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COUMARINS AND FLAVONES FROM *CASIMIROA PRINGLEI*

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Abstract – A chemical study of the leaves of *Casimiroa pringlei* led to the isolation of six coumarins, six flavones and the sesquiterpene, cryptomeridiol. Four of these compounds are new natural products which, on the basis of spectroscopic analyses, were characterized as (Z)-trichoclin acetate, 5,3',5'-trimethoxyflavone, 5,2',3',4'-tetramethoxyflavone, and 5,2',3',4',5',6'-hexamethoxyflavone. Although 5,2',3',4'-tetramethoxyflavone has been obtained by synthesis, this is the first report of its isolation from a natural source.

INTRODUCTION

The genus *Casimiroa* (fam. Rutaceae) contains nine recognized species.[1] One of them, *C. edulis*, is used in Mexican folk medicine mainly as sleep inducer, hipotensive and sedative.[2] As a consequence, numerous pharmacological and chemical studies on this species were published in the last century.[3,4] Less attention has been focused on the other *Casimiroa* species, of which only *C. greggii* (formerly *Sargentia greggii*),[5,6] *C. pubescens*,[7,8] *C. calderoniae*,[8] and *C. tetrameria*[9] have been studied. These species, as *C. edulis*, contain alkaloids,[4,7] coumarins,[5,8,9] limonoids,[7] and flavones.[5-7,9] In this paper, we describe the isolation of coumarins and flavones from the leaves of *C. pringlei*, as well as the elucidation of the structure of the new compounds.

RESULTS AND DISCUSSION

Chromatographic purifications of the acetone extracts of *C. pringlei* led to the isolation of six furocoumarins, 5-geranyloxypsoralen (**1**),[10] 8-geranylpsoralen (**2**),[10] isopimpinellin (**3**),[11] imperatorin (**4**),[12] (*Z*)-trichoclin acetate (**5**), and xanthotoxol (**6**),[13] together with the flavones 5,6-dimethoxyflavone (**7**),[5]

5,6,3'-trimethoxyflavone (**8**),[14] 5,6,2',3',5',6'-hexamethoxyflavone (**9**),[9] 5,2',3',4',5',6'-hexamethoxyflavone (**10**), 5,3',5'-trimethoxyflavone (**11**), 5,2',3',4'-tetramethoxyflavone (**12**), and one sesquiterpene identified as cryptomeridiol (**13**).[15] Compounds **5**, **10**, **11** and **12** are new natural products (Figure 1).

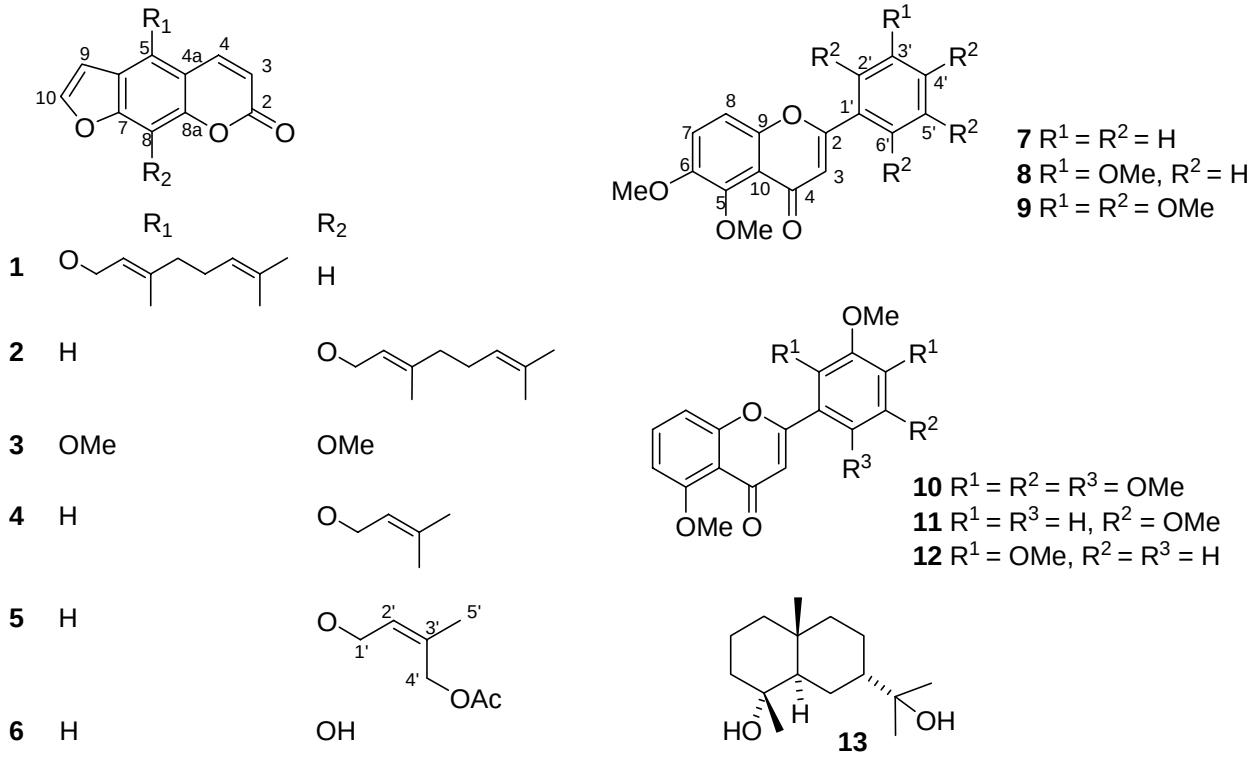

Figure 1

The HRFAB-MS of compound **5** showed the $[M + H]^+$ ion at *m/z* 329.1035, corresponding to the molecular formula $C_{18}H_{16}O_6$. The ^1H NMR spectrum showed signals for two furan protons (δ 6.78, d, *J* = 2.1 Hz, H-9 and δ 7.66, d, *J* = 2.1 Hz, H-10), one aromatic proton (δ 7.34, s, H-5), and for the α and β protons of an α,β-unsaturated-δ-lactone (δ 6.33, d, *J* = 9.6 Hz, H-3 and δ 7.74, d, *J* = 9.6 Hz, H-4). These signals and the observed NOE interactions between H-4 and H-5 indicate an 8-substituted furocoumarin. The substituent at C-8 was identified as a 4-acetoxy-3-methyl-2-butenyl group by the signals at δ 5.05 (2H, br d, *J* = 6.9 Hz, H-1'), 5.83 (1H, br t, *J* = 6.9 Hz, H-2'), 4.63 (2H, s, H-4'), 1.78 (3H, br s, H-5') and 2.01 (3H, s MeCO). NOE correlations between H-1' and H-4', and between H-2' and H-5' indicated a *Z*-configuration of the C-2'-double bond, thus establishing the structure of the new coumarin **5** as (*Z*)-8-[(4-acetoxy-3-methyl-2-butenyl)oxy]psoralen or (*Z*)-trichoclin acetate. The (*E*)-isomer of this compound has been previously isolated from *Phebalium* aff. *tuberculosum* (Rutaceae)[16] and obtained by acetylation of (*E*)-trichoclin ((*E*)-8-[(4-acetoxy-3-methyl-2-butenyl)oxy]psoralen). Its structure was confirmed by synthesis.[17]

Recently, (*E*)-and (*Z*)-trichoclins were described as two of the biotransformation products of imperatorin (**7**) by *Aspergillus flavus*.[18] However, this biosynthesized (*Z*)-trichoclin showed the H-4 signal at δ 8.12, thus indicating a C-5 rather than a C-8 substitution.[19] The same is true for the other biotransformation products.[18]

The HRFAB-MS spectrum of flavone **10** exhibited a $[M + H]^+$ ion at *m/z* 403.1385, which, together with the presence of twenty-one carbon signals in the ^{13}C NMR spectrum, established the molecular formula as $C_{21}H_{22}O_8$. Six of the carbon signals are due to methoxyl groups, one to a carbonyl group, and the others to twelve aromatic and two vinylic carbons, thus indicating the presence of a hexamethoxyflavone. The ^1H (Table 1) and COSY NMR spectra of **10** showed signals for six methoxyl groups, a singlet signal at δ 6.34, which was attributed to H-3, and three coupled aromatic protons. The first one was a triplet (δ 7.54, *J* = 8.0 Hz) and it was ortho-coupled with the signals at δ 7.02 (dd, *J* = 8.0, 1.0 Hz) and 6.82 (dd, *J* = 8.0, 1.0 Hz). These signals were assigned to H-7, H-8 and H-6, respectively. The last was supported by the HMBC spectrum which exhibited correlations between C-10 (δ 114.7) and H-3, H-6 and H-8, and between C-5 (δ 56.5) and H-6, H-7 and the methoxyl signal at δ 4.00. These correlations also revealed a 5-methoxy ring A. Consequently, the other five methoxyl groups are bonded to ring B, thus producing a symmetrical substituted aromatic ring, which is in agreement with the presence of three ^1H NMR signals for the methoxyl groups at δ 4.01 (3H), 3.83 (6H) and 3.90 (6H). In the HMBC spectrum, the signal at δ 4.01 correlated with the carbon signal at δ 149.8 which was assigned to C-4'. The signal at δ 3.90 was attributed to the C-3',C-5'-methoxyl groups, since it showed, in addition to the correlation with the carbon signal at δ 143.1 (C-3',C-5'), a very weak correlation with C-4'. The signal at δ 3.83 was assigned to the C-2',C-6'-methoxyl groups, because it correlated with the signal at δ 147.8 (C-2', C-6'), which in turn showed a weak correlation with H-3. The signal for C-1' appeared at δ 116.9. This established the identity of **10** as 5,2',3',4',5',6'-hexamethoxyflavone.

As in compound **10**, the other new flavones **11** and **12**, possess a 5-methoxy ring A. This was evident from the signals at δ 6.84 (dd, *J* = 8.5, 1.0 Hz, H-6), 7.58 (t, *J* = 8.5 Hz, H-7), 7.14 (dd, *J* = 8.5, 1.0 Hz, H-8) and 4.01 (3H, s, 5-OMe) for compound **11**, and δ 6.82 (br d, *J* = 8.5 Hz, H-6), 7.56 (t, *J* = 8.5 Hz, H-7), 7.09 (dd, *J* = 8.5, 1.0 Hz, H-8) and 4.00 (3H, s, 5-OMe) for compound **12**, which were observed in their respective spectra. Accordingly, the ^{13}C NMR signals for the C-5 to C-10 carbon atoms are almost the same in the three flavones (Table 1), thus indicating that flavones **10**-**12** differ only in the substitution pattern of ring B.

Compound **11** showed a $[M + H]^+$ peak at *m/z* 313.1070 (HRFAB-MS), which is in agreement with the molecular formula $C_{18}H_{16}O_5$, also supported by the presence of eighteen signals in the ^{13}C NMR spectrum. In addition to the above mentioned signals for ring A and that of ring C (δ 6.72, H-3), the ^1H NMR

spectrum of **11** showed signals for three aromatic, *meta*-coupled protons at δ 7.02 (2H, d, *J* = 2.5 Hz) and 6.61 (1H, t, *J*= 2.5 Hz). This and the signal for two equivalent methoxyl groups at δ 3.87 (6H), indicated a symmetrical 3',5'-dimethoxy ring B, like that present in cerrosillin (5,6,3',5'-tetramethoxyflavone), a flavone isolated from *C. edulis*[6,14]. As a consequence, **11** was identified as 5,3',5'-trimethoxyflavone.

Table 1. ^{1}H and ^{13}C NMR data for flavones **10 -12** ($CDCl_3$).

Position	10		11		12	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		157.6		160.9		159.5
3	6.34 s	115.9	6.72 s	109.5	7.00 s	112.4
4		178.0		178.3		178.7
5		159.9		159.8		159.8
6	6.82 dd (8.0, 1.0)	106.2	6.84 dd (8.5, 1.0)	106.5	6.82 br d (8.5)	106.3
7	7.54 t (8.0)	133.5	7.58 t (8.5)	133.8	7.56 t (8.5)	133.6
8	7.02 dd (8.0, 1.0)	110.2	7.14 dd (8.5, 1.0)	110.2	7.09 dd (8.5, 1.0)	110.1
9		159.0		158.3		158.5
10		114.7		114.7		114.3
1'		116.9		133.4		118.7
2'		147.8	7.02 d (2.5)	104.2		153.2
3'		143.1		161.2		142.9
4'		149.8	6.61 t (2.5)	103.5		156.2
5'		143.1		161.2	6.79 d (9)	107.4
6'		147.8	7.02 d (2.5)	104.2	7.53 d (9)	124.0
5-OMe	4.00 s	56.5	4.01 s	56.5	4.00 s	56.5
2'-OMe	3.83 s	61.8			3.96 s	61.2
3'-OMe	3.90 s	61.3	3.87 s	55.6	3.91 s	61.0
4'-OMe	4.01 s	61.5			3.94 s	56.1
5'-OMe	3.90 s	61.3	3.87 s	55.6		
6'-OMe	3.83 s	61.8				

Compound **12** is a tetramethoxyflavone with a molecular formula $C_{19}H_{18}O_6$, as deduced from the pseudomolecular ion at *m/z* 343.1185 (HRFAB-MS) and from the presence of nineteen carbon signals in the ^{13}C NMR spectrum. In addition to the signals corresponding to rings A and C, the ^{1}H NMR spectrum

of flavone **12** exhibited signals for three methoxyl groups and two *ortho*-coupled protons (δ 6.79, d, *J* = 9 Hz; δ7.53, d, *J* = 9 Hz), which were identified as H-5' and H6' because the HMBC spectrum, showed correlations between H-5' and C-1', C-3' and C-4', while H-6' correlated with C-2, C-2' and C-4', thus establishing the structure of **12** as 5,2',3',4'-tetramethoxyflavone. NOESY cross-peak between H-5' and the methoxyl signal at δ 3.94 (C-4'-OMe) gave further support to this structure. Furthermore, quite similar signals for ring B [δ_H 6.78 (d, *J* = 9 Hz, H-5'), 7.51 (d, *J* = 9 Hz, H-6'), 3.98 (s, C-5-OMe), 3.96 (s, C-2'-OMe), 3.934 (s, C-4'-OMe), 3.931 (s, C-6-OMe), 3.91 (s, C-3'-OMe); δ_C 118.9, 153.1, 142.9, 156.2, 107.4, 124.0 (C-1' to C-6'), 61.9 (C-5-OMe), 61.2 (C-2'-OMe), 61.0 (C-3'-OMe), 57.2 (C-6-OMe), 56.1 (C-4'-OMe)] were observed in the ^{1}H and ^{13}C NMR spectra of 5,6,2',3',4'-pentamethoxyflavone, which we isolated from another *Casimiroa* species.[20] This last compound was isolated for the first time from *Ardisia floribunda* (Myrsinaceae).[21] More recently it was isolated from *C. pubescens* and its ^{1}H and ^{13}C NMR data were published,[7] but those concerning the methoxyl groups should be revised. Although compound **12** has been obtained by synthesis[22,23] and its cytotoxicity has been explored,[24] to our knowledge this is the first report of its isolation from a natural source.

EXPERIMENTAL

General Experimental Procedures. The melting points (uncorrected) were determined in a Fisher-Johns apparatus. Column chromatographies were performed on silica gel 60 (Merck G). TLC was carried out on precoated Macherey-Nagel Sil G/UV_{254} plates. The UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were obtained either on a Varian XR-300 (^{1}H at 300 MHz, ^{13}C at 75 MHz) or a Varian Unity Plus 500 (^{1}H 500 MHz; ^{13}C 125 MHz) spectrometers with TMS as internal standard. EI-MS were recorded on a JEOL JMS-AX505HA mass spectrometer. Positive mode HR-FAB-MS (*m*-nitrobenzyl alcohol) were measured on a JEOL JMS-SX102A mass spectrometer.

Plant material. Leaves of *Casimiroa pringlei* (S. Watson) Engl. were collected in the vicinity of Villar town, 6.3 mi from the junction San Luis Potosí-Matehuala, on the road to Cerritos, San Luis Potosí, México. Voucher specimen (Chiang-1269) was identified by F. Chiang and deposited at the Herbario Nacional de México (MEXU).

Extraction and Isolation. Dried and ground leaves (602 g) were extracted with Me_2CO at rt. The extract was concentrated *in vacuo* to give 56.5 g of residue. This residue was chromatographed over a silica gel column, eluted with hexane-EtOAc mixtures of increasing polarity (1:0 to 0:1) to afford ten fractions (A1-A10). Fraction A2 (eluted with hexane-EtOAc 95:5) was chromatographed on a silica gel column eluted with hexane-EtOAc (95:5), to obtain fractions B1-B48. Crystallization of fraction B7-B11 gave **1** (809 mg). Fraction A3 (eluted with hexane-EtOAc 95:5) was subjected to column chromatography (silica

gel, hexane-Me_2CO 95:5) to obtain fractions C1-C5. Fraction C2 was purified by column chromatography (hexane-Me_2CO 96:4) to obtain compound **2** (93.3 mg). Column chromatography (hexane-EtOAc 95:5) of fraction C3 afforded **3** (8.2 mg). Fraction A4 (eluted with hexane-EtOAc 9:1) was decolorized with activated charcoal and subjected to a silica gel chromatography eluted with hexane-EtOAc (9:1 to 4:1), to obtain **3** (4.2 mg) and **4** (141.1 mg). Fraction A5 (eluted with hexane-EtOAc 17:3 to 4:1) was purified by column chromatography using hexane-EtOAc (4:1) as eluent to obtain compounds **5** (88.8 mg), **6** (52.1 mg) and **7** (31.6 mg). Fraction A6 (eluted with hexane-EtOAc 4:1 to 3:1) was chromatographed on a silica gel column eluted with hexane-EtOAc (3:1) to obtain fractions D1-D3. Fraction D2 was purified by column chromatography (silica gel, hexane-EtOAc 3:1), followed by preparative TLC (hexane-EtOAc 3:2) to obtain **13** (33.2 mg). Column chromatography (silica gel, hexane- $CHCl_3$-Me_2CO 71:24:5) of fraction D3 gave **8** (594.4 mg). Compound **10** (623.5 mg) was obtained by crystallization of fraction A8 (eluted with hexane-EtOAc 3:2 to 1:1). Mother liquors of **10** were decolorized with activated charcoal and chromatographed over a silica gel column (column E), eluted with hexane-EtOAc (3:2) to obtain fractions E1-E4. Crystallization of fraction E2 gave **9** (15.8 mg), while compound **10** (353.6 mg) was obtained from fraction E3. Compound **11** (296.4 mg) crystallized from fraction A9 (eluted with hexane-EtOAc 3:7). Its mother liquors were combined with fraction A10 (eluted with EtOAc) and subjected to a column chromatography eluted with $CHCl_3$-MeOH (19:1) to obtain compounds **11** (237.4 mg) and **12** (98.2 mg).

(*Z*)-Trichoclin acetate (**5**): Whitish crystals; mp 92-95 °C; UV λ_{max} (MeOH) nm (log ε) 219 (3.44), 248 (3.39), 299 (3.10); IR ($CHCl_3$) ν_{max}: 1730, 1629, 1590, 1403, 1152, 1092, 1029 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.74 (1H, d, *J* = 9.6 Hz, H-4), 7.66 (1H, d, *J* = 2.1 Hz, H-10), 7.34 (1H, s, H-5), 6.78 (1H, d, *J* = 2.1 Hz, H-9), 6.33 (1H, d, *J* = 9.6 Hz, H-3), 5.83 (1H, br t, *J* = 6.9 Hz, H-2'), 5.05 (2H, br d, *J* = 6.9 Hz, H-1'), 4.63 (2H, s, H-4'), 2.01 (3H, s, OAc), 1.78 (3H, br s, H-5'); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8 (OAc), 160.4 (C-2), 148.3 (C-7), 146.7 (C-10), 144.2 (C-4), 143.6 (C-8a), 136.5 (C-3'), 131.3 (C-8), 125.9 (C-6), 125.1 (C-2'), 116.5 (C-4a), 114.7 (C-3), 113.3 (C-5), 106.7 (C-9), 69.7 (C-1'), 62.7 (C-4'), 21.4 (C-5'), 20.8 (OAc); EI-MS *m/z* 328 $[M]^+$ (2), 269 (2), 202 (100), 174 (26), 145 (7), 127 (86), 89 (13), 85 (11), 67 (8), 57 (8), 43 (61). HR-FAB-MS: *m/z* 329.1035 (Calcd for $C_{18}H_{17}O_6$ $[M+H]^+$, 329.1025).

5,2',3',4',5',6'-Hexamethoxyflavone (**10**): Colorless crystals; mp 129-131 °C; UV λ_{max} (MeOH) nm (log ε): 257 (4.30), 317 (4.08); IR ($CHCl_3$) ν_{max}: 1649, 1608, 1578, 1478, 955, 860 cm^{-1}; ^{1}H NMR and ^{13}C NMR see Table 1; EI-MS *m/z* 402 $[M]^+$ (100), 387 (7), 373 (8), 356 (7), 344 (8), 301 (6), 273 (3), 201 (4), 172 (4), 151 (5), 143 (3), 107 (2), 93 (1), 57 (1). HR-FAB-MS: *m/z* 403.1385 (Calcd for $C_{21}H_{23}O_8$ $[M+H]^+$, 403.1393).

5,3',5'-Trimethoxyflavone (**11**): Whitish crystals; mp 173-175 °C; UV λ_{max} (MeOH) nm (log ε) 269 (4.34), 296 (4.21), 321 (4.19); IR ($CHCl_3$) ν_{max}: 1643, 1607, 1476, 955, 851 cm^{-1}; ^{1}H NMR and ^{13}C NMR

see Table 1; EI-MS *m/z* 312 $[M]^+$ (100), 295 (6), 283 (31), 266 (44), 253 (5), 225 (3), 208 (2), 162 (8), 133 (3), 121 (3), 107 (3), 57 (2). HR-FAB-MS: *m/z* 313.1070 (Calcd for $C_{18}H_{17}O_5$ $[M+H]^+$, 313.1076).

5,2',3',4'-Tetramethoxyflavone (**12**): Colorless crystals; mp 182-185 °C; UV λ_{max} (MeOH) nm (log ε) 217 (4.47), 246 (4.25), 265 (4.24), 325 (4.33) ; IR ($CHCl_3$) ν_{max}: 1637, 1600, 1472, 954, 860 cm^{-1}; ^1H NMR and ^{13}C NMR see Table 1; EIMS *m/z* 342 $[M]^+$ (100), 313 (18), 296 (27), 283 (5), 279 (8), 266 (4), 192 (5), 177 (5), 167 (18), 121 (8), 113 (5), 107 (3), 83 (3), 71 (8),57 (9). HR-FAB-MS: *m/z* 343.1185 (Calcd for $C_{19}H_{19}O_6$ $[M+H]^+$, 343.1182).

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