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# POLYPHENOLS FROM *ERIOSEMA TUBEROSUM*

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**Key Word Index**—*Eriosema tuberosum*; Leguminosae; roots; polyphenols; antifungal compounds.

**Abstract**—A dichloromethane extract of the roots of *Eriosema tuberosum* exhibited antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* using TLC bioautography. Bioassay-directed fractionation led to the isolation of four new compounds, eriosemaones A–D, together with a known compound, flemichin-D, as the active constituents. Three inactive polyphenols were also isolated after methylation, together with one new chromone, eriosematin. Structures were determined by spectroscopic analysis and from chemical evidence.

## INTRODUCTION

The genus *Eriosema* is composed of *ca* 140 species, most of them distributed in tropical areas [1]. Indians around Kunana, Venezuela, use the root decoction of *E. rufum* G. Don. against sterility in women and give it to accelerate delivery in childbirth [2]. Miao, Tai and Yi minority peoples living in Yunnan Province, China, use the root decoction of *E. tuberosum* (Ham.) Wang et Tang to treat diarrhoea, orchitis, hydrophobia and as a detoxifying medicine [3]. Until now, no studies on the chemical constituents of this genus have been reported. A dichloromethane extract of the roots of *E. tuberosum* exhibited antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* in bioautographic assays on silica gel TLC plates [4]. The isolation and structural determination is described for the fungicidal constituents **2**–**6**, as well as for **1** and the three methylated derivatives **7**–**9**, which are not active in this biological test.

## RESULTS AND DISCUSSION

Compound **1**, eriosematin, was obtained as yellow crystals. The $^{13}C$ NMR spectrum of **1** revealed 19 carbon atoms (DEPT: 9 × C, 5 × CH, 1 × $CH_2$ and 4 × Me). The EI and thermospray (TSP) mass spectral data ($[M]^+$ 312) together with the $^{13}C$ NMR data suggested the molecular formula to be $C_{19}H_{20}O_4$. The presence of a $\gamma,\gamma$-dimethylallyl ( = *iso*prenyl) group [$\delta$1.62, 1.67 (2 × Me), 3.34 (1 × $CH_2$) and 5.13 (1 × CH)], a chelated hydroxyl group ($\delta$12.78) and a dimethylchromene ring [$\delta$1.42 (2 × Me) and 5.59, 6.69 ($J$ = 10.0 Hz) assigned to the *cis*-olefinic protons] were indicated from the $^1H$ NMR spectrum. Two olefinic proton signals appearing at $\delta$7.75 and 6.16 ($J$ = 6 Hz) were also observed in the $^1H$ NMR spectrum. Thus, **1** is a prenylated chromone with a dimethylchromene ring [5, 6]. With the exception of the $[M]^+$ at *m/z* 312, the other fragment ion peaks at *m/z* 297 $[M - Me]^+$, 269 $[297 - C{=}O]^+$, 241 $[297 - C_4H_8]^+$, 215 $[241 - C_2H_2]^+$(RDA-cleavage) and 187 $[215 - C{=}O]^+$ in the EI-mass spectrum of **1** also coincided with those of typical chromone compounds [6, 7]. In order to determine the fusion pattern of the dimethylchromene ring on the A ring, SELECTIVE INEPT and FLOCK experiments were carried out. The long-range couplings observed in these experiments are shown in Figs 1 and 2. The couplings between H-4″ and C-5, 6, 7, H-1‴ and C-7, C-9, C-5–OH and C-5, 6 in the selective INEPT spectrum and H-4″ and C-5, H-5″ and C-6, C-5–OH and C-5, 6 in the FLOCK spectrum, confirmed that the chromene ring was fused at the C-6 and C-7 positions on the A ring, and also that C-8 connected with the *iso*prenyl group. Therefore, the structure of **1** was elucidated as 5-hydroxy-8-$\gamma$, $\gamma$-dimethylallyl-6″, 6″-dimethyl-pyrano (3″, 2″:6, 7) chromone. Since **1** produced suitable crystals from ethyl acetate, the structure was finally confirmed by a single crystal X-ray diffraction analysis (Fig. 3 shows a perspective view).

Compound **2** was obtained as a yellow powder. Its molecular formula was found to be $C_{25}H_{26}O_6$ by EI-mass spectrometry, together with analysis of its $^{13}C$ NMR spectrum. In the $^1H$ NMR spectrum, three typical one-proton double doublets at $\delta$5.54 ($J$ = 12.6 and 4.0 Hz), 2.87 ($J$ = 17.6 and 4.0 Hz) and 3.08

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Table 1. $^1$H NMR spectral data of **1**, **2**, **2a**, **2b**, **2c**, **3**, **4a**, **4b**, **4c**, **4d**, **5**, **6**, **8** and **9** (in $CDCl_3$)

| H | 1 | 2 | 2a | 2b | 2c | 3 | 4 |
|---|---|---|---|---|---|---|---|
| 2 | 7.75 *d* (6.0) | 5.54 *dd* (12.6; 4.0) | 5.45 *dd* (13.4; 4.2) | 5.52 *dd* (13.6; 4.1) | 5.65 *t* (8.0) | 5.55 *dd* (12.1; 3.0) | 5.55 *t* (8.4) |
| 3 | 6.16 *d* (6.0) | 2.87 *dd* (17.6; 4.0 H-*cis*) 3.08 *dd* (17.6; 12.6 H-*trans*) | 2.75 *dd* (18.0; 4.2 H-*cis*) 2.98 *dd* (18.0; 13.4 H-*trans*) | 2.65 *dd* (18.2; 4.1 H-*cis*) 2.91 *dd* (18.2; 13.6 H-*trans*) | 2.82 *d* (8.0) | 2.83 *dd* (17.4; 3.0 H-*cis*) 3.12 *dd* (17.4; 14.0 H-*trans*) | 2.92 *d* (8.4) |
| 5(–OH) | 12.78 s | 12.19 *s* | 12.22 *s* | | | 12.1 *s* | 12.14 *s* |
| 6 | | | | | | | |
| 7 | | | | | | | |
| 8 | | | | | | | |
| 2′ | | | | | | | 6.65 *br s* |
| 3′ | | 6.42 *br s* | 6.99 *d* (1.2) | 6.98 *d* (1.2) | 6.48 *d* (1.2) | 6.39 *br s* | |
| 4′ | | | | | | | 8.84 *br* s |
| 5′ | | 7.15 *d* (8.2) | 7.09 *dd* (8.4; 1.2) | 7.11 *dd* (8.5; 1.2) | 6.55 *dd* (8.4; 1.2) | 6.42 *d* (8.0) | |
| 6′ | | 6.43 *d* (8.2) | 7.65 *d* (8.4) | 7.65 *d* (8.5) | 7.49 *d* (8.4) | 7.14 *d* (8.0) | 6.65 *br s* |
| 3′′′′ | | | | | | | |
| 5′′′′ | | | | | | | |
| 6′′′′ | | | | | | | |
| Chromene ring | | | | | | | |
| 4″ | 6.69 *d* (10.0) | 6.62 *d* (10.0) | 6.62 *d* (10.0) | 6.36 *d* (10.0) | 6.62 *d* (10.0) | 6.50 *d* (10.0) | 6.57 *d* (10.0) |
| 5″ | 5.59 *d* (10.0) | 5.51 *d* (10.0) | 5.50 *d* (10.0) | 5.64 *d* (10.0) | 5.57 *d* (10.0) | 5.48 *d* (10.0) | 5.48 *d* (10.0) |
| 6″(–Me) | 1.42 *s* | 1.44 *s* | 1.41 *s* | 1.41 *s* | 1.41 *s* | 1.39 *s* | 1.41 *s* |
| 6″(–Me) | 1.42 *s* | 1.45 *s* | 1.42 *s* | 1.42 *s* | 1.42 *s* | 1.40 *s* | 1.42 *s* |
| *isoprenyl* | | | | | | | |
| 1‴ | 3.34 *d* (7.4) | 3.21 *d* (7.6) | 3.19 *d* (8.4) | 3.20 *d* (8.2) | 3.27 *d*(8.0) | 3.21 *d* (8.0) | 3.19 *d* (8.2) |
| 2‴ | 5.13 *t* (7.4) | 5.10 *t* (7.6) | 5.12 *t* (8.4) | 5.11 *t* (8.2) | 5.20 *t* (8.0) | 5.15 *t* (8.0) | 5.09 *t* (8.2) |
| 4‴ | 1.62 *s* | 1.67 *s* | 1.63 *s* | 1.63 *s* | 1.63 *s* | 1.68 *s* | 1.61 *s* |
| 5‴ | 1.67 *s* | 1.67 *s* | 1.63 *s* | 1.63 *s* | 1.64 *s* | 1.71 *s* | 1.62 *s* |
| 5 (–OAc) | | | | 2.41 *s* | | | |
| 2′(–OAc) | | | 2.25 *s* | 2.25 *s* | | | |
| 3′(–OAc) | | | | | | | |
| 4′(–OAc) | | | 2.26 *s* | 2.28 *s* | | | |
| 5′(–OAc) | | | | | | | |
| 5 (–OMe) | | | | | 3.79 *s* | | |
| 6 (–OMe) | | | | | | | |
| 2′(–OMe) | | | | | 3.83 *s* | | |
| 3′(–OMe) | | | | | | | |
| 4′(–OMe) | | | | | 3.84 *s* | | |
| 5′(–OMe) | | | | | | | |

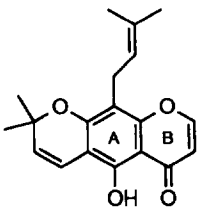
Coupling constants ($J$ values in Hz) are shown in parentheses.
* Data in $CD_3OD$.

($J$ = 17.6 and 12.6 Hz) assignable to H-2 and H-3 of a flavanone skeleton were observed [8]. The configuration at C-2 was *S*, as determined by its negative optical rotation value $\{[\alpha]_D - 16.3°$ ($CHC_3$; *c* 0.7)$\}$ [9]. Signals corresponding to a $\gamma,\gamma$-dimethylallyl group, a chelated hydroxyl group, a dimethylchromene ring and three aromatic protons were also found in the spectrum. The fragment ions at *m/z* 286 and 136 due to RDA-cleavage in the mass spectrum indicated that the $\gamma,\gamma$-dimethylallyl group and the dimethylchromene ring are on the A ring and that two hydroxyl groups are on ring B. The fusion pattern of the dimethylchromene ring on the A ring was determined to be linear by comparing the $^1$H NMR chemical shifts of hydrogen-bonded hydroxyl groups and olefinic protons on the dimethylchromene ring with those of known compounds (Tables 1–3) [5, 8]. In the $^1$H NMR spectrum, three aromatic protons on ring B resonated at $\delta$7.15 for one proton and 6.40 for two protons. This information gave two possible hydroxyl group substitution patterns on ring B: one is 2′,6′-dihydroxyl, another is 2′,4′-dihydroxyl. In order to determine the hydroxyl group substitution patterns on the B ring,

Table 1. *Continued*

| 4a | 4b | 4c | 4d | 5* | 6 | 8 | 9 |
|---|---|---|---|---|---|---|---|
| 5.45 *dd* (12.1; 1.1) | 5.46 *dd* (12.4; 1.8) | 5.71 *dd* (15.4; 4.2) | 5.68 *dd* (15.4; 3.8) | 5.65 *dd* (14.2; 3.8) | 7.94 *s* | 5.71 *dd* (14.1; 3.2) | 5.72 *t*(8.0) |
| 2.75 *dd* (16.8; 1.1 H-*cis*) | 2.76 *dd* (18.2; 1.8 H-*cis*) | 2.81 *dd* (18.8; 15.4 H-*trans*) | 2.62 *dd* (18.8; 15.4 H-*trans*) | 2.85 *dd* (17.2; 3.3 H-*cis*) | | 2.72 *dd* (18.6; 14.1 H-*trans*) | 2.81 *d* (8.0) |
| 2.85 *dd* (16.8; 12.1 H-*trans*) | 2.87 *dd* (18.2; 12.4 H-*trans*) | 2.95 *dd* (18.8; 4.2 H-*cis*) | 2.88 *dd* (18.8; 3.8 H-*cis*) | 3.16 *dd* (17.2; 14.2 H-*trans*) | | 2.85 *dd* (18.6; 3.2 H-*cis*) | |
| 12.18 *s* | | 12.31 *s* | | 6.49 *s* | 12.25 *s* | | |
| | | | | | 6.29 *d* (2.0) | | |
| | | | | | | 7.21 *br s* | |
| | | | | | 6.38 *d* (2.0) | | |
| 7.14 *s* | 7.17 *s* | 6.84 *d* (0.6) | 2.83 *d* (0.6) | | | 6.82 *d* (1.5) | |
| | | | | | 6.75 *s* | | 6.48 *d* (3.6) |
| 7.49 *s* | 7.42 *s* | 7.20 *d* (0.6) | 7.19 *d* (0.6) | 7.38 *s* | | | |
| | | | | | | | 6.55 *dd* (8.2; 3.6) |
| 4.14 *s* | 7.17 *s* | 6.84 *d* (0.6) | 6.83 *d* (0.6) | | 6.52 *s* | 6.83 *d* (1.5) | 7.49 *d* (8.2) |
| | | | | 6.72 *dd* (2.1; 0.2) | | | |
| | | | | 6.77 *dd* (8.2; 0.2) | | | |
| | | | | 6.65 *dd* (8.2; 2.1) | | | |
| 6.60 *d* (10.0) | 6.57 *d* (10.0) | 6.62 *d* (10.0) | 6.62 *d* (10.0) | 6.68 *d* (10.0) | 6.26 *d* (10.0) | 6.62 *d* (10.0) | 6.61 *d* (10.0) |
| 5.49 *d* (10.0) | 5.52 *d* (10.0) | 5.48 *d* (10.0) | 5.59 *d* (10.0) | 5.56 *d* (10.0) | 5.52 *d* (10.0) | 5.58 *d* (10.0) | 5.48 *d* (10.0) |
| 1.41 *s* | 1.42 *s* | 1.41 *s* | 1.41 *s* | 1.40 *s* | 1.41 *s* | 1.41 *s* | 1.41 *s* |
| 1.42 *s* | 1.43 *s* | 1.42 *s* | 1.42 *s* | 1.41 *s* | 1.41 *s* | 1.42 *s* | 1.44 *s* |
| 3.19 *d* (8.6) | 3.24 *d* (9.0) | 3.23 *d* (8.0) | 3.24 *d* (8.0) | 3.19 *d* (8.0) | | 3.29 *t* (7.4) | 3.25 *t* (7.8) |
| 5.10 *t* (8.6) | 5.22 *t* (9.0) | 5.19 *t* (8.0) | 5.20 *t* (8.0) | 5.12 *t* (8.0) | | 5.21 *t* (7.4) | 5.15 *t* (7.8) |
| 1.61 *s* | 1.70 *s* | 1.62 *s* | 1.59 *s* | 1.55 *s* | | 1.65 *s* | 1.65 *s* |
| 1.61 *s* | 1.70 *s* | 1.64 *s* | 1.60 *s* | 1.58 *s* | | 1.70 *s* | 1.78 *s* |
| | 2.42 *s* | | | | | | |
| 2.29 *s* | 2.25 *s* | | | | | | |
| 2.30 *s* | 2.26 *s* | | | | | | |
| | | | 3.82 *s* | | | 3.84 *s* | 3.79 *s* |
| | | | | | | 3.76 *s* | |
| | | | | | | | 3.81 *s* |
| | | 3.79 *s* | 3.78 *s* | | | | |
| | | | | | | | 3.82 *s* |
| | | 3.79 *s* | 3.78 *s* | | | 3.75 *s* | |

and also for the more accurate assignment of its $^{13}$C NMR data (Table 2), **2** was acetylated and methylated (see Experimental). Acetylation of **2** for 1.4 hr afforded two derivatives, **2a** and **2b**. Compound **2a** was confirmed to have a molecular formula of $C_{29}H_{30}O_8$ from TSP mass spectral data ($[M]^+$ *m/z* 506), together with the analysis of its $^{13}$C NMR spectrum. In the $^1$H NMR, a chelated hydroxyl group at $\delta$12.22 implied that the 5-OH group had not been acetylated. Therefore, **2a** was a derivative with two acetylated hydroxyl groups on the B ring. Furthermore, three aromatic protons on the B ring revealed a clear ABX system [$\delta$7.65 ($J$ = 8.4 Hz for one proton), $\delta$7.09 ($J$ = 8.4, 1.2 Hz for one proton) and $\delta$6.99 ($J$ = 1.2 Hz for one proton)] typical of substitutions at the 2′- and 4′-positions on the B ring. Compound **2b** was found to be a derivative with three acetylated hydroxyl groups through analysis of its TSP mass spectrum ($C_{31}H_{32}O_8$ $[M]^+$*m/z* 548]), $^1$H and $^{13}$C NMR spectral data (Tables 1 and 2). Thus, the structure of **2** was established as 5,2′,4′-trihydroxy-8-$\gamma$,$\gamma$-dimethylallyl-6″, 6″ dimethyl-pyrano (3″, 2″:6, 7) flavanone. It is a known compound, named flemichin-D, which was previously isolated from *Flemingia macrophylla* (Leguminosae) [10]. The antifungal activity of this compound and its $^{13}$C NMR data have not been reported, previously.



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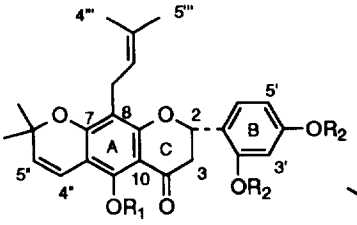
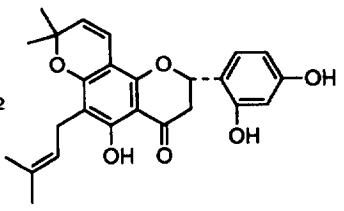


**2** : $R_1$=$R_2$=H
**2a** : $R_1$=H,$R_2$=Ac
**2b** : $R_1$=$R_2$=Ac
**2c** : $R_1$=$R_2$=$CH_3$



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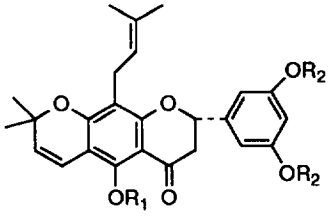
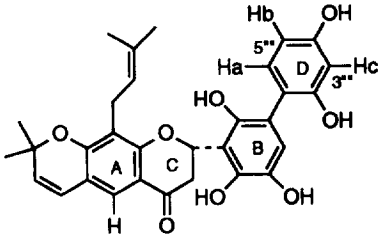


**4** : $R_1$=$R_2$=H
**4a** : $R_1$=H,$R_2$=Ac
**4b** : $R_1$=$R_2$=Ac
**4c** : $R_1$=H,$R_2$=Me
**4d** : $R_1$=$R_2$=Me



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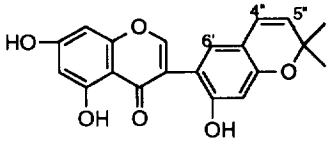

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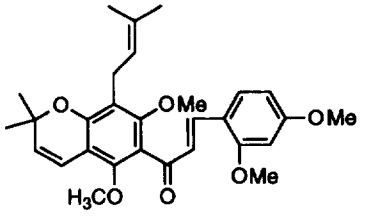

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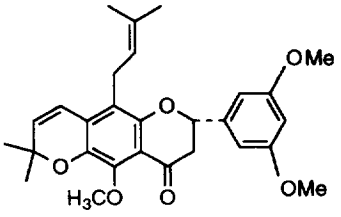

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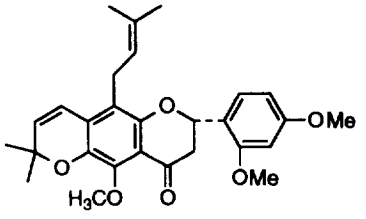

9

Compound **2c**, obtained by methylation of **2** with $Me_2SO_4$, was found to be a derivative with three methylated hydroxyl groups through analysis of its EI-mass spectrum ($C_{28}H_{32}O_6$, $[M]^+$ *m/z* 464]), $^1H$ and $^{13}C$NMR spectral data (Tables 1, 2). Moreover, in the $^1H$NMR spectrum, three typical one-proton double doublets belonging to H-2 and H-3 were changed into one doublet ($\delta$2.82, $J$ = 8.0 Hz; H-3) and one triplet ($\delta$5.65, $J$ = 8.0 Hz; H-2) due to the methylation reaction. This phenomenon can be explained by the fact that **2c** has a different conformation at C-3 than **2**. In the case of **2c**, H-2 revealed one triplet and the two protons at C-3 showed one doublet because they had the same dihedral angles with H-2. In the case of **2**, protons at C-3 have different dihedral angles with H-2, giving typical resonances for a flavanone (3 × one-proton double doublets).

Compound **3** was obtained as a yellow-brown powder. Its molecular formula was found to be $C_{25}H_{26}O_6$ by TSP and EI-mass spectrometry, together with analysis of the $^{13}C$NMR spectrum. The EI-mass spectrum of

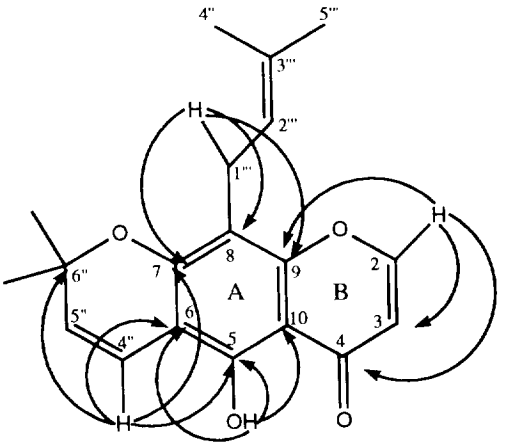


Fig. 1. Long-range couplings between protons and carbons observed by selective INEPT of **1**. Numbering scheme adopted for comparison with the other isolated compounds in Tables 1 and 2.

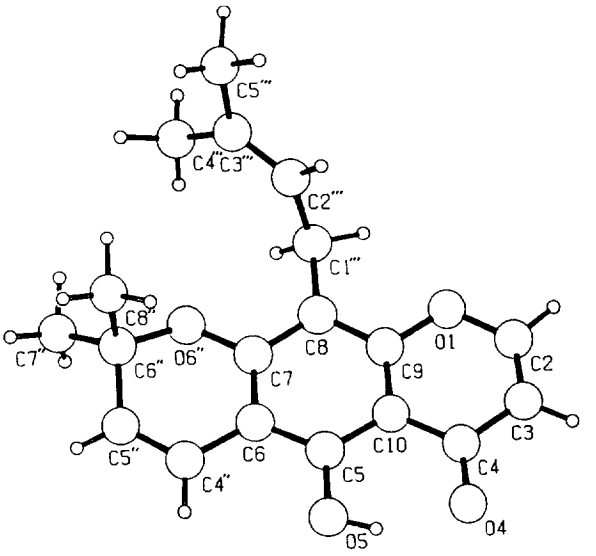


Fig. 3. Perspective view of **1**.

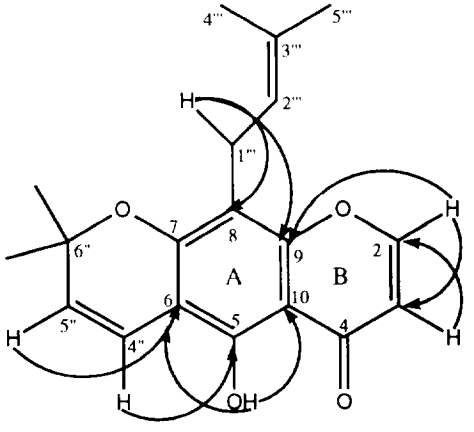


Fig. 2. Long-range couplings between protons and carbons observed in the FLOCK NMR spectrum of **1**. Numbering scheme adopted for comparison with the other isolated compounds in Tables 1 and 2.

**3** showed the same cleavage pattern as **2**. The $^1$H and $^{13}$C NMR spectral data (Tables 1, 2) were similar to those of **2**. However, the chemical shift of the hydrogen-bonded hydroxyl group was at $\delta$12.10, compared to $\delta$12.19 for **2**. The olefinic proton on the dimethylchromene ring (H-4″) appeared at $\delta$6.50 which was at a higher field than $\delta$6.62 of **2**. Comparison with $^1$H NMR data of known compounds (Table 3) [5, 8] suggested that **3** had the angular fusion pattern for the dimethylchromene ring on the A ring. Therefore, the structure of **3** (eriosemaone A) was established as 5,2′,4′-trihydroxy-8-$\gamma$,$\gamma$-dimethylallyl-6″,6″-dimethyl-pyrano (2″,3″:7,8) flavanone, a regioisomer of **2**.

Compound **4** was obtained as a yellow powder. Its molecular formula was found to be $C_{25}H_{26}O_6$ by EI-mass spectrometry, together with analysis of the $^{13}$C NMR spectrum. The EI-mass spectrum of **4** showed the same cleavage pattern as **2**. In the $^1$H NMR spectrum, the signals belonging to a $\gamma$,$\gamma$-dimethylallyl group, a chelated hydroxyl group, a dimethylchromene ring and three aromatic protons were also present. However, H-3 and H-2 showed the same coupling pattern as **2c**, implying H-3 to be the same as in **2c**. Furthermore, the aromatic protons on the B ring appeared at $\delta$8.84 (broad singlet for one proton) and 6.65 (broad singlet for two protons) which suggested a 3′,5′-substitution pattern on ring B. However, the $^{13}$C NMR chemical shift (Table 2) of C-3′ was different from that of C-5′. In order to confirm this substitution pattern, and also for a more accurate assignment of the $^{13}$C NMR data, **4** was transformed into two acetylated derivatives (**4a** and **4b**) and two methylated derivatives (**4c** and **4d**). A FLOCK experiment was chosen in order to check the substitution pattern on the B ring. Long-range couplings between H-4′ and C-2′, C-3′, C-5′ and C-6′ (Fig. 4) observed with **4** and long-range couplings between 3′-OMe and C-3′, 5′-OMe and C-5′, H-4′ and C-3′ (Fig. 5) observed with **4c** supported the 3′,5′-dihydroxyl substitution on ring B. Therefore, the structure of **4**, eriosemaone B, was established as 5, 3′, 5′-tetrahydroxy-8-$\gamma$, $\gamma$-dimethylallyl-6″, 6″-dimethylpyrano (3″,2″:6,7) flavanone. Compounds **4a** and **4b** were derivatives with two and three acetyl groups, respectively. Their structures were deduced from the analysis of the EI-mass spectral {[M]$^+$ *m/z*: 506 for **4a** ($C_{28}H_{30}O_8$), 548 for **4b** ($C_{31}H_{32}O_9$)}, and $^1$H and $^{13}$C NMR spectral data (Tables 1, 2). In the $^1$H NMR spectrum, three typical one-proton double doublets assignable to H-2 and 2 × H-3 of a flavanone skeleton were observed for both **4a** and **4b**. These data indicated that the conformation at C-3 was changed to that of **2** due to the acetylation reaction. Derivatives **4c** and **4d** had two

Table 2. $^{13}C$ NMR data of **1**, **2**, **2a**, **2b**, **2c**, **3**, **4**, **4a**, **4b**, **4c**, **4d**, **5**, **6**, **8** and **9**

| C | **1** | **2** | **2a** | **2b** | **2c** | **3** | **4** | **4a** | **4b** | **4c** | **4d** | **5*** | **6** | **8** | **9** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 155.3 | 76.7 | 74.1 | 74 | 73.9 | 76.4 | 76.1 | 73.9 | 74 | 74.2 | 74.1 | 75.8 | 155 | 74.1 | 73.8 |
| 3 | 110.9 | 41.4 | 42.2 | 44.1 | 44.2 | 42 | 41.5 | 42.5 | 44.3 | 42.5 | 44.3 | 43.3 | 122.9 | 44.5 | 44.2 |
| 4 | 182.3 | 197.1 | 197.1 | 189.1 | 190.4 | 196.5 | 197.1 | 195.6 | 188.9 | 197 | 190.2 | 199.2 | 181.9 | 190.2 | 190.1 |
| 5 | 154.4 | 159 | 159.4 | 151.4 | 155.2 | 159.9 | 156.6 | 156.6 | 148.4 | 156.7 | 150.1 | 118.7 | 162.3 | 155.1 | 157.2 |
| 6 | 106.3 | 103.1 | 103.1 | 109.6 | 111.2 | 110.3 | 103.1 | 103.1 | 109.6 | 102.8 | 111.9 | 109.9 | 100.2 | 157.5 | 157.9 |
| 7 | 156.9 | 157 | 157.7 | 157.8 | 157.1 | 157.1 | 159 | 158.8 | 157.3 | 159.6 | 160.5 | 157.5 | 163.3 | 116.5 | 117.2 |
| 8 | 107.6 | 108.9 | 108.9 | 115.2 | 113 | 102.6 | 108.9 | 108.8 | 115.3 | 108.6 | 115.4 | 103.5 | 94.2 | 108.9 | 108.5 |
| 9 | 154.7 | 160 | 160.1 | 160.6 | 160.4 | 161.3 | 160.1 | 159.9 | 160.5 | 159.7 | 160.7 | 160.7 | 156.5 | 161.4 | 159.5 |
| 10 | 105.4 | 102.6 | 102.6 | 107.6 | 109.2 | 101.9 | 102.6 | 102.4 | 107.2 | 102.7 | 107.2 | 128.5 | 105.3 | 109.4 | 105.9 |
| 1′ | | 117 | 128.1 | 128.1 | 120.2 | 116.9 | 125.9 | 132.1 | 132.2 | 128.7 | 128.4 | 103.7 | 115.4 | 128.8 | 120.1 |
| 2′ | | 154.8 | 148.8 | 147.5 | 157 | 154.4 | 116.2 | 122.5 | 122.4 | 113.5 | 113.4 | 156.2 | 155.5 | 111.2 | 157.1 |
| 3′ | | 103.9 | 116.2 | 116.1 | 98.1 | 104 | 146.9 | 144.5 | 144.5 | 149.8 | 149.9 | 119.3 | 107.7 | 149.5 | 98.3 |
| 4′ | | 156.5 | 152.1 | 151.4 | 160.4 | 155.1 | 113.4 | 120.1 | 120 | 111.3 | 111.4 | 118.1 | 157.8 | 113.1 | 160.8 |
| 5′ | | 107.7 | 119.3 | 119.8 | 104.1 | 107.9 | 149.4 | 148.5 | 148.4 | 153.9 | 153.9 | 151.9 | 111.9 | 153.6 | 104.2 |
| 6′ | | 127.8 | 127.4 | 127.5 | 127.1 | 128 | 117.3 | 123.6 | 123.6 | 112.5 | 112.3 | 147.8 | 127.2 | 112.3 | 127.2 |
| 1‴ | | | | | | | | | | | | 119.3 | | | |
| 2‴ | | | | | | | | | | | | 156.1 | | | |
| 3‴ | | | | | | | | | | | | 104.3 | | | |
| 4‴ | | | | | | | | | | | | 161 | | | |
| 5‴ | | | | | | | | | | | | 116.6 | | | |
| 6‴ | | | | | | | | | | | | 130.7 | | | |
| Chromene ring | | | | | | | | | | | | | | | |
| 4″ | 128.1 | 126.2 | 126.3 | 129.3 | 128 | 126.7 | 126.2 | 126.1 | 129.9 | 126 | 128.1 | 127.1 | 128.1 | 128.9 | 127.1 |
| 5″ | 115.7 | 115.4 | 115.5 | 115.2 | 116.4 | 115.6 | 115.5 | 115.5 | 115.4 | 115.8 | 116.2 | 115.9 | 121.2 | 116.5 | 116.1 |
| 6″ | 78.1 | 78.3 | 78.1 | 78.1 | 77.8 | 78.1 | 78.3 | 78.2 | 78 | 78.1 | 77.8 | 79.2 | 76.7 | 77.9 | 77.8 |

Table 2. *Continued*

| C | 1 | 2 | 2a | 2b | 2c | 3 | 4 | 4a | 4b | 4c | 4d | 5* | 6 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6″(–Me) | 28.2 | 28.3 | 28.1 | 28.2 | 28.1 | 28.3 | 28.4 | 28.2 | 28.3 | 28.3 | 28.2 | 28.3 | 28.1 | 28.3 | 27.9 |
| 6″(–Me) | 28.2 | 28.3 | 28.2 | 28.2 | 28.2 | 28.5 | 28.5 | 28.3 | 28.4 | 28.4 | 28.3 | 28.6 | 28.1 | 28.4 | 28.2 |
| *I*soprenyl | | | | | | | | | | | | | | | |
| 1‴ | 21.9 | 21.2 | 21.5 | 21.7 | 22.2 | 21 | 21.5 | 21.4 | 21.9 | 21.5 | 21.9 | 22.3 | | 22.1 | 21.8 |
| 2‴ | 121.8 | 122.2 | 122.1 | 122.6 | 122.1 | 122.2 | 122.3 | 122.4 | 121.6 | 122.7 | 121.9 | 123.7 | | 122.3 | 122.5 |
| 3‴ | 131.5 | 131.7 | 131.6 | 131.2 | 131 | 131.5 | 131.7 | 131.1 | 131.6 | 131.1 | 131.2 | 131.9 | | 131.4 | 131.2 |
| 4‴ | 17.4 | 17.8 | 17.9 | 17.8 | 17.9 | 17.9 | 17.8 | 17.8 | 17.8 | 17.9 | 17.8 | 18.1 | | 17.9 | 17.8 |
| 5‴ | 25.7 | 25.7 | 25.7 | 25.8 | 25.8 | 25.8 | 25.8 | 25.7 | 25.7 | 25.8 | 25.5 | 25.9 | | 25.9 | 25.5 |
| 5(–OAc) | | | | 169.1 | | | | | 169.4 | | | | | | |
| | | | | 21.6 | | | | | 21 | | | | | | |
| 2′(–OAc) | | | 163.8 | 168.4 | | | | | | | | | | | |
| | | | 20.1 | 21.5 | | | | | | | | | | | |
| 3′(–OAc) | | | | | | | | 168.8 | 168.8 | | | | | | |
| | | | | | | | | 21 | 21 | | | | | | |
| 4′(–OAc) | | | 168.5 | 168.5 | | | | | | | | | | | |
| | | | 21 | 21.5 | | | | | | | | | | | |
| 5′(–OAc) | | | | | | | | 169.1 | 169 | | | | | | |
| | | | | | | | | 21 | 21 | | | | | | |
| 5(–OMe) | | | | | 62.1 | | | | | | 62.2 | | | 62.3 | 62.1 |
| 6(–OMe) | | | | | | | | | | | | | | | 55.2 |
| 2′(–OMe) | | | | | 55.2 | | | | | | | | | | |
| 3′(–OMe) | | | | | | | | | | 55.8 | 55.7 | | | 56.1 | |
| 4′(–OMe) | | | | | 55.1 | | | | | | | | | | 55.1 |
| 5′(–OMe) | | | | | | | | | | 55.8 | 55.7 | | | 55.8 | |

* Data in $CD_3OD$.

Table 3. $^1$H NMR spectral data and fusion patterns of the pyran rings on the A rings of the reference compounds, euchrenones $\mathbf{a}_{11}$, $\mathbf{a}_{12}$, $\mathbf{a}_{14}$, $\mathbf{a}_5$ and **2**–**4** (in $CDCl_3$) [5, 8]

| | $\mathbf{a}_{11}$ | $\mathbf{a}_{12}$ | $\mathbf{a}_{14}$ | $\mathbf{a}_5$ | **2** | **3** | **4** |
|---|---|---|---|---|---|---|---|
| Chelated OH | 12.24 | 12.38 | 12.25 | 12.29 | 12.19 | 12.30 | 12.14 |
| Methine H-4″ | 6.64 | 6.53 | 6.63 | 6.55 | 6.62 | 6.50 | 6.57 |
| H-5″ | 5.51 | 5.49 | 5.49 | 5.45 | 5.51 | 5.48 | 5.48 |
| Fusion pattern | Linear | Angular | Linear | Angular | Linear | Angular | Linear |

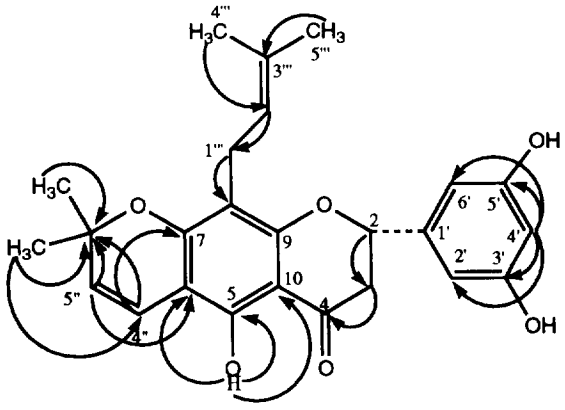

Fig. 4. Long-range correlations observed from the FLOCK NMR spectrum of **4**.

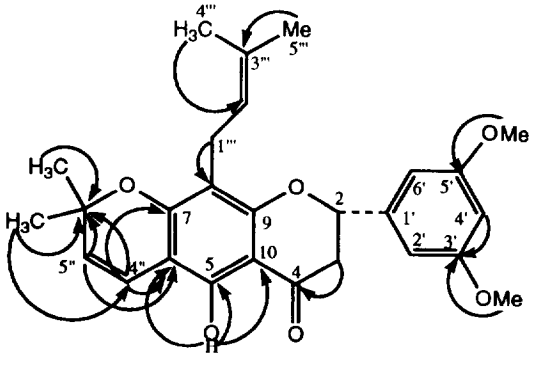

Fig. 5. Long-range correlations observed from the FLOCK NMR spectrum of **4c**.

methylated and three methylated groups, respectively. This was deduced from the analysis of their EI-mass spectra {$[M]^+$ *m/z*: 450 for **4c** ($C_{27}H_{30}O_6$), 464 for **4d** ($C_{28}H_{32}O_6$)} and $^1$H and $^{13}$C NMR spectral data (Tables 1, 2). In their $^1$H NMR spectra, three typical one-proton double doublets assignable to H-2 and 2 × H-3 of a flavanone skeleton were also observed. However, the H-3 at the *trans*-position resonated at lower fields [+2.81 (**4c**) and +2.62 (**4d**)] compared with those of **3**, **2**, **2a**, **2b**, **4a** and **4b** (Table 1). This phenomenon was caused by the methylation of the 3′ 5′-dihydroxyl groups of the B ring. Noteworthy was also the effect of the 5-OH, of the dimethyl-pyrano (3″, 2″:6, 7) flavanones on the chemical shift of C-4″ in the $^{13}$C NMR spectrum. Acetylation of the 5-OH caused a shift to lower fields of *ca* +3.3 ppm for C-4″. When 5-OH was methylated, the chemical shift of C-4″ had a lower field shift (*ca* +2.2 ppm) than the non-methylated derivative (Table 2).

The molecular formula of **5** was found to be $C_{31}H_{30}O_8$ by FAB ($[M]^+$ *m/z*: 529, negative ion mode; *m/z*: 531, positive ion mode), DCI ($[M + H]^+$ *m/z*: 531) and EI ($[M]^+$ *m/z*: 530) mass spectrometry, together with analysis of its $^{13}$C NMR spectrum. Comparison of $^{13}$C NMR spectral data (Table 2) with those of **2** and those of known compounds [11], showed the presence of one additional aromatic ring. The fragment ions at *m/z* 260 and 271 due to RDA-cleavage (Fig. 6) showed that an additional phenyl group was attached to the B ring. In the $^1$H NMR spectrum, three typical one-proton double doublets were assignable to H-2 and 2 × H-3 of a flavanone skeleton (Table 1). Signals corresponding to a $\gamma,\gamma$-dimethylallyl group and a dimethylchromene ring were also observed, but the absence of a signal for a chelated hydroxyl group indicated that there was no hydroxyl group at C-5; the C-5 proton appeared at $\delta$6.49 (singlet). The proton of the B ring appeared at $\delta$7.38, while the ABX system of ring D gave signals at $\delta$6.72 ($J_{cb}$ = 2.1, $J_{ca}$ = 0.2 Hz; H-c), 6.77 ($J_{ab}$ = 8.2, $J_{ac}$ = 0.2 Hz; H-a) and 6.65 ($J_{ba}$ = 8.2, $J_{bc}$ = 2.1 Hz; H-b)], typical of a 2‴, 4‴-dihydroxyl substitution. The presence of *ortho*-dihydroxyl substitution on the B ring was also supported by the UV spectral data, with a bathochromic shift (13 nm) with increase in the intensity of band I in NaOAc–$H_3BO_3$ relative to band I (302 nm) in methanol and a bathochromic shift (33 nm) with decreasing intensity of band I in $AlCl_3$ and $AlCl_3$–HCl relative to band I (302 nm) in methanol [12]. Therefore, the structure of **5** (eriosemaone C) was established as 2′, 3′, 6′, 2‴, 4‴-pentahydroxy-8-$\gamma,\gamma$-dimethylallyl-6″, 6″-dimethyl-pyrano (3″, 2″:6, 7) flavanone.

The molecular formula of **6** was found to be $C_{20}H_{16}O_6$ by EI-mass spectrometry ($[M]^+$ *m/z* 352) and analysis of $^{13}$C NMR spectral data (Table 2). In the $^1$H NMR spectrum, signals corresponding to a dimethylchromene ring and a chelated hydroxyl group were ob-

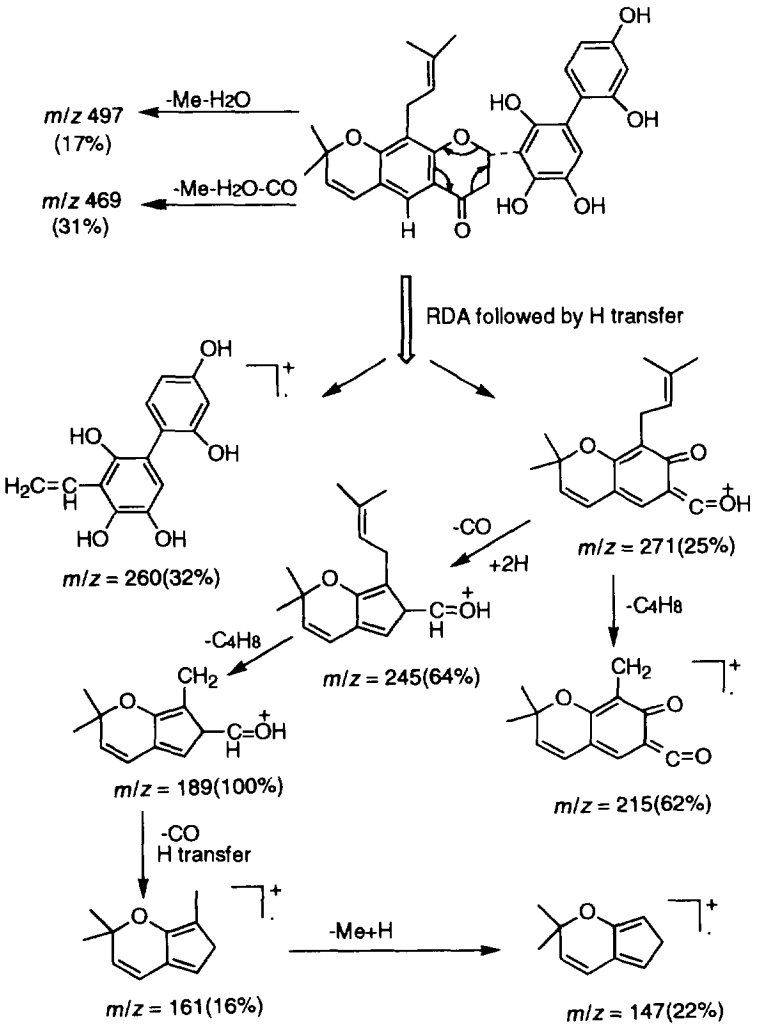


Fig. 6. Possible mass spectral fragmentations of **5** showing an additional phenyl substitution on the **B** ring.

served (Table 1). However, the signals due to the $\gamma,\gamma$-dimethylallyl group and a flavanone skeleton were absent, implying that **6** had a different skeleton from that of **2**. The proton singlet signal at $\delta$7.94 (H-2) was characteristic of an isoflavanone [13, 14]. Location of the dimethylchromene ring on the B ring was indicated by two characteristic fragment ions (*m/z* 153 and 185) in the mass spectrum, due to RDA-cleavage. These two fragments further suggested the location of the two hydroxyl groups on ring A. Two one-proton doublets at $\delta$6.29 ($J$ = 2.0 Hz) and 6.38 ($J$ = 2.0 Hz) in the $^1$H NMR spectrum suggested that the A ring had a 6,8-dihydroxyl substitution pattern. Finally, two one-proton singlets at $\delta$6.75 and 6.52, together with $^1$H–$^1$H coupling between the H-4″ and H-6′, observed in the $^1$H–$^1$H COSY spectrum, allowed the structure of **6** (eriosemaone D) to be deduced as 6,8,2′-trihydroxy-6″,6″-dimethyl-pyrano (2″,3″:4′,5′) isoflavanone.

Due to problems of separation, three other polyphenols could only be isolated as their methylated derivatives (**7**–**9**). Compound **7** was found to have a molecular formula of $C_{28}H_{34}O_6$ from EI([M]$^+$ *m/z*: 478) and DCI ([M + H]$^+$ *m/z*: 479) mass spectrometry, together with analysis of the $^{13}$C NMR spectrum (see Experimental). There were four methoxyl signals at $\delta$55.5( × 2), 62.5, 63.5 in the $^{13}$C NMR spectrum and at $\delta$3.65, 3.69, 3.81, 3.82 in the $^1$H NMR spectrum. Other signals corresponding to a $\gamma,\gamma$-dimethylallyl group, a dimethylchromene ring and an ABX system on ring B were also observed in the $^1$H NMR spectrum. Coupled signals ($^1$H–$^1$H COSY) were characteristic of the $\alpha$- and $\beta$-protons of a chalcone [15, 16]. In the EI-mass spectrum, the fragment ion at *m/z* 191 {[M − 287]$^+$, ($C_{18}H_{22}O_3$)} confirmed the dimethoxyl substitution on ring B. In the DCI-mass spectrum, the fragment ions at *m/z* 289 [$C_{18}H_{24}O_3$ + H]$^+$ and 167 [287 − $C_4H_8$ − OMe ( × 2)]$^+$ indicated another two methoxyl groups located on ring A. This information suggested **7** to be 2′,6′,2,4-tetramethoxy-5′-$\gamma,\gamma$-dimethylallyl-6″,6″ dimethyl-pyrano (3″,2″:3′,4′) chalcone.

Compound **8** had a molecular formula of $C_{28}H_{32}O_6$ as deduced from the EI-mass spectrum ($[M]^+$ *m/z*: 464) and analysis of $^{13}C$ NMR spectral data (Table 2). The signals at $\delta$55.8, 56.1, 62.3 in the $^{13}C$ NMR spectrum and $\delta$3.75, 3.76, 3.84 in the $^1H$ NMR spectrum indicated that it was a trimethylated derivative. Signals in the $^1H$ NMR spectrum corresponding to a $\gamma,\gamma$-dimethylallyl group and a dimethylchromene ring were present, as well as three typical one-proton double doublets (Table 1) assignable to H-2 and 2 × H-3 of a flavanone skeleton. Furthermore, H-3 at the *trans*-position also resonated at lower field, as observed in **4c** and **4d**. The fragment ions at *m/z* 300 and 164 due to the RDA-cleavage in the EI-mass spectrum suggested a structure similar to **4d**. Three aromatic protons of the B ring appeared at $\delta$7.21 ($J$ = 1.5 Hz; H-3'), 6.82 ($J$ = 1.5 Hz; H-2') and $\delta$6.83 ($J$ = 1.5 Hz; H-6') confirming the 3',5'-dimethoxyl substitution pattern on ring B. The signals of H-1''' of the $\gamma,\gamma$-dimethylallyl group exhibited a triplet ($\delta$3.29, $J$ = 7.4 Hz) instead of a doublet, as in the case of **4d**, or **2**–**4**. This phenomenon implied that H-1''' coupled with H-4'' (olefinic proton on the dimethylchromene ring). Therefore, the structure of **8** was determined as 5,3',5'-$\gamma,\gamma$-trimethoxy-8-dimethylallyl-6'',6''-dimethyl-pyrano (2'' 3'':6,7) flavanone.

The molecular formula of **9** was found to be $C_{28}H_{32}O_6$ from the EI-mass spectrum ($[M]^+$ *m/z* 464) and analysis of its $^{13}C$ NMR spectral data (Table 2). The signals at $\delta$55.1, 55.2, 62.1 in the $^{13}C$ NMR spectrum and $\delta$3.79, 3.81, 3.82 in the $^1H$ NMR spectrum indicated that it was a trimethylated derivative. The EI-mass spectrum gave the same peaks as **8** and **2c** which implied that **9** had the same substituents. Comparing the $^1H$ NMR spectrum with **2c**, the only difference was the triplet of H-1''' ($\delta$3.25, $J$ = 7.4 Hz) on the $\gamma,\gamma$-dimethylallyl group instead of the doublet in **2c**. This suggested that **9** had the same fusion pattern for the dimethylchromene ring on the A ring as **8**. Again, like **2c**, the 2',4'-dimethoxylation of the B ring gave a doublet in the $^1H$ NMR spectrum for the two protons at C-3. In order to confirm the fusion pattern of the dimethylchromene ring on the A ring, a NOE experiment was carried out. Thus, detection of NOEs between the olefinic protons (H-4'' and H-5'') and H-1''' of the $\gamma,\gamma$-dimethylallyl group confirmed that the fusion of the dimethylchromene ring on the A ring is the same as in **8**. Therefore, the structure of **9** was established as 5,2',4'-trimethoxy-8-$\gamma,\gamma$-dimethylallyl-6'',6''-dimethyl-pyrano (2'',3'':6,7) flavanone.

All the compounds (**1**–**9**) and their derivatives were tested for their antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* in TLC bioassays [17, 18]. Amounts of 1 $\mu$g of **2**–**6** deposited on a TLC plate were sufficient to prevent the growth of *C. albicans*. Under the same conditions, 0.01 $\mu$g of the commercially available miconazole was active against the fungus. In addition, **2**, **4**–**6** were fungitoxic at 5 $\mu$g against *C. cucumerinum*, while **3** displayed slight activity at 10 $\mu$g. In the same assay, the amount of the synthetic fungicide, propiconazole, necessary to inhibit the growth of *C. cucumerinum* was 0.01 $\mu$g. Compound **1** and all the derivatives were inactive at 50 $\mu$g against both fungi (Table 4).

Table 4. Antifungal activities of **1**–**9**

| Compounds | Activity against *Cladosporium cucumerinum* | Activity against *Candida albicans* |
|---|---|---|
| **1** | > 50 $\mu$g* | > 50 $\mu$g |
| **2** | 5 $\mu$g | 1 $\mu$g |
| **2a, b, c** | > 50 $\mu$g | > 50 $\mu$g |
| **3** | 10 $\mu$g | 1 $\mu$g |
| **4** | 5 $\mu$g | 5 $\mu$g |
| **4a, b, c, d** | > 50 $\mu$g | > 50 $\mu$g |
| **5** | 5 $\mu$g | 1 $\mu$g |
| **6** | 5 $\mu$g | 1 $\mu$g |
| **7** | > 50 $\mu$g | > 50 $\mu$g |
| **8** | > 50 $\mu$g | > 50 $\mu$g |
| **9** | > 50 $\mu$g | > 50 $\mu$g |
| Propiconazole | 0.001 $\mu$g | |
| Miconazole | | 0.01 $\mu$g |

* Minimum amount of compound needed to inhibit fungal growth on TLC plates.

This is the first phytochemical study of a species belonging to the genus *Eriosema*. The investigation afforded some new natural compounds; in particular, the derivatives **8** and **9** possess a novel flavanone skeleton where the C-2'' and C-3'' of the dimethylchromene ring are connected on positions 6 and 7 of the ring A. Although **2**–**6** were fungitoxic against *C. cucumerinum* and *C. albicans* using a TLC bioassay, they are less active than the reference compounds miconazole and propiconazole. Further quantitation of their antifungal properties has not been undertaken.

## EXPERIMENTAL

*General.* Mps: uncorr. For open CC, silica gel (40–63 $\mu$m) [Merck] was used. UV spectra were recorded in MeOH. Analytical HPLC was carried out on an instrument equipped with a photodiode array detector. Frs were analysed on Novapak C-18 columns, (4 $\mu$m, 150 × 3.9 mm i.d., Waters) at a flow rate of 1 ml $min^{-1}$. Semi-prep. HPLC was performed on LiChroprep RP-18 (7 $\mu$m, 250 × 16 mm i.d., Knauer), Lichrosorb Diol (7 $\mu$m, 250 × 20 mm i.d., Knauer), and Nucleosil RP-18 (7 $\mu$m, 250 × 20 mm, Macherey-Nagel) columns at a flow rate of 10 ml $min^{-1}$. MPLC was carried out on a LiChroprep RP-18 column (25–40 $\mu$m, i.d. 2.5 × 46 cm) at a flow rate of 10 ml $min^{-1}$ (20 bar). $^1H$ and $^{13}C$ NMR spectra were measured in $CDCl_3$ or in $CD_3OD$ at 200 MHz for proton and 50.30 MHz for carbon, respectively. TMS was used as int. standard. Selective INEPT [19] and FLOCK [20] were performed with delays optimized for $^nJ_{CH}$ = 4 or 8 Hz. FAB-MS (glycerol, negative and positive ion modes), EI-MS and DCI-MS analyses were recorded using a triple-stage quadrupole instrument.

*Plant material.* Roots of *E. tuberosum* (1670 g) 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 Academia of Science, Kumming.

*Extraction and isolation.* Powdered roots (1.67 kg) 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 elution (*n*-hexane–EtOAc, 90:10–0:100); 15 frs (A–O) were collected. Fr. D (5.4 g) was purified by repeated recrystallization from EtOAc and gave **1** (2.2 g). Fr. J(10 g) was dissolved in MeOH, the insoluble material filtered off and the MeOH-soluble part (6.5 g) submitted to MPLC on RP-18 (25–40 μm, Merck) eluting with a MeOH–$H_2O$ step gradient 70:30–100:0. Compounds **2** (1.8 g), **4** (500 mg), **5** (8 mg), **6** (15 mg) and sub-frs a (40 mg) and b (900 mg) were obtained. Sub-fr. a was submitted to semi-prep. HPLC on RP-18 (MeOH–$H_2O$, 17:3) to give **3** (7 mg). Sub-fr. b was methylated with $Me_2SO_4$ (20 ml) in $Me_2CO$ (200 ml) containing $K_2CO_3$ (33 g). The reaction soln was refluxed for 1.5 hr at 100°. The crude methylation products were submitted to semi-prep. HPLC on RP-18 (MeOH–$H_2O$, 9:1) giving **7** (13 mg) and **9** (14 mg) and sub-fr. c. Sub-fr. c was chromatographed by semi-prep. HPLC on a Diol column (*n*-hexane–EtOAc, 9:1), affording **8** (12 mg).

*Compound* **1** (*5-hydroxy-8-γ,γ-dimethylallyl-6″,6″-dimethyl-pyrano* (3″,2″:6,7) *chromone, eriosematin*). Yellow crystals, mp 114–115°. $[\alpha]_D$ − 52.8° ($CHCl_3$; *c* 0.009). HPTLC (Diol, petrol–EtOAc, 7:3): $R_f$ 0.67. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 280, (4.02), 335 (4.14); + NaOMe: 295, 350; + $AlCl_3$–HCl: 305, 350; + NaOAc–B(OH)$_3$: 280, 335. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3040, 2980, 2910, 1605, 1557, 1410, 1365, 1310, 1267, 1200, 1126, 856. TSP-MS (pos. mod.) *m/z* (rel. int.): 313 $[MH]^+$ (100). EIMS *m/z* (rel. int.): 312 $[M]^+$ (67), 297 (100), 269 (25), 241 (12), 215 (9), 187 (2). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Crystallographic data for **1**: $C_{19}H_{20}O_4$, $M_r$ = 312.35, monoclinic, $P2_1/n$, $a$ = 10.699(2), $b$ = 10.233(1), $c$ = 16.073(2) Å, $b$ = 105.59(1)°, $V$ = 1695.0(4) Å$^3$, $Z$ = 4, $D_x$ = 1.224 g$cm^{-3}$, $l$ = 0.71073 Å, $m$ = 0.085 $mm^{-1}$, $F$(OOO) = 660. 2979 unique reflections, 2963 reflections for refinement, 288 variables, 0 = restraints, $R1$ = 0.082, $wR2$ = 0.131 [for 1434 reflections with $I > 2s(I)$]; $R1$ = 0.181, $wR2$ = 0.186 [all data]. Max shift/sigma ratio 0.003, residual density (e/Å$^3$) max. 0.16, min. − 0.21. Intensity data were collected at room temp. on Stoe AED2 4-circle diffractometer using Mo-Kα graphite monochromated radiation and $W/Q$ scans out to 50° in 2Q. The structures were solved by Direct Methods using the programme SHELXS-90 [21] and refined using the programme SHELXL-93 [22]. Neutral complex-atom scattering factors are from ref. [23]. The H-atoms were located from difference maps and refined isotropically. The non-hydrogen atoms were refined anisotropically. The refinement method was full-matrix least-squares on $F^2$. The bond distances and angles are normal within experimental error. There is a strong intra-molecular hydrogen bond involving hydroxyl O5 and carbonyl O4. There are no short intermolecular ( < 3.2 Å) contacts between non-H-atoms in the crystal. Atomic parameters and complete tables of bond distances and angles have been deposited with the Cambridge Crystallographic Data Centre, Union Road, Cambridge CB2 2EZ, England. The numbering scheme used is illustrated in the PLUTON [24] plot, Fig. 3. Further details may be obtained from H. St-E.

*Compound* **2** (5, 2′, 4′-*tetrahydroxy-8-γ,γ-dimethylallyl-6″,6″-dimethylpyrano* (3″,2″:6,7) *flavanone, flemichin-D*). Yellow powder, mp 88–91°C. $[\alpha]_D$ − 16.3° ($CHCl_3$; *c* 0.7). TLC (silica gel, $CHCl_3$–MeOH, 9:1): $R_f$ 0.43. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 275 (3.19), 315 (4.01); + NaOMe: 275, 322; + $AlCl_3$: 275, 335; $AlCl_3$–HCl: 275, 314; + NaOAc–B(OH)$_3$: 275, 314. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3340, 2960, 2905, 1600, 1450, 1380, 1295, 1110, 980, 900, 840. TSP-MS (pos. mod.) *m/z*: 423 $[M + H]^+$ (100). FAB-MS (glycerol, pos. mod.) *m/z* (rel. int.): 423 $[M + H]^+$ (100), 407 (22), 367 (18), 333 (8), 287 (17), 231 (21), 185 (33). EIMS *m/z* (rel. int.): 422 $[M]^+$ (100), 407 (50), 389 (37), 361 (38), 333 (17), 286 (5), (285) (8), 271 (22), 243 (19), 215 (43), 136 (8). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Acetylation of **2**. Compound **2** (15 mg) was kept in pyridine–$Ac_2O$ (1:1, 4 ml) at room temp. for 1.4 hr. The mixt. was poured into ice and then partitioned into $Et_2O$. The residue, after evapn of $Et_2O$, was submitted to semi-prep. HPLC on RP-18 (MeOH–$H_2O$, 22:3), affording **2a** (10 mg) and **2b** (5 mg). Compound **2a**: powder, mp 54–59°. $[\alpha]_D$ − 6.6° ($CHCl_3$; *c* 0.01). UV: $\lambda_{max}^{MeOH}$ nm (log ε): 280 (4.22), 320 (4.12). EIMS *m/z* (rel. int.): 506 $[M]^+$ (69), 491 (100), 464 (18), 449 (16), 1431 (4), 271 (2), 215 (6). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Compound **2b**: powder, mp 77–81°. $[\alpha]_D$ − 5.6° ($CHCl_3$; *c* 0.005). UV: $\lambda_{max}^{MeOH}$ nm (log ε): 268 (4.23), 295 (3.10) (sh.), 350 (2.87). EIMS *m/z* (rel. int.): 548 $[M]^+$(32), 506 (73), 491 (100), 451 (8), 365 (2), 243 (2), 189 (4). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Methylation of **2**. Compound **2** (25 mg) was treated with excess $CH_2N_2$–$Et_2O$ at room temp. for 6 days. The reaction product was submitted to semi-prep. HPLC on RP-18 (MeOH–$H_2O$, 17:3) and diol (*n*-hexane–EtOAc, 9:1), giving **2c** (12 mg) as yellow-brown powder, mp 67–70°. $[\alpha]_D$ − 2.8° ($CHCl_3$; *c* 0.008). UV: $\lambda_{max}^{MeOH}$ nm (log ε): 265 (3.17), 300 (3.14) (sh.), 345 (2.16). EIMS *m/z* (rel. int.): 464 $[M]^+$ (100), 449 (61), 409 (12), 300 (2), 285 (16), 257 (6). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **3** (5, 2′, 4′-*trihydroxy-8-γ,γ-dimethylallyl-6″,6″-dimethylpyrano* (2″,3″:7,8) *flavanone, eriosemaone A*). Yellow-brown powder, mp 89–93°. $[\alpha]_D$ − 38.6° ($CHCl_3$; *c* 0.007). TLC (silica gel, $CHCl_3$–MeOH, 9:1): $R_f$ 0.40. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 275 (4.19), 315 (3.98); + NaOMe: 275, 322; + $AlCl_3$; 272, 330; $AlCl_3$–HCl: 275, 315; + NaOAc–B(OH)$_3$: 275, 315. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2950, 2900, 1625, 1450, 1390, 1300, 1015, 980, 890. TSP-MS (pos. mod.) *m/z*: 423 $[M + H]^+$ (100), 180 (21). DCI-MS ($NH_3$, pos.) *m/z* (rel. int.): 423 $[M + H]^+$ (100), 312 (23), 261 (78), 180 (73), 163 (27). EIMS *m/z* (rel. int.): 422 $[M]^+$ (100), 407 (58), 389 (31), 361 (40), 349 (17), 333 (14), 285 (7), 271 (22), 243 (15), 215 (55), 136 (5). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **4** (5, 3′, 5′-*tetrahydroxy-8-γ,γ-dimethylallyl-6″,6″-dimethylpyrano* (3″,2″:6,7) *flavanone, eriosemaone B*). Yellow powder, mp 85–87°. $[\alpha]_D$ − 79.3° ($CHCl_3$;

*c* 0.7). TLC (silica gel, $CHCl_3$–MeOH, 9:1): $R_f$ 0.37. UV $\lambda_{max}^{MeOH}$ nm (log ε): 277 (3.99), 306 (3.78); + NaOMe: 277, 395; + $AlCl_3$ 275, 325; $AlCl_3$–HCl: 275, 304; + NaOAc–B $(OH)_3$: 275, 304. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3340, 2890, 2605, 1645, 1460, 1380, 1263, 1210, 990, 860. EIMS *m/z* (rel. int.): 422 $[M]^+$ (100), 407 (48), 389 (16), 361 (21), 333 (14), 285 (4), 271 (6), 243 (28), 215 (23), 136 (7). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Acetylation of **4**. Compound **4** (70 mg) was kept in pyridine–$Ac_2O$ (1:1, 6 ml) at room temp. for 4 hr. The mixt. was poured into ice and then partitioned into $Et_2O$. The residue, after evapn of $Et_2O$, was submitted to semi-prep. HPLC on RP-18 (MeOH–$H_2O$, 17:3) affording **4a** (50 mg) and **4b** (30 mg). Compound **4a**: powder, mp 49–51°. $[\alpha]_D$ − 61.4° ($CHCl_3$; *c* 0.007). UV: $\lambda_{max}^{MeOH}$ nm (log ε): 275 (4.3), 310 (4.01). EIMS *m/z* (rel. int.): 506 $[M]^+$(82), 491 (100), 463 (8), 365 (4), 271 (2), 215 (3). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Compound **4b**: powder, mp 63–65°. $[\alpha]_D$ − 42.3° ($CHCl_3$; *c* 0.004). UV: $\lambda_{max}^{MeOH}$ nm (log ε): 270 (3.98), 300 (3.7) (sh.), 350. EIMS *m/z* (rel. int.): 548 $[M]^+$ (14), 506 (83), 491 (100), 463 (8), 451 (17), 365 (2), 243 (1.5), 189 (1.8). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Methylation of **4**. Compound **4** (78 mg) was treated with excess $CH_2N_2$–$Et_2O$ at room temp. for 5 days. The reaction product was submitted to semi-prep. HPLC on Nucleosil RP-18 (MeOH–$H_2O$, 47:3; flow rate 8 ml $min^{-1}$) affording **4c** and **4d**. Compound **4c** (10 mg), yellow-brown powder, mp 62–65°. $[\alpha]_D$ − 102° ($CHCl_3$; *c* 0.01). UV: $\lambda_{max}^{MeOH}$ nm (log ε): 275 (4.23), 300 (3.98) (sh.), 375. EIMS *m/z* (rel. int.): 450 $[M]^+$ (70), 435 (100), 271 (14), 215 (32), 149 (6). DCI-MS ($NH_3$, pos.) *m/z* (rel. int.): 451 $[M + H]^+$(86), 286 (2), 264 (8), 248 (100), 193 (18). $^1H$ and $^{13}C$ NMR: Tables 1 and 2. Compound **4d** (3 mg), yellow-brown powder, mp 70–73°. $[\alpha]_D$ − 40.0° ($CHCl_3$; *c* 0.003). UV: $\lambda_{max}^{MeOH}$ nm (log ε): 275 (4.13), 300 (sh.) (4.01), 350. DCI-MS ($NH_3$, pos.) *m/z* (rel. int.): 465 $[M + H]^+$ (100). EIMS *m/z* (rel. int.): 464 $[M]^+$ (97), 449 (100), 300 (12), 285 (71), 257 (42), 201 (8), 164 (14), 149 (17), 121 (9). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **5** (*2′,3′,6′,2‴,4‴-pentahydroxy-8-γ,γ-dimethylallyl-6″,6″-dimethyl-pyrano (3″,2″:6,7) flavanone, eriosemaone C*). Yellow-brown powder, mp 120–125°. $[\alpha]_D$ − 9.5° ($CHCl_3$; *c* 0.01). TLC (silica gel, $CHCl_3$–MeOH, 9:1): $R_f$ 0.28. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 275 (3.78), 300 (3.56); + NaOMe: 275, 317; + $AlCl_3$: 275, 335; $AlCl_3$–HCl: 275, 335; + NaOAc: 265, 320; + NaOAc– $B(OH)_3$: 265, 315. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3410, 2910, 2980, 1615, 1450, 1380, 1190, 1120. FAB-MS (glycerol, neg.) *m/z* (rel. int.): 529 $[M - H]^+$ (100), 285 (24), 269 (98), 243 (84). FAB-MS (glycerol, pos.) *m/z* (rel. int.): 531 $[M + H]^+$ (85), 515 (28), 469 (22), 457 (31), 287 (42), 287 (23), 285 (24), 271 (20), 231 (100), 215 (33), 189 (19), 133 (12). DCI-MS ($NH_3$, pos.) *m/z* (rel. int.): 531 $[M + H]^+$ (63), 313 (8), 261 (100), 180 (11). EIMS *m/z* (rel. int.): 530 $[M]^+$(28), 497 (17), 469 (31), 271 (25), 260 (32), 245 (64), 215 (62), 189 (100), 161 (16), 147 (22). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **6** (*6, 8, 2′-trihydroxy-6″,6″-dimethyl-pyrano (2″,3″: 4′,5′) isoflavanone, eriosemaone D*). Yellow powder, mp 118–122°. $[\alpha]_D$ − 10.1° ($CHCl_3$; *c* 0.02). TLC (silica gel, $CHCl_3$–MeOH, 9:1): $R_f$ 0.51. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 260 (4.1), 300 (3.98); + NaOMe: 273, 325; + $AlCl_3$–HCl: 272, 312; NaOAc-B $(OH)_3$: 262, 300. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2910, 1610, 1510, 1400, 1280, 1080. EIMS *m/z* (rel. int.): 352 $[M]^+$ (51), 338 (24), 337 (100), 284 (8), 185 (11), 153 (4). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **7** (*2′, 6′, 2, 4-tetramethoxy-5′-γ,γ-dimethylallyl-6″, 6″-dimethylpyrano (3″, 2″:3′,4′) chalcone*). Yellow- brown powder, mp 55–58°. $[\alpha]_D$ + 0.69° ($CHCl_3$; *c* 0.013). TLC (silica gel, $CHCl_3$–MeOH, 9:1): $R_f$ 0.45. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 235 (4.06), 363 (3.54). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1750, 1650, 1605, 1580, 1490, 1450, 1420, 1200, 1100, 1025, 980. DCI-MS ($NH_3$, pos.) *m/z* (rel. int.): 479 $[M + H]^+$ (71), 331 (6), 289 (14), 198 (18), 184 (18), 167 (100). EIMS *m/z* (rel. int.): 478 $[M]^+$ (36), 463 (100), 423 (11), 191 (4). $^1H$ NMR (200 MHz, $CDCl_3$): δ6.24 (1H, *d*, *J* = 3.8 Hz, H-3), 6.48 (1H, *dd*, *J* = 8.4, 3.8 Hz, H-5), 7.48 (1H, *d*, *J* = 8.4 Hz, H-6), 7.05 (1H, *d*, *J* = 16.0 Hz, H-α), 7.68 (1H, *d*, *J* = 16.0 Hz, H-β), 6.54 (1H, *d*, *J* = 8.1 Hz, H-4″), 5.59 (1H, *d*, *J* = 8.1 Hz, H-5″), 1.43 (6H, *s*, –OMe × 2, 6″), 3.28 (2H, *d*, *J* = 6.8 Hz, H-1‴), 5.19 (1H, *t*, *J* = 6.8 Hz, H-2‴), 1.68 (3H, *s*, H-4‴), 1.78 (3H, *s*, H-5‴), 3.65 (3H, *s*, –OMe), 3.69 (3H, *s*, –OMe), 3.81 (3H, *s*, –OMe), 3.82 (3H, *s*, –OMe). $^{13}C$ NMR (50 MHz, $CDCl_3$): δ111.1 (C-1′), 152.2 (C-2′), 116.9 (C-3′), 162.8 (C-4′), 119.2 (C-5′), 153.1 (C-6′), 195.2 (C=O), 129.9 (C-α), 141.1 (C-β), 121.3 (C-1), 156.2 (C-2), 98.3 (C-3), 160.1 (C-4), 105.2 (C-5), 127.2 (C-6), 129.1 (C-4″), 117.2 (C-5″), 77.9 (C-6″), 27.9 × 2 (C-6″-Me), 22.2 (C-1‴), 122.9 (C-2‴), 131.1 (C-3‴), 17.9 (C-4‴), 25.8 (C-5‴), 55.5 × 2 (C-2, 4-OMe), 62.5 (C-2′-OMe).

*Compound* **8** (*5, 3′, 5′-trimethoxy-8-γ,γ-dimethylallyl-6″, 6″-dimethylpyrano (2″, 3″ : 6, 7) flavanone*). Yellow-brown powder, mp 49–52°. $[\alpha]_D$ − 103.5° ($CHCl_3$: *c* 0.006). TLC (silica gel, $CHCl_3$–MeOH, 9:1): $R_f$ 0.39. HPTLC (diol, EtOAc–petrol, 1:4): $R_f$ 0.49. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 265 (4.1), 295 (3.89) (sh.), 350. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1680, 1595, 1485, 1450, 1425, 1210, 1100, 1057. EIMS *m/z* (rel. int.): 464 $[M]^+$ (84), 449 (100), 300 (2), 285 (18), 257 (8). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **9** (*5, 2′, 4′-trimethoxy-8-γ,γ-dimethylallyl-6″, 6″-dimethylpyrano (2″, 3″:6, 7) flavanone*). Yellow-brown powder, mp 48–51°. $[\alpha]_D$ − 4.9° ($CHCl_3$; *c* 0.014). HPTLC (diol, EtOAc–petrol, 1:4): $R_f$ 0.47. UV: $\lambda_{max}^{MeOH}$ nm (log ε): 270 (3.9), 298 (3.95) (sh.), 354. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2950, 2900, 1660, 1620, 1580, 1495, 1450, 1370, 1200, 1145, 1020, 830. EIMS *m/z* (rel. int.): 464 $[M]^+$ (100), 449 (75), 300 (6), 285 (86), 257 (49). $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

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