1′), 5.11 (1H, *t*, *J* = 8.0 Hz, H-2′), 1.64 (3H, *br s*, H-4′), 1.76 (3H, *br s* H-5′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 94.3 (C-2), 42.5 (C-3), 194.6 (C-4), 156.3 (C-5), 102.5 (C-6), 159.8 (C-7), 109.1 (C-8), 155.6 (C-9), 103.1 (C-10), 126.5 (C-4″), 115.5 (C-5″), 77.9 (C-6″), 28.3 (C-6″ × 2 Me), 21.4 (C-1′), 122.2 (C-2′), 131.2 (C-3′), 17.8 (C-4′), 25.7 (C-5′).

Compound **6** {5-*acetoxy*-8,8-*dimethyl*-10-(3-*methylbut*-2-*enyl*)-4*H*,8*H*-*pyrano*[2,3-*g*][1]*benzopyran*-4-*one*}. Yellow powder, mp 62–63°. TLC (silica gel, petrol–EtOAc, 2:1) R_f 0.27. UV λ_{max}^{MeOH} nm (log ε): 235 (3.3), 265 (4.8), 340 (3.2); IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1770, 1640, 1600, 1470, 1420, 1370, 1270, 1200, 900. EI MS *m/z* (rel. int): 354 (12), 312 (48), 297 (100), 269 (7), 241 (4). ^1H NMR (200 MHz, $CDCl_3$): δ 7.69 (1H, *d*, *J* = 6.0 Hz, H-2), 6.16 (1H, *d*, *J* = 6.0 Hz, H-3), 6.47 (1H, *d*, *J* = 10.0 Hz, H-4″), 5.74 (1H, *d*, *J* = 10.0 Hz, H-5″), 1.45 (6H, *s*, 6″-Me × 2), 3.43 (2H, *d*, *J* = 6.0 Hz, H-1′), 5.15 (1H, *t*, *J* = 6.0 Hz, H-2′), 1.66 (3H, *s*, H-4′), 1.66 (3H, *s*, H-5′), 2.42 (3H, *s*, OAc-5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 153.4 (C-2), 113.4 (C-3), 176.4 (C-4), 142.3 (C-5), 169.6 (C-5-$CH_3\underline{CO}$), 21.0 (C-5-$\underline{CH_3}CO$), 115.1 (C-6), 155.8 (C-7), 112.0 (C-8), 155.0 (C-9), 112.5 (C-10), 131.8 (C-4″), 115.4 (C-5″), 78.2 (C-6″), 28.3 (C-6″ × 2 Me), 21.9 (C-1′), 121.0 (C-2′), 132.2 (C-3′), 17.9 (C-4′), 25.7 (C-5′).

Compound **7** {5-*acetoxy*-8,8-*dimethyl*-6-(3-*methylbut*-2-*enyl*)-4*H*,8*H*-*pyrano*[2,3-*g*][1]*benzopyran*-4-*one*}. Yellow powder, mp 63–65°. TLC (silica gel, petrol–EtOAc, 2:1) R_f 0.30. UV λ_{max}^{MeOH} nm (log ε): 245 (3.4), 275 (4.3), 325 (3.2). IR ν_{max}^{KBr} cm^{-1}: 3550, 2900, 1750, 1610, 1580, 1440, 1350, 1250, 1180, 1100, 1000, 880. EI MS *m/z* (rel. int): 354 (42), 312 (100), 297 (63), 269 (21), 257 (26), 241 (13). ^1H NMR (200 MHz, $CDCl_3$): δ 7.67 (1H, *d*, *J* = 6.0 Hz, H-2), 6.12 (1H, *d*, *J* = 6.0 Hz, H-3), 6.71 (1H, *d*, *J* = 10.0 Hz, H-4″), 5.66 (1H, *d*, *J* = 10.0 Hz, H-5″), 1.46 (6H, *s*, 6″-Me × 2), 3.25 (2H, *d*, *J* = 6.0 Hz, H-1′), 5.06 (1H, *t*, *J* = 6.0 Hz, H-2′), 1.64 (3H, *s*, H-4′), 1.65 (3H, *s*, H-5′), 2.42 (3H, *s*, OAc-5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 153.1 (C-2), 113.7 (C-3), 175.9 (C-4), 146.8 (C-5), 169.6 (C-5-$CH_3\underline{CO}$), 21.1 (C-5-$\underline{CH_3}CO$), 111.5 (C-6), 155.5 (C-7), 111.5 (C-8), 151.6 (C-9), 107.3 (C-10), 129.5 (C-4″), 115.0 (C-5″), 78.2 (C-6″), 28.2 (C-6″ × 2 Me), 22.4 (C-1′), 121.1 (C-2′), 132.0 (C-3′), 17.9 (C-4′), 25.7 (C-5′).

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