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8-METHOXYKAEMPFEROL 3-NEOHESPERIDOSIDE AND OTHER FLAVONOIDS FROM BEE POLLEN OF *CRATAEGUS MONOGYNA*

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Key Word Index—*Crataegus monogyna*; Rosaceae; hawthorn; bee pollen; flavonols; 8-methoxykaempferol 3-neohesperidoside.

Abstract—A new flavonol glycoside, 8-methoxykaempferol 3-neohesperidoside and 8-methoxykaempferol 3-glucoside, 8-methoxykaempferol and kaempferol 3-neohesperidoside were identified from the bee pollen of *Crataegus monogyna*.

INTRODUCTION

Flavonoids occur in pollen of many angiosperm and gymnosperm species [1]. Flowers of the hawthorn, *Crataegus monogyna* Jacq. are widely used in herbal medicine and the flavonoid profile of this tissue has been determined previously [2]. Here we report the isolation and identification of the main flavonoids of the bee pollen of *C. monogyna* including the new glycoside **3**. This bee pollen was completely homogeneous and its methanolic extract similar, by 2D TLC, to an extract of pollen collected from the flowering plant. Such similarity in the flavonoid profiles of bee and natural pollen has been reported previously for other plants [3].

RESULTS AND DISCUSSION

The flavonoid aglycone 8-methoxykaempferol (sexangularetin) **1** and the flavonoid glycosides sexangularetin 3-glucoside **2**, sexangularetin 3-neohesperidoside **3** and kaempferol 3-neohesperidoside **4** were isolated and identified from *C. monogyna* bee pollen.

The aglycone **1** isolated in 0.23% yield was identified by comparison of EIMS, UV and ^{1}H NMR spectral data with literature values [2], which were in close agreement. The EIMS exhibited a molecular peak at *m/z* 316 and a base peak at M-15 characteristic of an 8-methoxy substitution [4, 5]. The oxygenation of carbon 8 was confirmed by the position of the ^{1}H NMR singlet assigned to H-6 (δ 6.25), which is at a higher field than that expected for a H-8 signal [6].

The glycoside **2** was the major flavonoid compound isolated from the pollen (1.6%). Its FABMS displayed a $[M+H]^+$ at *m/z* 479 and acid hydrolysis released glucose, which was identified by TLC, and the aglycone **1**. The position of glycosylation at the 3-hydroxyl was deduced from UV spectral analysis. Thus, in the UV spectrum of **2**, bathochromic shifts with sodium acetate and sodium methoxide indicated the presence of two free hydroxyl groups at C-7 and C-4′. In the ^{1}H NMR spectrum the free hydroxyl group at C-5 appeared as a singlet at δ 12.3 (exchanged with D_2O) and the β-linkage of the D-glucose was inferred from the signal of the anomeric proton at δ 5.45 (*d*, *J* = 7.Hz). The glycosylation of the 3-hydroxyl was confirmed in the ^{13}C NMR spectrum by the down-field shift of the C-2 signal in **2** compared with **1** (Table 1) [7]. On the basis of these results, **2** was identified as 8-methoxykaempferol 3-*O*-β-D-glucoside and its spectral data, especially the ^{13}C NMR spectrum, were in good agreement with reported values [2, 8]. Compound **2** has been isolated previously from *C. monogyna* flowers in very low yield (0.004%) [2].

The glycoside **3** was isolated in a very small amount (0.17% yield) and its structure unambiguously determined by spectral analysis. Thus, the FABMS/MS of **3** under collision-activated dissociation at 10 eV showed a peak at *m/z* 625 and other signals at 479 and 317 corresponding to a diglycoside quasi-molecular ion and a sequential loss, first of a deoxyhexose then of an hexose. The UV spectral data were similar to **2** and suggested it was a 3-*O*-diglycoside of 8-methoxykaempferol. In the ^{1}H and ^{13}C NMR spectra of **2** and **3**, analogy of the signals relating to the aglycone part confirmed our hypothesis. The interglycosidic linkage and the sequential arrangement of the sugars were deduced from the ^{1}H and ^{13}C NMR spectral data. Especially significant in ^{1}H NMR were the positions of the signals assigned to the anomeric protons at δ 5.75 (*d*, *J* = 7 Hz) and 5.22 (*d*, *J* = 2 Hz), respectively, and to a methyl group at δ 0.97 (*d*, *J* = 7 Hz) which indicated a rhamnosylglycoside sequence. In the ^{13}C NMR spectrum (Table 1), the position of the

glucose C-6 signal at δ 60.6 showed that the sugar linkage was not (1→6) as in rutinose [7, 9]. The presence of three signals at δ 77.4 and absence of any signal between 74 and 77 ppm indicated a down-field shift of the glucose C-2 signal and therefore inferred a rhamnosyl(1→2)glucoside [7]. Furthermore, in the ^{13}C NMR spectrum of **3**, positions of signals of the glycosidic part were in good agreement with the reported values of flavonoid neohesperidosides [9, 10]. The identity of the sugars was confirmed by total acid hydrolysis, which gave glucose, rhamnose and the aglycone **1**. From these data **3** was identified as 8-methoxykaempferol 3-neohesperidoside, a new flavonol glycoside.

The remaining flavonoid **4** was isolated in very low yield (0.18%) and identified by spectral analysis as kaempferol 3-neohesperidoside. The FABMS exhibited a $[M+H]^+$ at *m/z* 595 and UV, ^{1}H and ^{13}C NMR spectral data were similar to reported values for kaempferol 3-glycosides [9, 11]. In the ^{13}C NMR spectrum the positions of the signals for the glycosidic moiety were very similar to those for **3** indicating a neohesperidoside sequence.

EXPERIMENTAL

General. NMR spectra were measured at 200 MHz for ^{1}H and 50.3 MHz for ^{13}C in DMSO-d_6 or CD_3OD using TMS as int. reference.

Plant material. Bee pollen was collected by one of us (Dr A. Bekaert) from the area around Palaiseau (France) in June 1991. The sample was completely homogeneous, composed exclusively of pollen from *C. monogyna*. Comparison between bee pollen and flowering plant pollen composition was performed using 2D TLC on polyamide 6 UV_{254} with H_2O–EtOH–MeCOEt–HOAc (65:15:15:5, 1st direction) and $CHCl_3$–MeOH–MeCOEt (30:13:7, 2nd direction, spray reagent: $AlCl_3$).

Extraction and isolation. Dried bee pollen (6 g) of *C. monogyna* was extracted with MeOH under reflux and the concd extract suspended in H_2O. After partition with *n*-hexane, EtOAc and *n*-BuOH, the EtOAc-soluble portion was fractionated by prep. silica gel TLC in EtOAc–MeOH–H_2O (100:16.5:13.5) to yield **1** (R_f 0.81, 14 mg), **2** (R_f 0.52, 97 mg) and a mixt. of **3** and **4** (R_f 0.34, 14 mg). This mixt. was sepd by CC on polyamide MN CC6 (grain size < 0.07 mm) with $CHCl_3$–MeOH–MeCOEt (30:13:7) to give **3** (10 mg) and **4** (11 mg).

8-Methoxykaempferol (**1**). EIMS, *m/z* (rel. int.): 316$[M]^+$ (66), 301 $[M-Me]^+$ (100). UV λ_{max}^{MeOH} 222, 259sh, 275, 328, 378; +NaOMe, 287, 339, 432; +$AlCl_3$, 227, 263sh, 275, 310, 358, 434; +NaOAc, 282, 318, 401; +H_3BO_3, 274, 326, 380. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.82 (3H, *s*, OMe); 6.28 (1H, *s*, H-6); 6.94 (2H, *d*, *J* = 9 Hz, H-3′ and H-5′); 8.05 (2H, *d*, *J* = 9 Hz, H-2′ and H-6′). ^{13}C NMR (50.3 MHz, DMSO-d_6): see Table 1.

8-Methoxykaempferol 3-glucoside (**2**). Yellow crystals from EtOAc, mp 258–260° (lit. mp 264–265°) [8]. FABMS, *m/z*: 501 $[M+Na]^+$, 479 $[M+H]^+$, 317 [aglycone + H]$^+$. UV λ_{max}^{MeOH} 223, 272, 322, 359; +NaOMe, 285, 336, 418; +$AlCl_3$, 232, 282, 311, 353, 410; +NaOAc, 282, 312, 395; +H_3BO_3, 275, 318, 362. ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.82 (3H, *s*, OMe); 5.45 (1H, *d*, *J* = 7 Hz, H-1″); 6.30 (1H, *s*, H-6); 6.95 (2H, *d*, *J* = 9 Hz, H-3′ and H-5′); 8.05 (2H, *d*, *J* = 9 Hz, H-2′ and H-6′); 12.30 (1H, *s*, OH-5). ^{13}C NMR (50.3 MHz, DMSO-d_6): see Table 1.

Acid hydrolysis of **2**. Total hydrolysis was carried out with 2 M H_2SO_4 (30 min at 100°) and the aglycone identified as sexangularetin by comparison with **1**. D-glucose was identified by co-PC [pyridine–*n*-BuOH–H_2O (4:6:3)] and silica gel TLC [*n*-BuOH–iso-PrOH–H_2O (5:3:1)] with authentic D-glucose (spray reagent: aniline phthalate).

8-Methoxykaempferol 3-neohesperidoside (**3**). Yellow crystals from EtOAc, mp 173–175° $[\alpha]_D^{20} = -73.6°$ (MeOH, *c* 0.7). FABMS/MS under CAD (10 eV), *m/z*: 625 $[M+H]^+$, 479 $[M-C_6H_{10}O_4H]^+$, 317 [aglycone + H]$^+$. UV λ_{max}^{MeOH} 223, 273, 321, 358; +NaOMe, 284, 334, 414; +$AlCl_3$, 230, 282, 312, 354, 414; +NaOAc, 283, 312, 392; +H_3BO_3, 277, 318, 363. ^{1}H NMR (200 MHz, CD_3OD): δ 0.97 (3H, *d*, *J* = 7 Hz, Me-6‴); 3.80 (3H, *s*, OMe); 5.22 (1H, *d*, *J* = 2 Hz, H-1‴); 5.75 (1H, *d*, *J* = 7 Hz, H-1″); 6.25 (1H, *s*, H-6); 6.90 (2H, *d*, *J* = 9 Hz, H-3′ and H-5′); 8.10 (2H, *d*, *J* = 9 Hz, H-2′ and H-6′). ^{13}C NMR (50.3 MHz, DMSO-d_6): see Table 1.

Table 1. ^{13}C NMR data (50.3 MHz, DMSO-d_6) of **1**–**4**

C	1	2	3	4
2	146.8[a]	156.1[a]	156.0[a]	156.4
3	135.7	133.2	132.4	133.1
4	176.1	177.6	177.1	177.5
5	155.4	155.9[a]	155.3[a]	160.8
6	98.4	99.0	99.6	98.6[a]
7	156.5	157.1	159.4	164.7
8	127.4	127.5	127.8	94.1
9	148.4[a]	148.6	148.4	156.4
10	103.0	103.8	102.7	104.2
OMe	60.9	61.0	60.6	
1′	121.8	121.1	121.1	121.3
2′	129.3	130.8	130.5	131.1
3′	115.6	115.3	114.2	115.4
4′	159.3	160 1	160.0	160.0
5′	115.6	115.3	114.2	115.4
6′	129.3	130.8	130.5	131.1
Glucose				
1″		100.9	98.3	99.1[a]
2″		74.2	77.4	78.0[b]
3″		77.6	77.4	77.6[b]
4″		69.9	70.2[b]	70.4[c]
5″		76.4	77.4	77.5[b]
6″		60.9	60.6	61.0
Rhamnose				
1‴			100.5	101.0
2‴			70.5[b]	70.8[c]
3‴			70.5[b]	70.8[c]
4‴			71.8	72.0
5‴			68.2	68.6
6‴			17.2	17.5

[a–c] Assignments bearing the same superscript may be observed.

Acid hydrolysis of **3**. Total hydrolysis of **3** was carried out with 2 M CF_3COOH (1 hr at 100°) and aglycone identified as sexangularetin by comparison with **1**. D-glucose and L-rhamnose were identified by silica gel TLC [*n*-BuOH–iso-PrOH–H_2O (5:3:1)].

Kaempferol 3-neohesperidoside (**4**). FABMS, *m/z*: 617 $[M+Na]^+$, 595 $[M+H]^+$, 287 $[aglycone+H]^+$. UV λ_{max}^{MeOH} 219, 267, 303, 350; +NaOMe, 276, 330, 398; +$AlCl_3$, 226, 276, 305, 352, 400; +NaOAc, 276, 303, 368; +H_3BO_3, 268, 302, 352. ^1H NMR (200 MHz, DMSO-d_6): δ0.80 (3H, *d*, $J=7$ Hz, Me-6‴); 5.05 (1H, *br s*, H-1‴); 5.65 (1H, *d*, $J=7$ Hz, H-1″); 6.18 (1H, *d*, $J=2$ Hz, H-6); 6.40 (1H, *d*, $J=2$ Hz, H-8); 6.86 (2H, *d*, $J=9$ Hz, H-3′ and H-5′); 8.02 (2H, *d*, $J=9$ Hz, H-2′ and H-6′). ^{13}C NMR (50.3 MHz, DMSO-d_6): see Table 1.

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