New Acetophenone Glucosides Isolated from Extracts of *Helichrysum italicum* with Antiinflammatory Activity

Araceli Sala, María del Carmen Recio, Rosa María Giner, Salvador Máñez, and José-Luis Ríos*

Departament de Farmacologia, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

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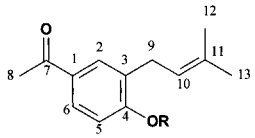
Three new acetophenone glucosides (**4**−**6**), three known aglycons (**1**−**3**), and a benzo-γ-pyrone glucoside (**7**) were isolated from the CH_2Cl_2, EtOAc, and BuOH extracts from the aerial parts of *Helichrysum italicum*. All the compounds tested showed antiinflammatory activity in a 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema test, and the ID_{50} value of compound **2**, the most active compound, was determined.

Helichrysum italicum (Roth) G. Don f. in Loudon (Asteraceae) is a characteristic species of the Mediterranean area. In a previous study its extracts showed antiinflammatory and antioxidative properties in different experimental in vivo and in vitro models.[1−3] Chromatographic analysis indicated that the compounds present in the active fraction are phenolics, principally flavonoids and acetophenone derivatives. In the present work we have isolated and identified the major compounds present in the active fractions of *H. italicum* (CH_2Cl_2, EtOAc, and BuOH) and have determined the activity of the isolated compounds in the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema model.

The active CH_3OH extract was fractionated in turn with hexane, CH_2Cl_2, EtOAc, and BuOH, and the fractions showed antiinflammatory activity.[2] By column chromatography on Sephadex LH-20, reversed-phase C_{18} Si gel (RP-18), and Si gel, we have isolated the most relevant principles present in these extracts. The compounds were identified by spectroscopic analysis. Known compounds isolated from the CH_2Cl_2 extract were 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (**1**), 4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone (**2**), and 12-hydroxytremetone (bitalin A) (**3**). In addition, the known compounds ursolic acid (triterpene) and gnaphaliin (flavone) were isolated. They were identified by comparison of their spectral data with values previously reported.[4−6]

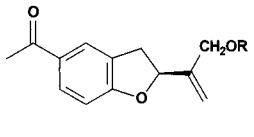
The EtOAc extract yielded two new acetophenone glycosides, **4** and **5**. The spectral analysis of **4** showed the same ^{13}C NMR signals as compound **1**, but there were six characteristic signals of a glucose moiety. The HMBC spectrum led to the connectivity between the sugar moiety and the aglycon on the basis of the cross-peak between C-4 (δ160.8) and H-1′ (δ 5.04). The analysis of the signal shift and comparison with the spectral data of acetophenone **1** demonstrated that compound **4** is the 4β-D-glucoside of compound **1**, or 3-(3-methyl-2-butenyl)acetophenone-4-*O*-β-glucopyranoside.

The ^{13}C NMR spectrum of **5** showed signals similar to that of compound **3**, with the addition of six characteristic signals of a glucose moiety. In compound **5** the linkage between the aglycon and the sugar is through the CH_2OH affixed to position C-13, which showed a clear ^{13}C NMR shift from δ 62.4 to 67.6 ppm. Enzymatic hydrolysis gave compound **3** and D-glucose. Compound **5** was identified as



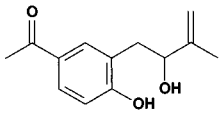
1 R = H

4 R = glucose



3 R = H

5 R = glucose



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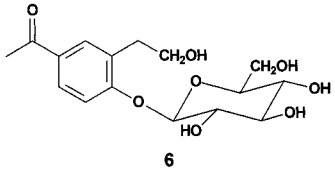

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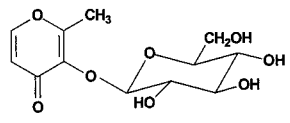

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12-hydroxytremetone-12-*O*-β-D-glucopyranoside (bitalin A-12-*O*-β-D-glucopyranoside).

The BuOH extract gave two substances, a new glucoside (**6**) and the known compound **7** (maltol β-D-glucopyranoside), previously described by Numata et al.[7] from *Osmunda japonica*. The ^1H NMR spectrum of **6** showed three aromatic protons, two of them in an *ortho* position and the other in a *meta* position. The corresponding signals in ppm were H-2 (δ 7.87, d, J = 2.0 Hz), H-5 (δ 7.22, d, J = 8.4 Hz), and H-6 (δ 7.84, dd, J = 8.4 Hz and J = 2.0 Hz). In addition, a singlet at δ 2.54 (3H) indicated the presence of an acetophenone group, and a doublet at δ 5.01 (J = 7.6 Hz) corresponded to the anomeric proton of a sugar moiety. The ^{13}C NMR signals were consistent with an acetophenone structure,[6] but in contrast to **4**, the side chain in C-3 was determined to be a hydroxyethyl group (C-9 at δ 34.5, and C-10 at δ 62.7, respectively). The linkage with glucose occurred through the phenolic OH at position C-4, which is characteristic of this class of compound.[8] Compound **6** was assigned as 3-(2-hydroxyethyl) acetophenone-4-*O*-β-D-glucopyranoside.

This is the first time that these acetophenone glucosides (**4**−**6**) have been reported as natural products. The ^{13}C NMR data of these compounds are provided in Table 1.

All the compounds inhibited acute mouse ear edema induced by TPA, but acetophenone **2** was the most active,

* To whom correspondence should be addressed. Tel and Fax: + 34 96 386 4973. E-mail: riosjl@UV.es.

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Table 1. ^{13}C NMR Data for Compounds **4**−**7**[a]

carbon	**4** (CD_3OD)	**5** (DMSO-d_6)	**6** (CD_3OD)	**7** (CD_3OD)
1	131.3	130.4	129.7	
2	130.6	130.1	132.4	144.0
3	132.3	127.6	132.4	165.0
4	160.8	163.1	161.3	177.6
5	115.0	108.7	115.4	117.7
6	129.7	125.6	130.1	157.2
7	199.7	196.1	199.7	16.2
8	26.4	26.5	26.5	
9	29.2	33.3	34.5	
10	123.1	83.8	62.7	
11	134.1	144.2		
12	25.9	112.3		
13	17.9	67.6		
1′	101.6	102.3	101.9	105.8
2′	74.5	73.4	74.9	75.8
3′	78.2	76.6	78.3	78.9
4′	71.2	70.0	71.2	71.5
5′	78.2	76.9	78.1	78.4
6′	62.4	61.0	62.5	62.9

[a] Spectra are recorded at 100 MHz. Chemical shifts are in δ (ppm) with TMS as internal standard. The assigments were based on 1D and 2D NMR experiments.

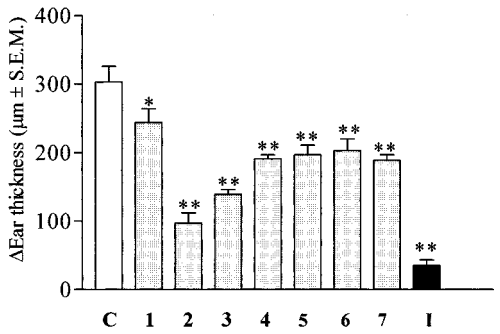

Figure 1. Effect of isolated compounds (**1**−**7**) on TPA-induced ear edema. Edema inhibition (% inhibition) was expressed as the reduction in thickness (in μm) with respect to the control group treated only with TPA (mean ± SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. * $p < 0.05$; ** $p < 0.01$ ($n = 6$ animals). C = control group; I = indomethacin.

with a 68% edema reduction. Of the rest of the compounds, only **3** reached an edema inhibition of at least 50% at the doses tested (Figure 1). These experimental data explain in part the biological activities of the *H. italicum* extracts, because the CH_2Cl_2 extract has a large quantity of ursolic acid and acetophenone **1** and a smaller amount of other active known principles such as gnaphaliin. The activity of these principles has been reported in different experimental models of inflammation,[9,10] but in those studies, the compounds were isolated from other sources. In a complementary experiment, the ID_{50} of acetophenone **2** was established to be 0.63 μmol/ear (coefficient of determination, r^2, for the lineal regression was 0.9856 and $p = 0.0423$ considered significant).

Only a few reports on the antiinflammatory effects of natural and synthetic acetophenone derivatives have been published. For example, Favier et al. reported the antiinflammatory activity of eight acetophenones, including tremetone, the dehydroxy derivative of acetophenone **3** (12-hydroxytremetone). Tremetone inhibited the mouse hind paw edema induced by carrageenan with a range of activity similar to that of phenylbutazone.[11] This finding is in agreement with the effects obtained in our study, although the acetophenones from *H. italicum* were assayed in a different experimental model of inflammation.

Experimental Section

General Experimental Procedures. Enzymatic hydrolysis was performed according to the method described by Markham.[12] Optical rotations were measured with a Perkin-Elmer 241 polarimeter using MeOH and/or $CHCl_3$. UV spectra were determined in spectroscopic grade MeOH on a Beckman DU640 spectrophotometer. IR spectra were measured on a Perkin-Elmer FTIR 1720 X spectrophotometer. ^1H and ^{13}C NMR spectra were run on a 400 MHz (Varian Unity 400) instrument in CD_3OD or DMSO-d_6. Complete proton and carbon assignments were based on 1D and 2D ($^1H-^1H$ COSY, $^1H-^{13}C$ HMQC, and $^1H-^{13}C$ HMBC) NMR experiments. FAB-MS and HREIMS were carried out in a VG Auto Spec (Fisons). Analytical TLC was carried out on Merck Si gel F_{254} and RP-18 aluminum sheets. Compounds were visualized with 1% sulfuric acid−anisaldehyde and then heated at 120 °C for 10 min.

Plant Material. Aerial parts of *Helichrysum italicum* (Roth) G. Don f. in Loudon (Asteraceae) were collected in Chiva (Valencia, Spain) in July 1997. A specimen (voucher number DF-1) was deposited in the herbarium of the Department of Pharmacology (Burjassot, Spain).

Chemicals. β-Glucosidase, indomethacin, and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) were purchased from Sigma Chemical Co. (St. Louis, MO). Solvents (analytical grade) were obtained from Panreac (Barcelona, Spain).

Extraction and Isolation. Air-dried and powdered aerial parts of *H. italicum* (400 g) were percolated with MeOH (3500 mL) at room temperature. The solution obtained was evaporated under reduced pressure, giving a residue of 55 g, which was redissolved in water and then fractionated with *n*-C_6H_{14}, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2 extract (22 g) was subjected to gel filtration over Sephadex LH-20 and eluted with MeOH to yield 13 fractions (I−XIII). Fraction IV was purified by precipitation and gave a compound that was identified as ursolic acid.[13] Fraction VI (4.7 g) was fractionated by vacuum-liquid chromatography (VLC) on a Si gel column and eluted with CH_2Cl_2−EtOAc mixtures to obtain 10 fractions (VI_1-VI_{10}). From fraction VI_5 eluted with pure CH_2Cl_2, compound **1** (2 g) was isolated by precipitation. Fraction VI_8 (448 mg) eluted with CH_2Cl_2−EtOAc (9:1) was further purified by VLC on Si gel with mixtures of the same mobile phase. The fraction that eluted with CH_2Cl_2−EtOAc (95:5) yielded two pure compounds, **2** (20 mg) and **3** (100 mg). Fraction IX (440 mg) was purified by column chromatography on Si gel and eluted with CH_2Cl_2 to obtain seven fractions (IX_1-IX_7). Fraction IX_4 gave a pure compound that was identified as gnaphaliin (120 mg).[14]

The EtOAc extract (10 g) was subjected to gel filtration over Sephadex LH-20 and eluted with MeOH to yield 10 fractions (I−X). Fraction II was fractionated on a Si gel column and eluted with CH_2Cl_2−MeOH mixtures to obtain 17 fractions (II_1-II_{17}). Fraction II_{10} gave a pure compound (**4**) directly by precipitation (100 mg). Fraction II_{11} (241 mg) was further purified on a Lobar LiChroprep RP-18 (Merck) column with a MeOH−H_2O (4:6) mixture. Fraction II_{11}-7 yielded **5** (50 mg) and fraction X_{11}-12 yielded an additional quantity of **4** (20 mg).

The BuOH extract (6 g) was subjected to gel filtration over Sephadex LH-20 and eluted with MeOH to yield 10 fractions (I−X). Fraction III (2.5 g) was fractionated by VLC on a Si gel column eluting with CH_2Cl_2−MeOH mixtures to obtain six fractions (III_1-III_6). Fraction III_4 (450 mg) was purified on a Lobar LiChroprep RP-18 (Merck) column with a MeOH−H_2O (2:8) mixture. Fraction III_4-2 yielded **7** (70 mg) and fraction III_4-3 yielded **6** (35 mg).

3-(3-Methyl-2-butenyl)acetophenone-4-*O*-β-D-glucopyranoside (4): mp 160 °C; $[\alpha]_D$ −45° (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} 221 and 266 nm; IR (dry film) ν_{max} 3340, 2923, 1668, 1599, 1253, 1074 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.75 (1H, d, $J = 2.0$ Hz, H-2), 7.18 (1H, d, $J = 8.4$ Hz, H-5), 7.83 (1H, dd, $J = 8.4$, 2.0 Hz, H-6), 2.52 (3H, s, CH_3−CO), 3.48 (2H, d, $J = 7.2$ Hz, H-9), 5.33 (1H, t, H-10), 1.71 (3H, s, CH_3-12), 1.73 (3H, s, CH_3-13), 5.04 (1H, d, $J = 7.2$ Hz, H-1′), 3.3−

3.8 (4H, m, H-2′- H-5′), 3.3−3.8 (2H, m, H-6′); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; EIMS *m*/*z* 203 [M − glucose + H]$^+$, 189 [aglycon − CH_3]$^+$; FABMS (+) *m*/*z* 389 [M + Na]$^+$, 367 [M + H]$^+$, 188 [M − glucose + H]$^+$; HREIMS *m*/*z* 204.1168 (calcd for $C_{13}H_{16}O_2$ (aglycon) 204.1150).

12-Hydroxytremetone-12-*O*-*β*-D-glucopyranoside or bitalin A-12-*O*-*β*-D-glucopyranoside (5): mp 155 °C, $[\alpha]_D$ −53° (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} 233 and 287 nm; IR (dry film) ν_{max} 3401, 2918, 1660, 1026 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.80 (1H, d, *J* = 2.0 Hz, H-2), 6.88 (1H, d, *J* = 8.4 Hz, H-5), 7.79 (1H, dd, *J* = 8.4, 2.0 Hz, H-6), 2.48 (3H, s, CH_3−CO), 3.15 (1H, dd, *J* = 14.0, 9.0 Hz, H-9a), 3.43 (1H, dd, *J* = 16.0, 9.9 Hz, H-9b), 5.46 (1H, t, H-10), 4.13 (1H, d, *J* = 13.5 Hz, H-12a), 4.35 (1H, d, *J* = 13.5 Hz, H-12b), 5.21 (1H, s, H-13a), 5.30 (1H, s, H-13b), 4.15 (1H, d, *J* = 7.8 Hz, H-1′), 3.3−3.7 (4H, m, H-2′- H-5′), 3.3−3.7 (2H, m, H-6′); ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 1; FABMS (+) *m*/*z* 403 [M + Na]$^+$, 381 [M + H]$^+$, 218 [M − glucose + H]$^+$; HREIMS *m*/*z* 380.1468 (calcd for $C_{19}H_{24}O_8$ 380.1471).

3-(2-Hydroxyethyl)acetophenone-4-*O*-*β*-D-glucopyranoside (6): mp 177 °C, $[\alpha]_D$ +1° (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} 230, 246 and 286 nm; IR (dry film) ν_{max} 3409, 2924, 1650, 1098 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.87 (1H, d, *J* = 2.0 Hz, H-2), 7.22 (1H, d, *J* = 8.4 Hz, H-5), 7.84 (1H, dd, *J* = 8.4, 2.0 Hz, H-6), 2.54 (3H, s, CH_3−CO), 2.94 (2H, dt, H-9), 3.20−4.00 (2H, m, H-10), 4.01 (1H, d, *J* = 7.8 Hz, H-1′), 3.2−4.0 (4H, m, H-2′−H-5′), 3.2−4.0 (2H, m, H-6′); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; FABMS (+) *m*/*z* 341 [M − H]$^+$; HREIMS *m*/*z* 180.0790 (calcd for $C_{16}H_{22}O_8$ (aglycon) 180.0786).

TPA-Induced Mouse Ear Edema.[15] Edema was induced by topical application of TPA (2.5 μg/ear in 20 μL of acetone). The test compounds and the standard drug indomethacin were applied topically (0.5 mg/ear), simultaneously with TPA. The 50% inhibitory dose (ID_{50}) was determined by applying the acetophenone **2** at five different doses ranging from 50 to 250 μg/ear.

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