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FLAVONOIDS FROM *ARTEMISIA CAMPESTRIS* SUBSP. *MARÍTIMA*

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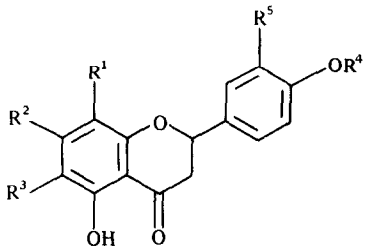
Key Word Index: *Artemisia campestris* subsp. *maritima*; Compositae; flavonoids; flavanones; 5,8,4′-trihydroxyflavanone; 5,6-dihydroxy-4′-methoxyflavanone.

Abstract—Five flavonoids and an acetophenone derivative were isolated from *Artemisia campestris* subsp. *marítima*, including a new flavanone, 5,8,4′-trihydroxyflavanone, and 5,6-dihydroxy-4′-methoxyflavanone, obtained for the first time from a natural source. The known natural products were 5,4′-dihydroxy-7,3′-dimethoxyflavanone,7,3′-dimethoxy-3,5,4′-trihydroxyflavanone, 5,4′-dihydroxy-6,7-dimethoxyflavone and 3-(isopent-2-enyl)-4-hydroxyacetophenone.

INTRODUCTION

As part of our research on metabolites of the Compositae we report here five flavonoids and an acetophenone derivative from *Artemisia campestris* subsp *marítima*. One of the flavonoids is the new compound, 5,8,4′-trihydroxyflavanone (**1**). Also, the new naturally occurring product 5,6-dihydroxy-4′-methoxyflavanone (**2**), previously known as a synthetic compound [1], was also isolated. Of the remaining flavonoids, one of them was found on *Artemisia* species for the first time, namely 5,4′-dihydroxy-,7,3′-dimethoxyflavanone (**3**). The dihydroflavonol 7,3′-dimethoxy-3,5,4′-trihydroxyflavanone (**4**) was previously isolated from *Artemisia pygmaea* [2], 5,4′-dihydroxy-6,7-dimethoxyflavone (**5**) from *Artemisia capillaris* [3] and 3-(isopent-2-enyl)-4-hydroxyacetophenone (**6**) from *Artemisia campestris* subsp. *glutinosa* [4].



1 R^1 = OH; R^2 = R^3 = R^4 = R^5 = H
2 R^1 = R^2 = R^5 = H; R^3 = OH; R^4 = Me
3 R^1 = R^3 = R^4 = H; R^2 = R^5 = OMe

RESULTS AND DISCUSSION

A chloroform leaf wash of *A. campestris* subsp. *marítima* afforded compounds **1**–**6**. Compound **1**, mp 262–264°, had IR absorptions at ν_{max}^{KBr} cm^{-1}: 3448, 1646 and 1575, corresponding to hydrogen bonded OH, hydrogen bonded conjugated carbonyl group and aromatic ring, respectively. The UV spectrum of **1** exhibited a major band at 295 nm which did not shift after addition of sodium acetate. A bathochromic shift of 13 nm after addition of aluminium chloride/hydrochloric acid suggested the presence of a free hydroxyl group at 5-position. This was confirmed by the ^{1}H NMR spectrum of **1**, which showed a singlet at δ 12.23 typical from a hydrogen bonded 5-hydroxyl. The ABX type signals characteristic of a flavanone appeared at δ 5.39 for H-2 as a doublet of doublets with coupling constants of 13.2 Hz and 4.2 Hz with H-3$_{trans}$ and H-3$_{cis}$ respectively; the H-3 protons each presented a doublet of doublets at δ 3.27 (H-3$_{trans}$) and 2.66 (H-3$_{cis}$) with coupling constants of 13.2 and 17.4 Hz for H-3$_{trans}$, and 4.2 and 17.4 Hz for H-3$_{cis}$. The substitution at 4′-position of ring B was also confirmed by the presence of two doublets at δ 7.37 and 6.88, each one corresponding to two protons, H-2′, H-6′ and H-3′, H-5′, respectively, with a coupling contant of 8.4 Hz. At δ 5.89 a singlet appeared corresponding to two protons, H-6 and H-7, which are equivalent in this 5,8-dihydroxyflavanone. The mass spectrum of **1** exhibited a molecular ion at *m/z* 272 for $C_{15}H_{12}O_5$ as the base peak. Fragment ions at *m/z* 179, 166 and 153 were consistent with a ring A containing two hydroxyl groups. Fragment ions at these *m/z* values were also obtained from naringenin [5, 6]. The protonated ion at *m/z* 153 and the ion at *m/z* 120 are typical for a retro-Diels–Alder cleavage and the presence of the

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fragment ion at m/z 120 also suggested that ring B had only one hydroxyl group. The relative intensity of the $[M-H]^+$ fragment (55%) was consistent with the expected loss of a proton from a hydroxyl group at 8-position [7].

Compound **2**, mp 176–177°, (lit [1] 175–176°), also showed a ^{1}H NMR spectrum with signals characteristic of a flavanone, with the H-2 proton exhibiting a doublet of doublets at δ 5.36 with coupling constants of 12.8 Hz and 2.9 Hz with the H-3 protons in *trans* and *cis* positions, respectively. The H-3_{trans} proton presented a doublet of doublets at δ 3.10 with a coupling constant of 17.2 Hz with the H-3_{cis} proton which signal appeared at δ 2.75 as a doublet of doublets. The presence of the free OH-5 was also detected by the singlet at δ 12.00. Besides one singlet at δ 3.83 corresponding to one methoxyl group present in the molecule, a multiplet appeared at δ 6.08–6.03, corresponding to H-7 and H-8. The substitution at 4′-position of ring B was also confirmed by the presence of two doublets at δ 7.37 and 6.95, each one corresponding to two protons, H-2′, H-6′ and H-3′, H-5′, respectively, with a coupling constant of 8.6 Hz.

The bathochromic shifts observed in the UV spectrum in methanol on addition of $AlCl_3$ and $AlCl_3$/HCl indicated the presence of a hydroxyl group at the 5-position. Also a bathochromic shift of 27 nm of band II was observed on addition of NaOAc. Compound **2** showed IR absorptions at ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1640 (CO) and 1600 (aromatic ring).

The proposed structure was confirmed by the ^{13}C NMR spectrum and by the mass spectrum. The ^{13}C NMR spectrum showed a signal due to the methoxyl group at δ 55.6, and signals at δ 43.5 and 79.7 characteristic for C-3 and C-2 respectively. This spectrum also presented a signal at δ 196 for the carbonyl group and four oxygenated aromatic carbons (δ 160.9, 164.2, 165.2 and 167.4) corresponding to C-4′, C-9, C-5 and C-6. The mass spectrum presented a molecular ion peak at m/z 286 for $C_{16}H_{14}O_5$ as the base peak. The fragments at m/z 179 and m/z 153 confirmed the structure of ring A with two hydroxyl groups. A signal at m/z 134 corresponded to the ion due to the retro-Diels–Alder cleavage of the flavanone and suggested that ring B had one methoxyl group, as did the fragment ion at m/z 121 typical of a tropylium ion [8]. The relative intensity of the $[M-H]^+$ fragment (60%) was in accordance with the presence of a hydroxyl group in 6-position [7]. Compound **2** gave the intensive green colour (which did not fade) wth alcoholic ferric chloride, which is consistent with the presence of the two vicinal hydroxyl groups (OH-5 and OH-6).

Compound **3**, mp 149–150° (lit. [9] 148–150°), had IR, UV, ^{1}H NMR and MS data identical to those given in the literature for 5,4′-dihydroxy-7,3′-dimethoxyflavanone [9, 10]. Its ^{13}C NMR spectrum was in agreement with the proposed structure and had not been reported previously. The signals for two methoxyl groups appeared at δ 56.0 and 56.9, and those characteristic for C-2, C-3 and C-4 were also present at δ 79.4, 43.4 and 199.1, respectively. Also the signals corresponding to five oxygenated aromatic carbons were present at δ 146.3, 146.8 (ring B), 162.9, 164.2 and 168.2.

The dihydroflavonol, mp 180° (lit. [11] 180°), was identified as 7,3′-dimethoxy-3,5,4′-trihydroxyflavanone (**4**) from its IR, UV, ^{1}H NMR and MS spectral properties, which agreed with those reported earlier [2, 8, 11].

The flavone, mp 259–261° (lit. [12] 259–261°), exhibited UV maxima in methanol and shifts with diagnostic reagents in agreement with those given in the literature for compound **5** [12, 13]. The observed bathochromic shift of 20 nm of band I in the presence of $AlCl_3$/HCl is typical for 5-hydroxy-6-methoxyflavones [14]. The mass spectrum of **5** showed a molecular ion at m/z 314 as the base peak and a $[M-Me]^+$ ion at m/z 299 with 75% relative intensity, typical for the loss of a methyl group from a methoxyl function at C-6 [15]. In addition, the low intensity of the $[M-H]^+$ ion (10%) confirmed the absence of a C-6 or C-8 hydroxyl function [7]. Also IR and ^{1}H NMR spectroscopic data were in accordance with the proposed structure.

The *p*-hydroxyacetophenone derivative obtained, mp 91–92° (lit. [4] 90°) presented UV, ^{1}H NMR and MS data in agreement with those given in the literature [4, 16].

EXPERIMENTAL

Plant material. The aerial parts of *Artemisia campestris* subsp. *maritima*, growing wild on the beach sands near Cabo Espichel, were collected in October 1986 and identified by Prof. M. S. Pais. Voucher specimens are on deposit at the Herbarium of Faculdade de Ciências of Universidade de Lisboa.

General. Mps: uncorr. CC employed silica gel 60 35–70 mesh. TLC was performed on silica gel 60 F_{254} and prep. TLC on silica gel 60 F_{254} with layer thickness of 0.5 mm. Compounds were identified with shortwave UV and with vanillin reagent. ^{1}H and ^{13}C NMR were recorded using TMS as int. standard.

Extraction and isolation. Ground air-dried aerial parts (0.4 kg) were extracted with $CHCl_3$. The extract (35 g) was purified over a silica gel column eluting with EtOAc–*n*-hexane (1 : 4). Fractions containing compounds **2** and **3** were further separated by CC with EtOAc–C_6H_6 (1 : 9). Compound **1** was purified by prep. TLC with EtOAc–toluene (1 : 2) and recovered from the absorbent with Me_2CO.

The yields of pure compounds were as follows: **1** 10 mg; **2** 100 mg; **3** 50 mg; **4** 12 mg; **5**, 15 mg and **6** 65 mg.

5,8,4′-Trihydroxyflavanone (**1**). Mp. 262–264° ($MeOH-H_2O$); R_f 0.46 (EtOAc–toluene, 1 : 2); UV λ_{max}^{MeOH} nm (log ε): 295 (4.99), 328sh (4.41); + NaOMe 295 (4.97), 364 (4.55); + $AlCl_3$ 313 (5.15), 380 (4.49); + $AlCl_3$/HCl 308 (5.09), 380 (4.46); + NaOAc 295 (4.89), 328 sh (4.49); + H_3BO_3/NaOAc 295 (5.02), 328 sh (4.55); ^{1}H NMR (300 MHz, Me_2CO-d_6): δ 2.66 (1H, *dd*, J = 4.2 Hz, J = 17.4 Hz, H-3_{cis}), 3.27 (1H, *dd*, J = 13.2 Hz, J = 17.4 Hz, H-3_{trans}), 5.39 (1H, *dd*, J = 4.2 Hz, J = 13.2 Hz, H-2), 5.89 (2H, *s*, H-6 and H-7), 6.88 (2H, *d*, J = 8.4 Hz, H-3′ and H-5′), 7.37 (2H, *d*, J = 8.4 Hz, H-2′ and H-6′), 12.23 (1H, *s*, OH-5); EIMS 70 eV, m/z (rel. int.): 272 $[M]^+$ (100), 271 $[M-H]^+$ (55), 179 $[M-C_6H_5O]^+$ (28), 166 $[M-C_7H_6O]^+$ (28), 153 $[M-C_8H_7O]^+$ (96), 120 $[M-C_7H_4O_4]^+$ (64).

5,6-Dihydroxy-4′-methoxyflavanone (**2**). Mp 176–177° (C_6H_6); R_f 0.70 (EtOAc–toluene, 1 : 2) UV λ_{max}^{MeOH} nm (log ε): 294 (4.77), 324 sh (4.17); + NaOMe 320 (5.10), + $AlCl_3$ 311 (5.04), 378 (4.36); + $AlCl_3$/HCl 309 (5.04), 378 (4.36); + NaOAc 321 (5.06), + NaOAc/H_3BO_3 294 (4.96), 324 (4.65); ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.75 (1H, *dd*, J = 2.9 Hz, J = 17.2 Hz, H-3_{cis}), 3.10 (1H, *dd*, J = 12.8 Hz, J = 17.2 Hz, H-3_{trans}), 3.83 (3H, *s*, OMe), 5.36 (1H, *dd*, J = 2.9 Hz, J = 12.8 Hz, H-2), 6.03–6.08 (2H, *m*, H-7 and H-8), 6.95 (2H, *d*, J = 8.6 Hz, H-3′ and H-5′), 7.37 (2H, *d*, J = 8.6 Hz, H-2′ and H-6′), 12.00 (1H, *s*, OH-5); ^{13}C NMR (22.5 MHz, Me_2CO-d_6): δ 43.5 (*t*, C-3), 55.6 (*q*, OMe), 79.7 (*d*, C-2), 95.9 (*d*, C-7 or C-8), 96.8 (*d*, C-8 or C-7), 102.7 (*s*, C-10), 114.7 (*d*, C-3′ and C-5′), 128.8 (*d*, C-2′ and C-6′), 131.8 (*s*, C-1′), 160.9 (*s*, C-4′), 164.2 (*s*, C-9), 165.2 (*s*, C-5 or C-6), 167.4 (*s*, C-6 or C-5), 196.0 (*s*, C-4); EIMS 70 eV, m/z (rel. int.): 286 $[M]^+$ (100), 285 $[M-H]^+$ (60), 179 [M

$-C_7H_7O]^+$ (16), 153 $[M-C_9H_9O]^+$ (7), 152 $[M-C_9H_{10}O]^+$ (8), 134 $[M-C_7H_4O_4]^+$ (82), 121 $[M-C_8H_5O_4]^+$ (51).

5,4′-Dihydroxy-7,3′-dimethoxyflavanone (**3**). Mp 149–150° (see text); R_f 0.63 (EtOAc–toluene, 1:2), IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1660 (CO), 1590 (aromatic ring): UV λ_{max}^{MeOH} nm 290, 330 sh, +NaOMe 290, 360, +$AlCl_3$ 311, 380, +$AlCl_3$/HCl 309, 378, +NaOAc 291, 330 sh, NaOAc/H_3BO_3 291, 330 sh; ^1H NMR (200 MHz, $CDCl_3$): δ 2.75 (1H, *dd*, $J=2.9$ and 17.5 Hz, H-3_{cis}), 3.08 (1H, *dd*, $J=12.8$ and 17.5 Hz, H-3_{trans}), 3.78 (3H, *s*, OMe-3′), 3.90 (3H, *s*, OMe-7), 5.30 (1H, *dd*, $J=2.9$ and 12.8 Hz, H-2), 6.06 (1H, *d*, $J=2.1$ Hz, H-6), 6.08 (1H, *d*, $J=2.1$ Hz, H.8), 6.87–6.98 (3H, *m*, H-2′, H-5′ and H-6′), 12.02 (1H, *s*, OH-5); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 43.4 (*t*, C-3), 56.0 (*q*, OMe), 56.9 (*q*, OMe), 79.4 (*d*, C-2), 95.1 (*d*, C-6 or C-8), 95.3 (*d*., C-8 or C-6), 103.5 (*s*, C-10), 108.8 (*d*, C-2′), 114.6 (*d*, C-5′), 119.6 (*d*, C-6′), 130.3 (*s*, C-1′), 146.3 (*s*, C-3′ or C-4′), 146.8 (*s*, C-4′ or C-3′), 162.9 (*s*, C-9), 164.2 (*s*, C-5), 168.2 (*s*, C-7), 199.1 (*s*, C-4); EIMS 70 eV, *m/z* (rel. int.): 316 $[M]^+$ (76), 179 $[M-C_8H_9O_2]^+$ (39), 167 $[M-C_9H_9O_2]^+$ (100), 150 $[M-C_8H_6O_4]^+$ (85), 137 $[M-C_9H_7O_4]^+$ (48).

7,3′-Dimethoxy-3,5,4′-trihydroxyflavanone (**4**). Mp 180° (see text); R_f 0.44 (EtOAc–toluene, 1:2); IR ν_{max}^{KBr} cm^{-1}: 3444 (OH), 1648 (CO), 1580 (aromatic ring) UV λ_{max}^{MeOH} nm: 290, 325 sh, 375 sh, +NaOMe 290, 353, +$AlCl_3$ 315, 390, +$AlCl_3$/HCl 315, 390, +NaOAc 290, 320 sh, 360 sh, +NaOAc/H_3BO_3 290, 325 sh, 375 sh; ^1H NMR (300 MHz, Me_2CO-d_6): δ 3.86 (3H, *s*, OMe-3′), 3.88 (3H, *s*, OMe-7), 4.71 (1H, *dd*, $J=11.4$ Hz, $J=3.6$ Hz, H-3), 4.82 (1H, *d*, $J=3.6$ Hz, OH-3), 5.11 (1H, *d*, $J=11.4$ Hz, H-2), 6.05 (1H, *d*, $J=2.7$ Hz, H-6), 6.08 (1H, *d*, $J=2.7$ Hz, H-8), 6.88 (1H, *d*, $J=8.7$ Hz, H-5′), 7.02 (1H, *dd*, $J=2.1$ and 8.7 Hz, H-6′), 7.22 (1H, *d*, $J=2.1$ Hz, H-2′), 11.70 (1H, *s*, OH-5); EIMS 70 eV, *m/z* (rel. int.): 332 $[M]^+$ (14), 314 $[M-H_2O]^+$ (18), 303 $[M-CHO]^+$ (18), 179 $[M-C_8H_9O_3]^+$ (16), 167 $[M-C_9H_9O_3]^+$ (100), 166 $[M-C_9H_{10}O_3]^+$ (28), 137 (16) $[M-C_9H_7O_5]^+$ (20).

5,4′-Dihydroxy-6,7-dimethoxyflavone (**5**). Mp 259–261° (see text); R_f 0.56 (EtOAc–toluene, 1:2); IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1640 (CO), 1580 (aromatic ring) UV λ_{max}^{MeOH} nm: 276, 328, +NaOMe 276, 364, +$AlCl_3$ 301, 356, +$AlCl_3$/HCl 300, 348, +NaOAc 276, 364, +NaOAc/H_3BO_3 276, 328; ^1H NMR (300 MHz, Me_2CO-d_6): δ 3.87 (3H, *s*, OMe-6), 3.92 (3H, *s*, OMe-7), 6.64 (1H, *s*, H-3), 6.70 (1H, *s*, H-8), 7.13 (2H, *d*, $J=8.7$ Hz, H-3′ and H-5′), 8.29 (2H, *d*, $J=8.7$ Hz, H-2′ and H-6′), 13.21 (1H, *s*, OH-5); EIMS 70 eV, *m/z* (rel. int.): 315 $[M+H]^+$ (21), 314 $[M]^+$ (100), 313 $[M-H]^+$ (10), 299 $[M-Me]^+$ (75), 296 $[M-H_2O]^+$ (55), 285 $[M-CHO]^+$ (8), 271 $[M-Me-CO]^+$ (33), 197 $[M-C_8H_5O]^+$ (4), 196 $[M-C_8H_6O]^+$ (6), 181 $[M-C_9H_9O]^+$ (6), 167 $[M-C_{10}H_{11}O]^+$ (14), 153 $[M-C_{10}H_9O_2]$ (5), 118 $[M-C_9H_8O_5]^+$ (10).

3-(isopent-2-enyl)-4-Hydroxyacetophenone (**6**). Mp 91–92° (see text); R_f 0.63 (EtOAc–toluene, 1:2); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1700 (CO), 1580 (aromatic ring); UV λ_{max}^{MeOH} nm: 226, 280; ^1H NMR (200 MHz, $CDCl_3$): δ 1.77 (6H, *s*, $Me_2C=$), 2.55 (3H, *s*, MeCO), 3.39 (2H, *d*, $J=7.2$ Hz, Ph–CH_2), 5.32 (1H, *t*, $J=7.2$ Hz, HC=), 6.88 (1H, *d*, $J=8$ Hz, H-5), 7.72 (1H, *d*, $J=2.3$ Hz, H-2), 7.75 (1H, *dd*,, $J=2.3$ and 8 Hz, H-6); EIMS 70 eV, *m/z* (rel. int.): 204 $[M]^+$ (38), 189 $[M-Me]^+$ (49), 149 $[M-Me_2C=CH]^+$ (52), 161 $[M-Me-CO]^+$ (11), 133 $[M-Me-2CO]^+$ (33).

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