Pubesamides A, B, and C, Three New N-Benzoyltyramide Derivatives Isolated from *Casimiroa pubescens*

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Three new N-benzoyltyramides derivatives named pubesamides A, B and C together with the known skimmianine, β-sitosterol and daucosterol were isolated from seeds of *C. pubescens*. Their structures were determined by spectroscopic data.

Key words: Casimiroa pubescens, N-Benzoyltyramides, Pubesamides A, B, and C

Introduction

The small genus *Casimiroa* Llave et Lex. (Rutaceae) comprises only 9 species [1] and grows especially on the tropical and subtropical areas of Central America and México. Previous phytochemical studies on *Casimiroa* have shown the presence of flavones, coumarins and limonoids [2 – 4]. Within the flavones, the 5,6 di-substituted pattern is quite often found in the genus [5 – 7]. Due to its pleasant flavour *C. edulis* is cultivated and sold in several markets of Central México. It has been known since prehispanic times for its interesting sedative-like effect and its use as a sleep inducer [4]. In folk medicine, a concoction of leaves, and less frequently of seeds, is administered for this purpose. Furthermore, the seeds are used in the treatment of dermatological conditions [8].

As part of our ongoing investigation on biologically active compounds from Mexican plants [9] and especially from *Casimiroa* genus [10], we have studied the seeds of *Casimiroa pubescens* (Rutaceae). To our knowledge this is the first phytochemical study on *C. pubescens*.

Results and Discussion

From the EtOAc extract of *C. pubescens* seeds, pubesamides A (**1**), C (**3**), and a mixture of **1** and pubesamide B (**2**) were isolated. On the other hand, skimmianine and β-sitosterol were isolated from the hexanic extract and daucosterol and a mixture of **1** and **2** were isolated from the methanolic extract.

The presence of a *N*-benzoyltyramide core in **1**, **2**, and **3** was easily deduced by means of their IR absorptions due to a secondary amide (NH and CO), as well as their ^{1}H and ^{13}C NMR spectra (Table 1) and MS spectral data. Then, the structural differences between **1** – **3** result from the alkyl residue of the side chain on the oxygen atom of the 1,4-disubstituted benzene ring of the *N*-benzoyltyramide nucleus.

The high resolution mass spectrum of pubesamide (**1**), showed its $[M]^+$ at 391.2220 for a $C_{25}H_{29}O_3N$ formula. The peaks at *m/z* 135, 120 and 105 were attributed to the *N*-benzoyltyramide residue. The presence of a oxymonoterpene moiety ($C_{10}H_{15}O$) in the side chain was indicated by the mass difference between **1** and the *N*-benzoyltyramide moiety. It contains an isopropylidene moiety at the end of monoterpene residue (C-17/C-20), conjugated to a carbonyl group at C-16 as deduced by their signals in the ^{1}H and ^{13}C NMR spectra [δ ^{1}H: 1.88, s (3H), H-19; 2.17, s (3H), H-20 and 6.13, brs (1H), H-17; δ ^{13}C: 27.8, C-19; 20.6, C-20; 127.4, C-17; 153.0, C-18 and 191.4, C-16] and by the fragment at *m/z* 83 in the MS spectrum. Also the presence of a vinylic methyl group at C-21 and a methylene residue at C-13, both linked to a trisubstituted double bond at C-14/C-15, conjugated with the carbonyl group at C-16 was deduced [δ ^{1}H: 2.22, s (3H), H-21; 6.08, s (1H), H-15; 2.59, t, $J = 7.1$ (2H),

Table 1. ^{1}H-^{13}C NMR spectral data of **1**–**4**.

	1		**2**		**3**		**4**	
No.	^{1}**H**	^{13}**C**	^{1}**H**	^{13}**C**	^{1}**H**	^{13}**C**	^{1}**H**	^{13}**C**
1	3.69 (t, $J = 6.9$),	41.3	3.69 (t, $J = 6.9$),	41.3	3.67 (t, $J = 6.9$),	41.3	3.69 (t, $J = 6.9$),	41.2
2	2.87 (t, $J = 6.9$),	34.8	2.86 (t, $J = 6.9$),	34.8	2.86 (t, $J = 6.9$),	34.8	2.87 (t, $J = 6.9$),	34.8
3	*	131.2	*	130.7	*	131.3	*	130.8
4	7.14 (d, $J = 8.7$),	129.8	7.13 (d, $J = 8.7$),	129.7	7.14 (d, $J = 8.7$),	129.8	7.15 (d, $J = 8.7$),	129.7
5	6.85 (d, $J = 8.7$),	114.9	6.86 (d, $J = 8.7$),	114.8	6.85 (d, $J = 8.7$),	114.9	6.85 (d, $J = 8.7$),	114.7
6	*	157.5	*	157.7	*	157.2	*	157.7
7	*	167.4	*	167.4	*	167.4	*	167.4
8	*	134.7	*	134.7	*	134.6	*	134.7
9	7.69 (brd),	126.8	7.68 (brd),	126.1	7.68 (brd),	126.8	7.69 (brd),	126.8
10	7.45 m,	128.5	7.45 m,	128.5	7.41 m,	128.5	7.38 m,	128.5
11	7.38 m,	131.4	7.41 m,	131.4	7.47 m,	131.4	7.45 m,	131.6
12	4.09 (t, $J = 7.1$),	65.9	4.16 (t, $J = 6.6$),	67.2	4.12 m,	65.4	3.97 (t, $J = 6.9$),	66.0
13a	2.59 (t, $J = 7.1$),	40.6	3.06 (t, $J = 6.6$),	33.7	2.14 (dd, $J = 15,6$),	40.9	1.78 m,	36.0
13b	*		*		1.97 (dd, $J = 15,8$)		1.67 m	
14	*	154.9	*	155.0	*	72.7	2.26 m,	26.4
15	6.08(brs),	126.2	6.08 m,	127.4	5.63 (d, $J = 15$),	136.6	2.41 m,	50.6
16	*	191.4	*	190.8	6.52 (dd, $J = 11,15$),	124.5	*	210.4
17	6.13(brs),	127.4	6.13 m,	126.0	5.82 (d, $J = 11$),	124.3	2.26 m,	52.3
18	*	153.0	*	153.0	*	135.5	2.15 (hep, $J = 6$),	24.5
19	1.88 s,	27.8	1.89 s,	27.8	1.73 s,	18.3	0.90 (d, $J = 6$),	22.6
20	2.17 s,	20.6	2.15 s,	20.6	1.76 s,	26.0	0.91 (d, $J = 6$),	22.6
21	2.22 s,	19.3	2.01 s,	26.8	1.37 s,	29.0	0.97 (d, $J = 6$),	19.9

HETCOR correlation of: **1**: 19.3/2.22, 20.6/2.17, 27.8/1.88, 34.8/2.87, 40.6/2.59, 41.3/3.69, 65.9/4.09, 114.9/6.85, 126.2/6.08, 126.8/7.68, 127.4/5.63, 128.5/7.45, 129.8/7.14, 131.4/7.38. **3**: 18.3/1.73, 26.0/1.76, 29.0/1.37, 34.8/2.86, 40.9/2.14 and 1.97, 41.3/3.67, 65.4/4.12, 114.9/6.85, 124.3/5.82, 124.5/6.52, 126.8/7.68, 128.5/7.41, 129.8/7.14, 131.4/7.47, 136.6/5.63. **4**: 19.9/0.97, 22.6/0.90 and 0.91, 24.5/2.15, 26.4/2.26, 34.8/2.87, 36.01/1.78 and 1.67, 41.2/3.69, 50.6/2.41, 52.3/2.26, 66.0/3.97, 114.7/6.85, 126.8/7.69, 128.5/7.45, 129.7/7.15, 131.6/7.38.

H-13; δ ^{13}C: 19.3, C-21; 126.2, C-15; 154.9, C-14 and 40.6, C-13]. Finally, the presence of an oxymethylene moiety at C-12 vicinal to the vinylic methylene at C-13 was observed [δ ^{1}H: 4.09, t, $J = 7.1$ (2H); δ ^{13}C: 65.9]. All the assignments were supported by HETCOR and COSY experiments. The *Z* stereochemistry of the double bond at C-14/C-15 in **1** was deduced by NOESY and COLOC experiments.

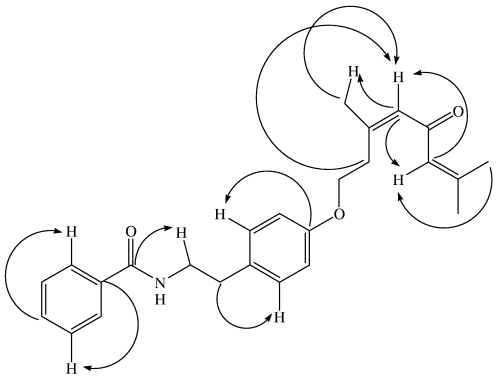
A detailed analysis of the NMR spectral data, IR adsorptions and MS fragmentations of the mixture of **1** and **2**, clearly showed a *Z*, *E* isomeric relationship between them at the C-14/C-15 double bond. Then **2** correspond to be the *E* isomer (Table 1).

Additional experimental support for the structural proposal of **1** and **2** causes the spontaneous transformation of **1** to a mixture of **1** and **2** **(**1:1) during the time of acquisition of NMR data (approx 10 h). Thus, the less stable isomer (*Z*)-**1** (heat of formation −230.8822 KJ/mole, determined by MOPAC calculations [11]) is transformed into the more stable isomer (*E*)-**2** (heat of formation −239.8344 KJ/mole, determined by MOPAC calculations). Probably, this transformation is due to the acidity of $CDCl_3$. Accordingly, **2** could be an artifact produced during the chromatographic procedures.

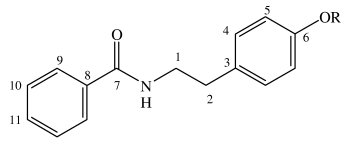
When **1** and **2** mixture was treated with hydrogen in presence of PtO_2, tetrahydro-pubesamide A (**4**) was obtained.

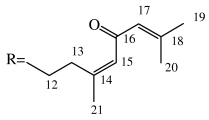
As expected, the high-resolution mass spectrum of **4** showed four hydrogen atoms more than present in **1** ($[M]^+$ at 395.2537 for a $C_{25}H_{33}O_3N$ formula). The only difference between **4** and **1** is found in the monoterpenoid side chain. (Table 1). Thus, the ^{13}C NMR spectrum of **4** revealed the signals for three methyl, six methylene and two methine atom carbons in the sp^3 region and at δ210.4 ppm the signal for the carbonyl at C-16. The correlation between the signals of ^{1}H and ^{13}C NMR spectra of **4** are shown in Table 1.

The high-resolution mass spectrum of **3** showed its $[M]^+$ at 393.2333 for a $C_{25}H_{31}O_3N$ formula. The presence of an unsaturated monoterpene moiety ($C_{10}H_{17}O$) in the side chain was indicated by the mass difference between **1** and the *N*-benzoyltyramide residue. As, in **1** and **2**, the presence of an isopropylidene moiety at the end of the monoterpenic residue in **3** was evident

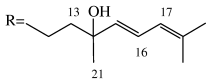


Principal COLOC correlations in **1**

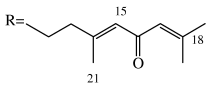




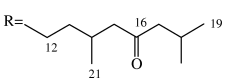
Pubesamide A (**1**)



Pubesamide C (**3**)



Pubesamide B (**2**)



Tetrahydropubesamide A (**4**)

(Table 1). The presence of an additional double bond at C-15/C-16 conjugated to the isopropylidene moiety was deduced by the signals in the ^{1}H and ^{13}C NMR spectra [$\delta\,^1$H: 5.63, d, $J = 15$, H-15 6.52, dd, $J = 11$, 15, H-16; 5.82, d, $J = 11$, H-17; $\delta\,^{13}$C: 136.6, C-15; 124.5, C-16; 124.3, C-17]. The presence of a hydroxyl group and a methyl group at C-14 was deduced by their signals in the ^{1}H and ^{13}C NMR spectra [$\delta\,^1$H: 1.37, s (3H), H-21; $\delta\,^{13}$C: 29.0, C-21; 72.7, C-14] and the signals at 40.9 and 65.4 were assigned to the methylene groups at C-13 and C-12 respectively. All the assignments were supported by COSY, HETCOR and COLOC experiments. During the time of acquisition of NMR data (approx 10 h), **3** did not suffer isomerization as **1**, but hydrolysis affording *N*-benzoyltyramide. As it can be seen, *N*-benzoyltyramides derivatives **1**–**3** are sensitive to mild acidic conditions. The presence of *N*-benzoyltyramide was also detected in a hexanic extract of *C. edulis*. However, this extract was previously treated with an aqueous solution of HCl [12]. Then, according to our results, it is quite probable that compounds similar to **1**–**3** were present in *C. edulis*.

Experimental Section

Plant material

Seeds of *C. pubescens* were collected from plants growing in the surroundings of Ixmiquilpan Hidalgo, México. A voucher specimen was deposited in the Herbarium of Facultad de Ciencias UNAM (FCME 84833).

Dried and milled seeds (3834 g) were extracted with hexane, EtOAc and MeOH successively (51×3 times, each) at room temperature for 48 h.

The hexanic extract (62.53 g, residue dry weight) was absorbed on 4 g of silica gel and then chromatographed on a column packed with 130 g of silica gel 60 (Merck). Elution with solvent mixtures of increasing polarity afforded β-sitosterol (35 mg, Hex:EtOAc, 9:1 v/v) [13], isopimpinellin (29 mg, Hex:EtOAc, 6:4 v/v) [14] and skimmianine (82 mg, EtOAc:MeOH, 7:3 v/v) [15]. Chromatography of the EtOAc extract (181.5 g) absorbed on 6 g of Si-gel and using 370 g of silice gel afforded pubesamide **A** (**1**) (20 mg, Hex:EtOAc, 7:3 v/v), mixture of pubesamide **A** (**1**) and **B** (**2**) (297 mg, Hex:EtOAc, 6:4 v/v) and pubesamide **C** (**3**) (52 mg, Hex:EtOAc, 3:7 v/v). Mixture of **1** and **2** (108 mg, Hex:EtOAc, 7:3 v/v) and daucosterol (12 mg, EtOAc:MeOH, 8:2 v/v) [16] were isolated from the methanolic extract (61.2 g) chromatography.

The identification of the known compounds was achieved by comparison of their physical and spectroscopic data with those published in the literature.

*Pubesamide A (**1**)*

Yellow solid m.p. 88–91 °C HRMS: *m/z*: 391.2220 (rel. int.) ([M]$^+$), calcd. for $C_{25}H_{29}O_3N$: 391.2226). MS *m/z*: (rel. int.) 391 ([M$^+$]) (15), 254 (6), 151 (100), 135 (16), 120 (44), 105 (47), 83 (86), 77 (16). UV λ_{max} (nm) ($CHCl_3$) 269 ($\lg\varepsilon = 4.38$) IR (KBr) ν_{max} (cm^{-1}) 3452, 1657, 1513, 1030. ^{1}H, ^{13}C NMR see Table 1.

*Mixture of pubesamides A (**1**) and B (**2**)*

Yellow solid m.p. 89–92 °C. MS *m/z*: (rel int.) 391 ([M$^+$]) (5), 295 (10), 271 (8), 257 (2), 254 (5), 151 (100), 137 (11), 134 (6), 123 (12), 120 (43), 105 (60), 83 (86), 77 (22), 55 (13), 43 (8). IR (KBr) ν_{max} (cm^{-1}) 3317, 1634, 1537, 1242. ^{1}H, ^{13}C NMR see Table 1.

*Pubesamide C (**3**)*

Yellowish solid m.p. 83–84 °C HRMS: *m/z*: 393.2333 ([M]$^+$), calcd. for $C_{25}H_{31}O_3N$: (393.2304). MS *m/z*: (rel.

int.) 393 ([M^+]) (6), 375 (30), 272 (8), 254 (94), 232 (16), 202 (14), 193 (30), 168 (76), 166 (98), 135 (100), 120 (68), 105 (89), 93 (42), 77 (37), 55 (20). UV λ_{max} (nm) (MeOH) 230 ($\lg\varepsilon = 4.52$) and 202 ($\lg\varepsilon = 4.37$) IR (KBr) ν_{max} (cm^{-1}) 3455, 1655, 1512, 1486 and 1286. ^1H, ^{13}C NMR see Table 1.

*Tetrahydropubesamide A (**4**)*

From 1 and 2 mixture. A sample of the **1** and **2** mixture (70 mg) dissolved in CH_2Cl_2 was treated with hydrogen in presence of PtO_2 (7 mg). The reaction mixture was separated by preparative TLC, layer 2.0 mm, eluted with hexane-EtOAc, 3:2 mixtures, affording **4** (60 mg).

From 1. A sample of **1** (20 mg) dissolved in CH_2Cl_2 was treated with hydrogen in presence of PtO_2 (7 mg). Usual work up yielded **4** (16 mg).

White crystals m. p. 78 – 80 °C, HRMS: *m/z* 395.2537 ([$M]^+$), calcd. for $C_{25}H_{33}O_3N$: 395.2539). MS *m/z*: (rel int.) 395 ([M^+]) (5), 274 (13), 155 (100), 120 (10), 105 (26), 57 (23). UV λ_{max} (nm) ($CHCl_3$) 251 ($\lg\varepsilon = 3.62$) IR (KBr) ν_{max} (cm^{-1}): 3314, 2930, 1705, 1636, 1539, 1513, 1244. ^1H, ^{13}C NMR see Table 1.

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