*Article*

*J. Mex. Chem. Soc.* **2006**, *50(2),* 71-75

ISSN 1870-249X

# New Prenylated Flavanones from *Esenbeckia berlandieri* ssp. *acapulcensis*

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Recibido el 12 de enero del 2006; aceptado el 29 de junio del 2006

**Abstract**. Three new (**1-3**) and four known prenylated flavanones as well as friedelin were isolated from the aerial parts of *Esenbeckia berlandieri* ssp *acapulcensis* (Rutaceae). The structures of the new compounds were elucidated as 5,7-dihydroxy-8-(2,3-epoxy-3-methylbutyl)flavanone (**1**), 5,4´-dihydroxy-7-methoxy-8-(2,3-dihydroxy-3-methylbutyl)flavanone (**2**), and 5,7,4´-trihydroxy-8-(2,3-dihydroxy-3-methylbutyl)flavanone (**3**). The isolated flavanones possess diferentially functionalized isoprene units at C-8, and this is the first report of flavanones isolated from *Esenbeckia* species.
**Keywords**: *Esenbeckia berlandieri* ssp. *acapulcensis,* Rutaceae, prenylflavanones.

**Resumen**. Tres flavanonas preniladas novedosas (**1-3**), cuatro conocidas así como friedelina fueron aisladas de las partes aéreas de *Esenbeckia berlandieri* ssp *acapulcensis* (Rutaceae). Las estructuras de los compuestos nuevos fueron determinadas como 5,7-dihidroxi-8-(2,3-epoxi-3-metilbutil)flavanona (**1**), 5,4´-dihidroxi-7-metoxi-8-(2,3-dihidroxi-3-metilbutil)flavanona (**10**) y 5,7,4´-trihidroxi-8-(2,3-dihidroxi-3-metilbutil)flavanona (**11**). Las flavanonas aisladas poseen isoprenilos con diferente funcionalidad unidos a C-8, y este es el primer reporte de flavanonas aisladas de especies de *Esenbeckia*
**Palabras clave**: *Esenbeckia berlandieri* ssp. *acapulcensis,* Rutaceae, prenilflavanonas.

The genus *Esenbeckia* (family Rutaceae, subfamily Rutoideae) is known to be a source of metabolites such as limonoids [1-3], coumarins [2,4-10], phenylpropanoids [5], polyprenols, phloroglucinols [11], alkaloids [2,6-10,12-14], triterpenoids [3,15], and flavonoids [16]. In our research on the chemical constituents of this group of plants, new prenylated flavanones were isolated from *Esenbeckia berlandieri* ssp. *acapulcensis* and this is the first report of prenylated flavanones from *Esenbeckia* species. In this paper the isolation, structural determination and some biological evaluations of these compounds are described.

## Results and Discussion

The ethanolic extract of the leaves of *E. berlandieri* ssp. *acapulcensis* was chromatographed on a silica gel column eluted with a *n*-hexane-EtOAc gradient to give **1-3**, together with the known compounds 7-methylglabranin (**4**) [17], glabranin (**5**), [18-20], 8-prenylnaringenin (**6**) [21,22], and 7-methyl-8-prenylnaringenin (**7**) [23].

The totally decoupled $^{13}$C NMR spectrum of **1** (Tables 1 and 2) showed 20 resonances, sorted by DEPT experiments in two methyls, two methylenes, eight methines (six sp$^2$ and two sp$^3$ carbon bearing an oxygen atom) and eight quaternary carbons (including one carbonyl and one sp$^3$ carbon bearing oxygen), deducing a molecular formula of $C_{20}H_{20}O_5$, in agreement with the $^1$H NMR and the EIMS ($M^+$ 340). The IR spectrum established the presence of hydroxyl groups (3591-3066 cm$^{-1}$) and the carbonyl group of a γ-pyrone (1642 cm$^{-1}$), $^1$H-$^1$H NMR COSY correlations between H-2 ($\delta_H$ 5.44) and H-3 ($\delta_H$ 3.07 and 2.82) and between the aromatic protons H-2´ through H-6´ ($\delta_H$ 7.43 – 7.47) revealed the presence of a 2,8-disubstituted 5,7-dihydroxychroman-4-one ring. Because ten of eleven unsaturations were accounted for a flavanone nucleus, it was concluded the presence of an isoprenyl group. The presence of an epoxide in this fragment was confirmed by the $^{13}$C NMR resonances at $\delta_C$ 79.1 and 71.8 and by the signal at $\delta_H$ 4.71 in the $^1$H NMR spectrum. These results indicated that **1** is 5,7-dihydroxy-8-(2,3-epoxy-3-methylbutyl)flavanone (2´,3´´-epoxy-glabranin). Compound **2** had a molecular formula $C_{21}H_{24}O_7$ as deduced from EIMS *m/z* 370 ($M^+$ -$H_2O$), $^1$H, $^{13}$C NMR and DEPT spectral data. The IR spectrum of **2** indicated the presence of the carbonyl group of a γ-pyrone (1643 cm$^{-1}$) and hydroxyl groups (3585-3055 cm$^{-1}$). The flavanone nucleus was determined by the presence of the AMX system for H-2 ($\delta_H$ 5.38, dd, *J* = 12.7, 3.1) and H-3a ($\delta_H$ 3.09, *J* = 17.1, 12.7), H-3b ($\delta_H$ 2.79, *J* = 17.1, 3.1), and the hydroxyl at C-4´ was evident by the presence of an AA´BB´ proton doublets centered at $\delta_H$ 7.37 (2H, d, *J* = 9 Hz, H-2´, H-6´) and $\delta_H$ 6.94 (2H, d, *J* = 9 Hz, H-3´, H-5´) in the $^1$H NMR spectrum, and by the result of the methylation of **3** (*vide infra*). The $^{13}$C NMR signals at $\delta_C$ 90.5, 71.9, 29.7, 25.9 and 23.9 revealed the presence of a 2,3-dihydroxy-3-methylbutyl group at C-8. Thus, the structure of **2** was determined to be 5,4´-dihydroxy-7-methoxy-8-(2,3-dihydroxy-3-methylbutyl)flavanone (7-methyl-8-(2´´,3´´-dihydroxyprenyl)-naringenin).

The IR, $^1$H and $^{13}$C NMR spectra of **3** were similar to those of **2** except for the absence of a methyl group at C-7, in agreement with the molecular formula $C_{20}H_{22}O_7$ registered by EIMS ($M^+$ *m/z* 374). Reaction of **3** with diazomethane afforded **2**, identical by direct comparison with the natural product, con-

**Table 1.** $^{1}$H NMR Data (200 MHz, $CDCl_3$) for Compounds **1-3**

| H | **1** | **2** | **3** |
|---|---|---|---|
| H-2 | 5.44 (dd, 12.7, 3.2) | 5.38 (dd, 12.9, 3.3) | 5.36 (dd, 12.7, 3.3) |
| $H\text{-}3_{eq}$ | 2.82 (dd, 17.1, 3.2) | 2.79 (dd, 17.1, 3.3) | 2.78 (dd, 17.1, 3.3) |
| $H\text{-}3_{ax}$ | 3.07 (dd, 17.1, 12.7) | 3.09 (dd, 17.1, 12.9) | 3.05 (dd, 17.1, 12.7) |
| H-6 | 6.04 (s) | 6.03 (s) | 6.03 (s) |
| H-1´´ | 3.06-2.99 (m) | 3.09-2.98 (m) | 3.11-2.99 (m) |
| H-2´´ | 4.71 (t, 8.9) | 4.69 (dd, 10.2, 2.7) | 4.70 (tq, 8.1, 2.4) |
| H-4´´ | 1.21 (s) | 1.20 (d, 1.8) | 1.21 (d, 1.8) |
| H-5´´ | 1.19 (s) | 1.33 (d, 2.4) | 1.19 (d, 1.5) |
| $C_5$-OH | 12.10 (s) | 12.00 (s) | 12.31 (s)s |
| $C_7\text{-}OCH_3$ | | 3.86 (s) | |
| H-2´, H-6´ | 7.47-7.43 | | |
| ´ H-3´, H-5´ | (m) | | |
| H-4´ | | | |
| H-2´, H-6´ | | 7.37 (d, 9.0) | 7.31 (d, 8.4) |
| H-3´, H-5´ | | 6.94 (d, 9.0) | 6.89 (d, 8.4) |

**Table 2.** $^{13}$C NMR Data (50 MHz, $CDCl_3$) for Compounds **1-3**.

| C | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 79.1 | 79.0 | 79.2 |
| 3 | 43.6 | 43.2 | 43.5 |
| 4 | 195.1 | 195.4 | 196.2 |
| 5 | 165.1 | 165.2 | 162.2 |
| 6 | 91.7 | 91.6 | 96.6 |
| 7 | 168.6 | 168.6 | 163.6 |
| 8 | 104.8 | 104.7 | 106.0 |
| 9 | 157.1 | 160.1 | 159.2 |
| 10 | 103.1 | 103.1 | 103.7 |
| 1´ | 138.0 | 130.6 | 130.6 |
| 2´ | 126.1 | 127.7 | 127.7 |
| 3´ | 128.9 | 114.2 | 115.4 |
| 4´ | 126.1 | 157.3 | 155.8 |
| 5´ | 128.9 | 114.2 | 115.4 |
| 6´ | 126.1 | 127.7 | 127.5 |
| 1´´ | 26.8 | 29.7 | 29.5 |
| 2´´ | 29.7 | 90.5 | 91.0 |
| 3´´ | 71.8 | 71.9 | 71.9 |
| 4´´ | 23.9 | 24.0 | 23.9 |
| 5´´ | 25.9 | 25.9 | 25.8 |
| $Ph\text{-}O\text{-}CH_3$ | | 55.38 | |

firming the structure **3**. This result was similar to that of the methylation of **6** to afford **7** (vide infra).

The *S*- configuration at C(2) for compounds **1-3** was assigned following previous observations of the enantiodifferentiated biotransformation of chalcones to flavanones by the corresponding enzyme [24,25], establishing that optically active flavanones in higher plants possess *S*- configuration.

Regarding the configuration at C-2´´, it has been previously discussed the epoxidation of the prenyl residue proceeds with high stereoselectivity for quinoline alkaloids and coumarins in a given species of the Rutaceae and Umbelliferae [26], and the opening of the epoxide occurs through an acid catalyzed reaction not involving the chiral center. However, since this is only a working hypothesis, additional arguments are necessary to determine the stereochemistry of the chiral carbon at the prenyl fragment.

Methylation of **5** and **6** with diazomethane afforded **4** and **7,** respectively, which were identified by their physicochemical properties. Prolonged diazomethane treatment of **6** or **7** afforded **8**. Acetylation of 7-methylglabranin (**4**) under reflux afforded the prenyl acetylated derivatives **9** and **10**, while acetylation of glabranin (**5**) afforded **11**. These results were in agreement with placement of the prenyl group at C(8) (instead of C(6)) [27], and further confirmed by the observed $^{1}$H NMR

NOESY correlation between the vinylic hydrogen H-2´´ and the oxymethine hydrogen H-2 for **4** and **5** (see Figure 1). The analysis of the COLOC and HETCOR spectra allowed the assignments of the $^{1}H$ and $^{13}C$ NMR data, which are shown in Tables 1 and 2.

It is interesting to note the structural variation of the hemiterpenic fragment (olefin, epoxide, diol) in this species, in agreement with the proposed biogenesis of furo- and pyrano-rings. The occurrence of prenylated flavanones from *Esenbeckia* is unprecedented, although this type of compounds has been reported from other genera —*Phellodendron*, *Phebalium*, *Euodia* and *Flindersia*— of the Rutaceae [28]. Recently, various flavonoids possessing isopentenyl side chains in the A-ring have been prepared and evaluated for their capacity to bind estrogen receptor [29]. 8-Prenylnaringenin (**6**) isolated from *Marshallia grandiflora, Sophora tomentosa* and *Humulus lupulus* has recently been shown to be an extremely potent phytoestrogen [30].

With respect to the antimicrobial activity, the ethanolic extract and the pure compounds did not show significant inhibitory activity against *B. cereus*, *C. albicans*, *E. coli*, *E. cloacae*, *K. pneumoniae*, *M. fortuitum*, *S. pyogenes*, *Salmonella sp*., *P. aeruginosa*. Only glabranin (**5**) displayed activity against *Staphylococcus aureus* and *Candida albicans* [18].

The ethanolic extract of *E. berlandieri* ssp *acapulcensis* did not display toxicity in the brine shrimp bioassay, but some fractions that contained **1** and **5** showed activity. The toxicities for the pure compounds **1** and **5** were $LC_{50}$ 252 ppm and $LC_{50}$ 289 ppm, respectively.

The present and earlier investigations have resulted in the interesting observation that the constituents of the various *Esenbeckia* species differ fairly from one another. In particular, *E. berlandieri* ssp *acapulcensis* biosynthesized prenylated flavanones similar to those characterized from *Tephrosia* and *Lonchocarpus* (Fabaceae), suggesting a chemotaxonomic relationship.

## Experimental Section

**General Experimental Procedures**. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. IR: Nicolet Magna FT-IR 750 spectrometer. $^{1}H$ and $^{13}C$ NMR Spectra: Varian Unity 300 spectrometer and Varian Gemini spectrometer (at 300/75 MHz, and 200/50 MHz). EI-MS: Jeol JMS-AX505HA mass spectrometer. Column Chromatography (CC): silica gel (70-230). TLC silica gel 60 F254 (Merck) plates. PTLC silica gel 60 F254 (Merck) plates.

**Plant Material**. Aerial parts of *Esenbeckia berlandieri* ssp *acapulcenis* Rose were collected in "El Rebaljito", 20 Km from Melaque, Jalisco, Mexico. The plant was identified by Clara H. Ramos and a voucher specimen with the identification number CH-83 has been deposited at the Herbario Nacional (MEXU) of the Instituto de Biología de la Universidad Nacional Autónoma de México.

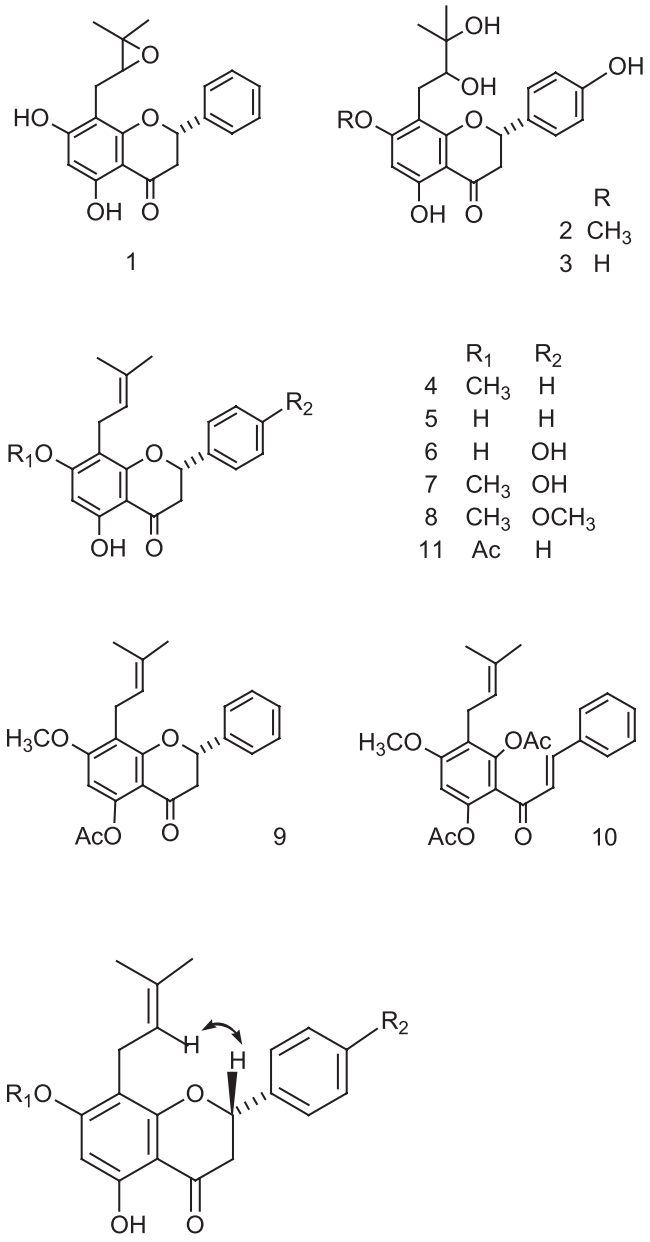


**Fig. 1**. H-2 / H-2´´ NOESY correlation

**Extraction and Isolation**. The ethanol extract (52 g) obtained by maceration of the aerial parts (1.63 Kg) was adsorbed in silica gel (50 g) and subjected to vaccum chromatography (VLC) using *n*-hexane and mixtures of *n*-hexane-EtOAc. This procedure allowed the isolation of methylglabranin (**4,** 261.7 mg), glabranin (**5**, 7.09 g), 8-prenylnaringenin (**6,** 28. 4 mg), friedelin (240 mg) and 7-methyl-8-(2´´, 3´´-dihydroxy-3´´-metilbutil)-naringenin (**3,** 5.4 mg)

The residue obtained of some fractions eluted with *n*-hexane-EtOAc 9:1 (106 mg) was rechromatographed and additionally purified by preparative TLC, to afford **1** (14.7 mg) and **7** (7-methyl-8-prenyl-naringenin, 8 mg). The residue obtained from some fractions eluted with *n*-hexane-EtOAc 4:1 (357

mg) was further purified by column chromatography and preparative TLC to afford **3** (7.2 mg) and **2** (23.4 mg).

Some fractions eluted from the initial chromatography, eluted with *n*-hexane-EtOAc 85:15, were treated with activated charcoal and then treated with ethereal diazomethane at 0°C to afford, after preparative TLC, additional amount of **3 (**23.4 mg).

From the fractions eluted with *n*-hexane-EtOAc 9:1 was obtained a residue (905 mg) which was dissolved in EtOH and treated with ethereal diazomethane at 0°C. The succesive chromatographies of the residue afforded **8** (77.3 mg).

**5,7-Dihydroxy-8-(2,3-epoxy-3-methylbutyl)flavanone (2´´,3´´-epoxy-glabranin) (1)**: yellow oil, $R_f$ 0.6 (*n*-hexane-EtOAc 3:2), $[\alpha]_D^{25}$ = -25 (*c* 0.7, $CHCl_3$), IR $n_{max}$ ($CHCl_3$): 3591, 3066, 2978, 2929, 1642, 1475, 1383, 1249, 1144 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$): see Table 1; $^{13}C$ NMR (50 MHz, $CDCl_3$): see Table 2; EIMS *m/z* (rel. int.): 340 (87), 307 (68), 282 (83), 262 (22), 203 (70), 191 (29), 178 (100), 177 (64), 176 (51), 150 (77).

**5,4´-Dihydroxy-7-methoxy-8-(2,3-dihydroxy-3-methylbutyl)flavanone (7-methyl-8-(2´´, 3´´-dihydroxyprenyl)-naringenin) (2)**, yellow oil, $R_f$ 0.5 (*n*-hexane-EtOAc 3:2), $[\alpha]_D^{25}$ = -15.2 (*c* 0.04, $CHCl_3$), IR $\nu_{max}$ ($CHCl_3$): 3585, 3055, 2976, 2931, 1643, 1516, 1474, 1383, 1250,1148 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$): see Table 1; $^{13}C$ NMR (50 MHz, $CDCl_3$): see Table 2; EIMS *m/z* (rel. int.): 371 [$M^+$+1-$H_2O$] (23), 370 [$M^+$-$H_2O$] (100), 369 (23), 337 (60), 312 (40), 311 (50), 219 (28), 203 (94), 178 (67), 177 (64), 176 (33), 150 (64), 134 (74), 121 (48) y 91 (31).

**5,7,4´-Trihydroxy-8-(2,3-dihydroxy-3-methylbutyl)flavanone (8-(2´´,3´´-dihydroxyprenyl)-naringenin) (3)**: yellow oil; $R_f$ 0.4 (*n*-hexane-EtOAc 3:2), IR $\nu_{max}$ ($CHCl_3$): 3595, 3037, 3007, 2981, 1645, 1476, 1385, 1338, 1144 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$): see Table 1; $^{13}C$ NMR (50 MHz, $CDCl_3$): see Table 2; EIMS *m/z* (rel. int.): 374 [$M^+$, 23], 356 (100), 323 (18), 203 (43), 178 (40), 165 (54), 150 (40) 149 (69), 120 (50), 97 (48) 83 (46), 81 (41), 59 (40), 57 (90), 55 (95), 43 (92).

**Acetylation of 7-methylglabranin (4)**. Compound **4** (140.3 mg) was refluxed with acetic anhydride (2 mL) and pyridine (0.5 mL) for 23 h to afford a mixture (107 mg) which was separated by preparative TLC to afford **9** (41 mg) and **10** (55 mg). **5-Acetoxy-7-methoxy-8-prenyl-flavanone** (**9**). Colorless amorphous solid, $R_f$ 0.6 (*n*-hexane-EtOAc 8:1), IR ($CHCl_3$) $\nu_{max}$: 3016, 3008, 2970, 1766, 1607, 1573, 1411, 1367, 1290, 1186, 1168, 1097 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz): δ 7.44-7.42 (s, m, 5H), 6.29 (s, 1H, H-6), 5.44 (1H, dd, $J_{2,3ax}$ = 13, $J_{2,3eq}$ = 3.1, Hz, H-2), 5.15 (1H, m, H-2´´), 3.32 (1H, d, *J* = 6.8 Hz, , H-1´´), 2.99 (1H, dd, $J_{3ax3eq}$ = 16.7, $J_{3ax2}$ = 13 Hz, H-$3_{ax}$), 2.75 (1H, dd, $J_{3ax3eq}$ = 16.8, $J_{3ax2}$ = 3 Hz, H-$3_{eq}$), 1.65 (s, 6H, 2$CH_3$-4´´, $CH_3$-5´´); $^{13}C$ NMR ($CDCl_3$, 75 MHz, assignments by DEPT): δ 189.6 (C-4), 162.8 (C-7), 150.5 (C-5), 149.9 (C-9), 138.9 (C-1´), 131.8 (C-3´´), 128.7 (C-3´ and C-5´), 128.5 (C-2´ and C-6´), 125.9 (C-4´), 121.8 (C-2´´), 116.0 (C-8), 108.1 (C-10), 100.2 (C-6), 78.0 (C-2), 45.3 (C-3), 22.2 (C-5´´), 21.2 (C-1´´), 17.7 (C-4´´), 56.0 ($CH_3O$-Ph), 169.8 (-$COOCH_3$) and 25.8 ($CH_3$-CO); EIMS *m/z* (rel. int.): 338 (100), 323(45), 295 (15), 283 (13), 270 (24), 219 (35), 191 (15), 179 (15).

The more polar compound **2-hydroxy-4-methoxy-6-acetoxy-3-prenil-chalcone** (**10**), yellow oil, $R_f$ 0.4 (*n*-hexane-EtOAc 4:1), IR ($CHCl_3$) $\nu_{max}$ : 3007, 2968, 1770, 1669, 1610, 1448, 1369, 1286, 1165, 1125, 1069 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz): δ 7.41-7.27 (br m, 5H), 7.48 (1H, d, *J* = 16.1 Hz, H-b), 6.94 (1H, d, *J* = 16.1 Hz, H-a), 6.58 (s, 1H, CH-6), 5.10 (m, 1H, CH-2´´), 3.87 (s, 3H, $CH_3$OPh), 3.24 (d, *J* = 7 Hz, 2H, $CH_2$-1´´), 1.72 (s, 3H, $CH_3$-5´´),1.67 (s, 3H, $CH_3$-4´´), 2.12 (s, 3H, $CH_3$CO), 2.17 (s, 3H, $CH_3$CO), EIMS *m/z* (rel. int.): 422 [$M^+$, 43] 379 (60), 362 (4), 337 (100), 323 (53), 295 (25), 283 (35), 233(43), 219 (30), 179 (32), 43 (20).

**Acetylation of the glabranin (5)**. Compound **5** (49.3 mg) in $CHCl_3$ (5 mL) was treated with $Ac_2O$ (1 mL) and Py (0.5 mL) a room temp. for 30 min. After usual work-up 40 mg of a residue was obtained which was purified by prep. TLC (eluting with *n*-hexane-EtOAc 9:1) to give **11** (22.5 mg) as yellow crystals, mp. 89-90°C; $R_f$ 0.7 (*n*-hexane-EtOAc 7:3), IR ($CHCl_3$) $\nu_{max}$: 2970, 2916, 1769, 1649, 1625, 1593, 1372, 1186, 1137, 1078, 1062 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 11.74 (1H, s, $C_5$-OH), 7.47-7.41 (m, 5H), 6.30 (1H, s, H-6), 5.44 (1H, dd, $J_{2,3ax}$ = 12.9, $J_{2,3ax}$ = 3.3 Hz, H-2), 5.05 (1H, tq, *J* = 7.1 and 1.4, CH-2"), 3.17 (2H, d, *J* = 7.7 Hz, H-1´´), 3.12 (1H, dd, $J_{3ax3eq}$ = 17.2, 12.9 Hz, H-$3_{ax}$), 2.88 (1H, dd, $J_{3eq3ax}$ = 17.2, $J_{3ax2}$ = 3.3 Hz, H-$3_{eq}$), 1.66 (3H, s, $CH_3$-5´´), 1.65 (3H, s, $CH_3$-4´´), 2.31 (3H, s, $CH_3COO$); $^{13}C$ NMR ($CDCl_3$, 75 MHz, assignments by DEPT): δ 197.5 (C-4), 160.8 (C-7), 160 (C-5), 156.7 (C-9), 138.3 (C-1´), 132 (C-3´´), 128.8 (C-3´ and C-5´´), 126 (C-2´, C-4´ and C-6´), 121.5 (C-2´´), 113.5 (C-8), 106.6 (C-10), 103.9 (C-6), 79.2 (C-2), 43.6 (C-3), 22.6 (C-5´´), 20.9 (C-1´´), 17.3 (C-4´´), 168.4 (-$COOCH_3$) y 25.7 ($CH_3$-CO). EIMS *m/z* (rel. int.): 367 [$M^+$ + 1, 20], 323 (100), 309 (29), 281 (18), 269 (22), 219 (36), 177 (25), 165 (21).

**Biological evaluations**. Antimicrobial assays were performed with cultures of *S. aureus* (ATCC, 6538), *B. cereus* (ATCC, 14737), *C. albicans* (ATCC, 10231), *E. coli* (ATCC, 8739), *E. cloacae* (ATCC; 29249), *K. pneumoniae* (ATCC, 10872), *M. fortuitum* (ATCC; 27408), *S. pyogenes* (ATCC, 10096), *Salmonella sp*., *P. aeruginosa* (ATCC, 9037). The bacteria were maintained in trypticase soy agar, and the yeast on Sabourand's dextrose agar. The screening method was performed in duplicates and based on the method of Mitscher [19] and disk assay procedures. Brine shrimp lethalities for the extracts were determined as described in the literature [31].

## Acknowledgements

The authors thank Rocío Patiño, María Isabel Chávez, Angeles Peña, Luis Velasco and Javier Pérez from the Instituto de Química de la UNAM for technical assistance, and Prof. Esteban M. Martínez from the Instituto de Biología de la UNAM, for helpful discussions

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27. It is interesting to note that several years ago Bohlmann [33] proposed that the placement of the prenyl group at C(6) or at C(8) in the flavanone nucleus could be determined by the difference in the chemical shifts of the methyl hydrogens H(4´´) and H(5´´). If this difference was near zero, the prenyl group would be at C(8), while a prenyl group at C(6) would produce different chemical shifts for the methyl groups. These observations are not followed by compounds **1**-**3**. Therefore, additional arguments should be considered for structural assignments.
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