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FIVE 8-*C*-BENZYLATED FLAVONOIDS FROM *THYMUS HIRTUS* (LABIATEAE)

RACHID MERGHEM, MAURICE JAY, MARIE-ROSE VIRICEL, CHRISTINE BAYET and BERNARD VOIRIN

Laboratoire de Biologie Micromoléculaire et Phytochimie, I.A.S.B.S.E., Université Lyon 1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne, France

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Abstract—From the aerial part of *Thymus hirtus*, eight flavonoids were extracted. Besides three common flavones, apigenin, luteolin and diosmetin, three derivatives of these flavones and two derivatives of quercetin and kaempferol, substituted by a *p*-hydroxybenzyl moiety, were isolated. The 8-*C*-substitution of apigenin, luteolin, diosmetin and quercetin was demonstrated by spectrometric data.

INTRODUCTION

As part of our program on the flavonoid study of Algerian plants, we have harvested *Thymus hirtus*, a Labiateae widely distributed in Eastern Algeria; this species, like most *Thymus* [1–5], synthesized many free flavone aglycones, among which some were common, apigenin (**1**), luteolin (**2**) and diosmetin (**3**), identified by classical procedures [6], whereas five others showed an original chromatographic behaviour compared to their spectral properties. Four were identified by spectrometric data as 8-*C*-*p*-hydroxybenzylluteolin (**4**), 8-*C*-*p*-hydroxybenzyldiosmetin (**5**), 8-*C*-*p*-hydroxybenzylapigenin (**6**) and 8-*C*-*p*-hydroxybenzylquercetin (**7**), respectively. The remaining compound, obtained in minute amount, was tentatively identified as 8-*C*-*p*-hydroxybenzylkaempferol (**8**).

RESULTS AND DISCUSSION

The mixture of flavone aglycones obtained from the ethyl acetate aerial extract of *Thymus hirtus* was purified by column, paper and thin layer chromatography to give eight compounds. The first three were readily identified as apigenin (**1**), luteolin (**2**) and diosmetin (**3**) from UV [6] and MS data and chromatographic properties (Table 1 and Experimental).

The UV spectra in methanol with classical reagents of the five remaining compounds suggested, respectively, the following parent aglycones: luteolin, diosmetin, apigenin, quercetin and kaempferol, i.e. three flavones and two flavonols. Nevertheless, by chromatographic comparisons with authentic samples of each aglycone, each of these native flavonoids showed much higher R_f values in 60% acetic acid on PC (Table 1). Owing to the fact that each of these compounds presented all the expected hydroxyl groups (UV data, Table 1) and that, in their respective EI mass spectra, they presented two unclassical and abundant ions at *m/z* 107 and the corresponding ion at [M − 106] (**4**, [M]: 392, [M − 106]: 286 (65%), *m/z* 107:22%; **5**, [M]: 406, [M − 106]: 300 (17.2%), *m/z* 107:17.5%; **6**, [M]: 376, [M − 106]: 270 (66%), *m/z* 107:20%; **7**, [M]: 408, [M − 106]: 302 (30.5%), *m/z* 107:15.9%; **8**, [M]: 392, [M − 106]: 286 (42%), *m/z* 107:20%), the presence of the same substituent linked to the flavone skeleton, corresponding to 107 mu, was suspected.

The ^{1}H NMR spectrum of the luteolin derivative (**4**) showed 11 protons, five of them were attributed to the flavone skeleton (H-2′, *d* 7.42 ppm, $J = 2$ Hz; H-5′, *d* 6.87 ppm, $J = 8.3$ Hz; H-6′, *dd* 7.33 ppm, $J = 2$ and 8 Hz; H-3, *s* 6.62 ppm; A-ring: 1H, *s* 6.32 ppm) and six to a benzyl moiety shared between two protons for $-CH_2$, *s* at 3.99 ppm, and four aromatic protons in a typical A_2B_2 system (H-2″, H-6″, *d* 7.06 ppm, $J = 8$ Hz; H-3″, H-5″, *d* 6.61 ppm, $J = 8$ Hz). These assignments of B-ring aromatic protons on the one hand and those of the benzyl substituent on the other hand were confirmed by decoupling experiments. Moreover, the irradiation at δ3.99 induced a smaller intensity of the doublet at δ7.06 showing a long-range coupling between the $-CH_2$ and the protons H-2″ and 6″. According to these data and the presence of ion fragment at 107 mu in the mass spectrum, the substituent was identified as the *C*-*p*-hydroxybenzyl moiety. Moreover, the value of the singlet at δ6.32, clearly distinguishable from the downfield 8-proton singlet at δ6.91, shown by 6-hydroxyquercetin 7-glucoside run under identical conditions (DMSO), proved that this substituent was linked to the flavone skeleton at C-8. The ^{13}C spectrum confirmed the identification of the aglycone as

Table 1. UV absorbance maxima and chromatographic properties for *Thymus hirtus* flavonoids

Compound	MeOH	$AlCl_3$	$AlCl_3+HCl$	NaOH	NaOAc	$NaOAc+H_3BO_3$	R_f values PC HOAc 60%
1	269	276	278	274	276	270	
Apigenin	294sh	302	303	325	300sh	300sh	0.65
	336	348	346	392	338	338	
		384	386				
6	274	280	280	283	278	274	
8-*C*-*p*-(OH)	326	308	308	330	386	326	0.78
Benzylapigenin	352sh	350	346	398		352sh	
		396	392				
2	254	272	262	268	268	262	
Luteolin	268	300sh	272	328	384	372	0.49
	348	330sh	294	404			
		424	360				
			386				
4	256	279	265sh	284	282	266	
8-*C*-(OH)	278	306	284	346sh	346	279	0.65
Benzylluteolin	346	430	358	410	406	376	
			394sh				
3	252	260	262	270	277	254sh	
Diosmetin	269	274	276	306	324	272	0.62
	290sh	294sh	296sh	388	368	348	
	346	360	358				
		388	388				
5	249	262sh	262	278	280	275	
8-*C*-(OH)	274	280	281	335sh	324	337	0.80
Benzyldiosmetin	338	301sh	299sh	396	392		
		359	353				
		396sh	394sh				
7	259	255sh	270	281	279	264	
8-*C*-(OH)	271sh	274	308sh	335	330	390	0.60
Benzylquercetin	332sh	301sh	364	429 dec.	412		
	376	452	439				
8	272	260sh	272	284	274	—	
8-*C*-(OH)	324	274	304sh	428	372	—	0.73
Benzylkaempferol	372	304sh	356				
		358	430				
		430					

luteolin and the acyl moiety as *C*-*p*-hydroxybenzyl. Moreover, careful comparisons of the chemical shifts of C-6 (98.8 ppm) and C-8 (106.9 ppm) of the flavonoid moiety with reported values for 5,7-dihydroxy-6-*C*-*p*-hydroxybenzylflavanone (C-6:110 ppm; C-8:95.4 ppm) and 5,7-dihydroxy-8-*C*-*p*-hydroxybenzylflavanone (C-6:96.5 ppm; C-8:108.9 ppm) [7] were in accordance with the C-8 substitution. In addition, the DEPT spectral data were also in accordance with the *p*-substitution of the benzyl group (C-2″, 6″ 128.9 ppm; C-3″, 5″ 115.1) and its location at C-8 (C-6 98.5). So, this new natural compound is 5,7,3′,4′-tetrahydroxy-8-*C*-*p*-hydroxybenzylflavone or 8-*C*-*p*-hydroxybenzylluteolin.

The fifth compound was identified as 8-*C*-*p*-hydroxybenzyldiosmetin at the same time—from the EI mass spectrum, which showed a molecular ion at *m/z* 406 ($C_{23}H_{18}O_7$), a fragment at 300 *m/z* due to the loss of *C*-*p*-hydroxybenzyl group—from the ^1H NMR spectrum which revealed the existence of a methoxy group (3H, *s* at δ3.85), of a *C*-*p*-hydroxybenzyl group (CH_2, *s* at δ3.99 ppm, H-2″, H-6″, *d* at δ7.05, $J = 8$ Hz; H-3″, H-5″, *d* at δ6.60, $J = 8$ Hz) and the five protons of flavone aglycone (H-3, *s* at δ6.69 ppm; H-6, *s* at δ6.33; H-2′, *m* at δ7.43; H-5′, *m* at δ7.05; H-6′, *m* at δ7.43) and—from the MeOH + NaOH spectrum ($\delta\lambda$ B1 + 58 nm and low intensity indicating a 4′ *O*-substitution, Table 1). In the same way, the structure of **6** was established: the UV spectra in methanol with classical reagents showed the existence of free hydroxyl groups at C-5, C-7 and C-4′ (Table 1), whereas the together data of EIMS (molecular ion at 376 mu and ion fragment at 270 mu) and ^1H NMR spectrum (H-3, *s* at δ6.60; H-6, *s* at δ6.17; H-2′, 6′, *d* at

δ7.76, J = 8.8 Hz; H-3′, 5′, d6.88, J = 8.8 Hz; H-2″, 6″, d at δ7.04, J = 8.5 Hz; H-3″, 5″, d at δ6.61, J = 8.5 Hz; CH_2, s at δ3.96) led to the identification of **6** as 8-*C*-*p*-hydroxybenzylapigenin. Moreover, the assignments of B-ring protons were confirmed by decoupling experiments.

From their UV and EI mass spectrum, **7** and **8** appeared, respectively, as *C*-hydroxybenzyl derivatives of quercetin and kaempferol. The ^{1}H NMR of **7** (H-6, s at δ6.30; H-2′, d at δ7.70, J = 2Hz; H-5′, d at δ6.85, J = 8 Hz; H-6′, dd at 7.43, J = 2 and 8 Hz; H-2″, 6″, d at δ7.03, J = 8.5 Hz; H-3″, 5″, d at δ6.59, J = 8.5 Hz) showed that this new natural compound was identified as 8-*C*-*p*-hydroxybenzylquercetin, although in the ^{1}H NMR spectrum, the singlet corresponding to the CH_2 was obscured by H_2O.

As there was an insufficient amount of **8** for ^{1}H NMR spectrum, it was not possible to locate unambiguously, as previously, the benzylated substituent at C-6 or C-8. Nevertheless, since 'the ratio of A band 1 and band 2, in the methanol spectrum, constitutes a criterion for distinguishing the 6- or 8-substituted position on the A-ring, 8-substitution particularly decreases this value, whereas 6-substitution increases it [8], and all the new compounds (**4**, ratio A band 1–band 2: 0.72, **5**: ratio 0.82, **6**: ratio 0.81 and **7**: ratio 0.66) presented a ratio lower than one, **8** (ratio 0.59) was tentatively identified as 8-*C*-*p*-hydroxybenzylkaempferol. The natural occurrence of these compounds has been controlled by extracting fresh material with a benzene–ethyl acetate mixture and by extemporaneous chromatographic analysis.

8-hydroxylation and 8-methoxylation have been described in *Thymus* species [1–5], but these results are the first report of 8-*C*-benzyl substitution in the Labiateae. Nevertheless, *C*-benzylflavonoids have been already described in Annonaceae by Hufford and Lasswell [7], but the aglycone was a flavanone (pinocembrin) substituted by one (8-*C*-substitution: chamanetin; 6-*C*-substitution: isochamanetin), two (6-8 di-*C*-substitution: dichamanetin,) or three *O*-hydroxybenzyl group(s) (5‴-*C*-*O*-hydroxybenzyldichamanetin; uvarinol). Moreover, a compound isolated from *Haplopapus* (Compositeae) has been identified as 5,7-dihydroxy-3,4′-dimethoxy-8-*C*-*p*-hydroxyphenylethylflavone [9]. It is noteworthy that this 8-*C*-substituted 3-methoxyflavone presents, in the methanol spectrum, a ratio band 1–band 2 inferior to one (0.6) as the 8-*C*-substituted flavones and flavonols described in this work.

EXPERIMENTAL

Plant material. Aerial part of *Thymus hirtus* was collected near Batna (Algeria).

Extraction. The air-dried powdered aerial part (*ca* 1 kg) was exhaustively extracted at room temp. with EtOH–H_2O (8:2). This extract concd under red. pres. was redissolved in H_2O and extracted successively with EtOAc and *n*-BuOH.

Isolation of compounds The EtOAc dried extract was dissolved in MeOH. CC of this extract on Polyamide SC6 with a gradient toluene–MeOH as eluent provided 30 frs of 100 ml each. All the frs were chromatographed over Whatman 3 with HOAc–H_2O 50%. The main band was eluted with MeOH and chromatographed on TLC Polyamide DC6. Frs 4–5 (gradient 70–30) main band R_f PC 50%:0.65, DC6, solvent toluene–butanone–MeOH(2:1:1): apigenin (**1**) R_f 0.45; diosmetin (**3**) 0.55; frs 6–7 (gradient 65–35) main band R_f PC 50%: 0.80, DC6, solvent toluene–butanone–MeOH 4:3:3: 8-*C*-*p*-hydroxybenzyldiosmetin (**5**) R_f 0.60: 8-*C*-*p*-hydroxybenzylapigenin (**6**) R_f 0.50; frs 8–9 (gradient 55–45) main band R_f PC 50%: 0.49, DC6, solvent toluene–butanone–MeOH 4:3:3: luteolin (**2**) R_f 0.29; frs 10–12 (gradient 40–60) main band R_f PC 50%: 0.70, DC6, solvent benzene–butanone–MeOH (2:1:1) : 8-*C*-*p*-hydroxybenzylluteolin (**4**) R_f 0.25; 8-*C*-*p*-hydroxybenzylkaempferol (**8**) 0.39; frs 13 (gradient 5–95) main band R_f PC 50%; 0.60, DC6, solvent toluene–butanone–MeOH 4:3:3 8-*C*-*p*-hydroxybenzylquercetin (**7**) 0.55. Final purification was achieved by chromatography on Sephadex LH 20 column.

The R_f values and the UV spectral data of **1**–**8** are given in Table 1. The ^{1}H NMR data of **4**–**7** are given in the text.

Compound **2**. EIMS *m/z* (rel. int.): 300 (100%), 153 (20%), 148 (10%).

Compound **3**. EIMS *m/z* (rel. int.): 286 (100%), 153 (40%), 137 (5%), 134 (17%).

Compound **4**. EIMS *m/z* (rel. int.): 392 (100%), 299 (26%) 298 (15%), 286 (65%), 270 (9%), 165 (22%), 153 (14%), 137 (10%), 134 (11%), 107 (22%), 94 (19%). ^{13}C NMR, 100 MHz DMSO-d_6, TMS as int. standard: δppm: 164.1 (C-2), 102.9 (C-3), 182.2 (C-4), 159.6 (C-5), 98.8 (C-6), 162.1 (C-7), 106.9 (C-8), 154.9 (C-9), 104 (C-10), 122.1 (C-1′), 113.7 (C-2′), 146.1 (C-3′), 150 (C-4′), 116.3 (C-5′), 119.1 (C-6′), 27.3 (CH_2), 131 (C-1″), 129.1 (C-2″, 6″), 115.3 (C-3″, 5″), 155.6 (C-4″). DEPT: 128.9 (C-2″, 6″), 118.9 (C-3″, 5″), 118.9 (C-6′), 116.1 (C-5′), 113.5 (C-2′), 102.7 (C-3), 98.5 (C-6), 27 (CH_2).

Compound **5**. EIMS *m/z* (rel. int.): 406 (46%), 391 (13%), 300 (17.2%), 287 (100%), 151 (5%), 148 (6%), 107 (17.5%), 106 (21%).

Compound **6**. SM: EIMS *m/z* (rel. int.): 376 (100%), 283 (24%), 282 (5%), 270 (66%), 121 (13%), 107 (20%), 94 (27%).

Compound **7**. EIMS *m/z* (rel. int.): 408 (2%), 302 (305%), 153 (7.5%), 137 (14%).

Compound **8**. EIMS *m/z* (rel. int.): 392 (15%), 299 (5%), 286 (42%), 258 (4%), 153 (13%), 121 (9%), 107 (20%), 94 (100%).

6-hydroxyquercetin 7-glucoside. ^{1}H NMR, DMSO-d_6 with TMS as int. standard: δ7.69 (H-2′, d, J = 2 Hz), 6.89 (H-5′, d, J = 8 Hz), 7.53 (dd, J = 2 and 8 Hz), 6.91 (H-6, s), 3.22–3.76 (6H, m, sugar protons), 5.0 (H-1″, d, J = 7 Hz).

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