Chemistry of Natural Compounds, Vol. 48, No. 2, May, 2012 [Russian original No. 2, March–April, 2012]

CHEMICAL INVESTIGATION OF THE POLLEN OF *Brassica napus*

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UDC 547.972

The pollen of *Brassica napus* L., distributed widely in China, has a long history of use as a well-tolerated treatment for prostate-related diseases such as benign prostatic hyperplasia (BPH) and prostatitis in men [1]. In previous studies, the EtOAc extract of the pollen of *Brassica napus* L. showed strong activity in decreasing the secretion of prostate specific antigen (PSA) in LNCaP cells [2]. Sterols, flavones, long-chain hydrocarbons, and brassinolide have been reported from the EtOAc-soluble fraction [2–4]. However, there was little systematic investigation of the chemical constituents of the pollen of *Brassica napus* L. We have previously reported sphingolipids from the pollen of *Brassica napus* [5]. Continuing our investigations into the constituents of the EtOAc extract of the pollen of *Brassica napus* by chromatographic and spectroscopic methods, we have isolated twelve flavonoids (**2**, **3**, **5**, **6**, **7**, **8**, **10**, **12**, **13**, **14**, **15**, and **16**), two sterides (**1**, **4**), and two nucleosides (**9**, **11**).

The pollen of *Brassica napus* L. was collected in September 2007 in Gansu, China. The material was identified by Prof. Huan-Yang Qi of Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, China. A voucher specimen (ZY200701) has been deposited at Key Laboratory of Chemistry of Northwestern Plant Resources, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, China.

The air-dried and powdered pollen of *B. napus* (5 kg) was extracted with 70% EtOH (3 × 20 L, 7 days each) at room temperature, and the percolate was evaporated in vacuum to yield the EtOH extract (1750 g), which was suspended in distilled water and extracted with *n*-hexane and EtOAc. The EtOAc extract (80 g) was subjected to column chromatography over silica gel (200–300 mesh, 2500 g) and eluted with $CHCl_3$, $CHCl_3$–MeOH (95:5), (90:10), (85:15), (80:20), (70:30), (50:50), (30:70), (10:90), and MeOH to yield 10 fractions (Fr.1–10). Fractions 1–7 were subjected to repeated chromatography on silica-gel columns eluting with a gradient mixture of *n*-hexane–EtOAc or $CHCl_3$–MeOH, Sephadex LH-20 eluting with $CHCl_3$–MeOH, MeOH, or a gradient mixture of MeOH–H_2O, and C-18 reverse silica-gel columns eluting with a gradient mixture of MeOH–H_2O. A total of sixteen compounds (**1–16**) was isolated and identified based on NMR and mass spectra, and by direct comparison with authentic samples and the literature. All the identified compounds are known compounds. Seven compounds (**8**, **9**, **10**, **11**, **12**, **14**, and **16**) are reported for the first time from the pollen of *Brassica napus*.

***β*-Sitosterol (1),** white needle crystals; TLC was identical to that of an authentic sample [6].

Naringenin (2), white crystalline powder, $C_{15}H_{12}O_5$ [7].

Kaempferol 3-*O*-α-L-(2″,3″-di-*E*-*p*-coumaroyl)rhamnoside (3), pale-yellow powder, $C_{39}H_{32}O_{14}$. ESI-MS *m/z:* 747 $[M + Na]^+$, 723 $[M - H]^-$. ^1H NMR (400 MHz, acetone-d_6, δ, ppm, J/Hz): 1.01 (3H, d, J = 6.0, Me-6″), 5.67 (1H, d, J = 1.6, H-1″), 6.25 (1H, d, J = 2.0, H-6), 6.31 (1H, d, J = 15.9, H-2‴), 6.37 (1H, d, J = 2.0, H-8), 6.40 (1H, d, J = 15.9, H-2‴), 6.72 (2H, d, J = 8.6, H-6‴, 8‴) 6.75 (2H, d, J = 8.6, H-6⁗, 8⁗), 6.88 (2H, d, J = 8.8, H-3′, 5′), 7.43 (2H, d, J = 8.6, H-5‴, 9‴), 7.45 (2H, d, J = 8.6, H-5⁗, 9⁗), 7.58 (1H, d, J = 15.9, H-3‴), 7.63 (1H, d, J = 15.9, H-3⁗), 7.88 (2H, d, J = 8.8, H-2′, 6′).

^{13}C NMR (100 MHz, acetone-d_6, δ): 157.4 (C-2), 133.0 (C-3), 178.0 (C-4), 161.9 (C-5), 98.7 (C-6), 164.4 (C-7), 93.7 (C-8), 157.1 (C-9), 104.6 (C-10), 121.2 (C-1′), 130.8 (C-2′, 6′), 115.8 (C-3′, 5′), 160.3 (C-4′), 98.7 (C-1″), 69.3 (C-2″), 71.7 (C-3″), 69.4 (C-4″), 71.0 (C-5″), 16.9 (C-6″), 165.6 (C-1‴), 114.8 (C-2‴), 145.1 (C-3‴), 126.0 (C-4‴), 130.1 (C-5‴, 9‴), 115.2 (C-6‴, 8‴), 159.9 (C-7‴), 166.3 (C-1⁗), 114.9 (C-2⁗), 145.8 (C-3⁗), 126.2 (C-4⁗), 130.3 (C-5⁗, 9⁗), 115.6 (C-6⁗, 8⁗), 160.3 (C-7⁗) [8].

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 0009-3130/12/4802-0310

β-Daucosterol (4), white powder; TLC was identical to that of an authentic sample [9].

Kaempferol (5), yellow powder, $C_{15}H_{10}O_6$ [10].

Luteolin (6), pale-yellow crystals, $C_{15}H_{10}O_6$ [11].

Kaempferol 3-*O*-α-L-rhamnopyranoside (7), yellow powder, $C_{21}H_{20}O_{10}$, ESI-MS *m/z:* 455 $[M + Na]^+$, 433 $[M + H]^+$ [12].

Kaempferol 3-*O*-β-D-glucoside (8), yellow needle-like crystals, $C_{21}H_{20}O_{11}$ [13].

Uridine (9), white needle-like crystals, $C_9H_{12}N_2O_6$ [14].

Quercetin 3-*O*-β-D-glucopyranoside (10), yellow powder, $C_{21}H_{20}O_{12}$, ESI-MS *m/z:* 465 $[M + H]^+$, 487 $[M + Na]^+$ [13].

Adenosine (11), white powder, ESI-MS *m/z* 268 $[M + H]^+$, $C_{10}H_{12}N_5O_4$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 5.86 (1H, d, J = 6.0, H-1′) , 7.36 (1H, s, H-6), 8.12 (1H, s, H-8), 8.34 (1H, s, H-2).

^{13}C NMR (100 MHz, DMSO-d_6, δ): 152.4 (C-2), 149.1 (C-4), 119.4 (C-5), 156.2 (C-6), 140.0 (C-8), 87.9 (C-1′), 70.7 (C-2′), 73.4 (C-3′), 85.9 (C-4′), 61.7 (C-5′) [15].

Kaempferol 3-*O*-β-D-(2-*O*-β-D-6-*O*-acetylglucosyl)-glucopyranoside (12), pale-yellow powder, $C_{29}H_{32}O_{17}$. ESI-MS *m/z* 653 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.18 (3H, s, acetyl CH_3), 4.10 (1H, d, J = 7.6, H-1′′′), 5.53 (1H, d, J = 7.6, H-1′′), 6.19 (1H, d, J = 2.0, H-6), 6.43 (1H, d, J = 2.0, H-8), 6.89 (2H, d, J = 8.5, H-3′, 5′), 8.04 (2H, d, J = 8.5, H-2′, 6′).

^{13}C NMR (100 MHz, DMSO-d_6, δ): 156.3 (C-2), 132.7 (C-3), 177.4 (C-4), 161.3 (C-5), 98.6 (C-6), 164.1 (C-7), 93.6 (C-8), 155.9 (C-9), 104.1 (C-10), 120.8 (C-1′), 130.9 (C-2′, 6′), 115.3 (C-3′, 5′), 160.0 (C-4′), 97.9 (C-1′′), 82.2 (C-2′′), 76.5 (C-3′′), 69.6 (C-4′′), 77.1 (C-5′′), 60.8 (C-6′′), 103.8 (C-1′′′), 74.3 (C-2′′′), 76.4 (C-3′′′), 69.6 (C-4′′′), 74.0 (C-5′′′), 62.6 (C-6′′′), 169.7 (acetyl-CO), 19.9 (acetyl CH_3) [16].

Kaempferol 3-*O*-β-D-(2-*O*-β-D-glucosyl)glucopyranoside (13), yellow powder, $C_{27}H_{30}O_{16}$. ESI-MS *m/z*: 633 $[M + Na]^+$, 611 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 4.28 (1H, d, J = 7.6, H-1′′′), 5.69 (1H, d, J = 7.6, H-1′′), 6.18 (1H, d, J = 2.0, H-6), 6.42 (1H, d, J = 2.0, H-8), 6.90 (2H, d, J = 8.5, H-3′, 5′), 8.03 (2H, d, J = 8.5, H-2′, 6′).

^{13}C NMR (100 MHz, DMSO-d_6, δ): 156.3 (C-2), 132.8 (C-3), 177.5 (C-4), 161.2 (C-5), 98.6 (C-6), 164.0 (C-7), 93.6 (C-8), 155.6 (C-9), 104.1 (C-10), 120.9 (C-1′), 130.9 (C-2′, 6′), 115.3 (C-3′, 5′), 159.9 (C-4′), 97.9 (C-1′′), 82.4 (C-2′′), 76.6 (C-3′′), 69.5 (C-4′′), 77.5 (C-5′′), 60.8 (C-6′′), 103.9 (C-1′′′), 74.3 (C-2′′′), 76.0 (C-3′′′), 69.7 (C-4′′′), 77.0 (C-5′′′), 60.5 (C-6′′′) [10].

Kaempferol 3-neohesperidoside (14), yellow powder, $C_{27}H_{30}O_{15}$. ESI-MS *m/z* 595 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.74 (3H, d, J = 6, Me-6′′′), 5.05 (1H, br.s, H-1′′′), 5.47 (1H, d, J = 7.6, H-1′′), 6.19 (1H, d, J = 2, H-6), 6.42 (1H, d, J = 2, H-8), 6.87 (2H, d, J = 8.8, H-3′, H-5′), 8.03 (2H, d, J = 8.8, H-2′, H-6′).

^{13}C NMR (100 MHz, DMSO-d_6, δ): 156.4 (C-2), 132.7 (C-3), 177.4 (C-4), 161.3 (C-5), 98.7 (C-6), 164.1 (C-7), 93.7 (C-8), 156.1 (C-9), 104.0 (C-10), 120.9 (C-1′), 130.8 (C-2′, 6′), 115.1 (C-3′, 5′), 159.9 (C-4′), 98.3 (Glc-1′′), 77.5 (Glc-2′′), 77.5 (Glc-3′′), 70.2 (Glc-4′′), 77.3 (Glc-5′′), 60.8 (Glc-6′′), 100.6 (Rha-1′′′), 70.6 (Rha-2′′′), 70.5 (Rha-3′′′), 71.8 (Rha-4′′′), 68.3 (Rha-5′′′), 17.3 (Rha-6′′′) [17].

Kaempferol-3,4′-di-*O*-β-D-glucoside (15), yellow powder, $C_{27}H_{30}O_{16}$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 5.06 (1H, d, J = 7.0, H-1′′′), 5.47 (1H, d, J = 7.6, H-1′′), 6.21 (1H, s, H-6), 6.45 (1H, s, H-8), 7.14 (2H, d, J = 9.0, H-3′, 5′), 8.11 (2H, d, J = 9.0, H-2′, 6′), 10.91 (1H, s, 7-OH), 12.56 (1H, s, 5-OH).

^{13}C NMR (100 MHz, DMSO-d_6, δ): 156.4 (C-2), 133.7 (C-3), 177.7 (C-4), 161.2 (C-5), 98.7 (C-6), 164.2 (C-7), 93.7 (C-8), 155.6 (C-9), 104.1 (C-10), 123.7 (C-1′), 130.6 (C-2′, 6′), 115.8 (C-3′, 5′), 159.2 (C-4′), 100.8 (C-1′′), 74.2 (C-2′′), 76.5 (C-3′′), 69.9 (C-4′′), 77.6 (C-5′′), 60.9 (C-6′′), 99.9 (C-1′′′), 73.2 (C-2′′′), 76.4 (C-3′′′), 69.6 (C-4′′′), 77.1 (C-5′′′), 60.6 (C-6′′′) [18].

Kaempferol 3-*O*-(6-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside (16), yellow powder, $C_{27}H_{30}O_{16}$. ESI-MS *m/z* 633 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 4.38 (1H, d, J = 7.7, H-1′′′), 5.40 (1H, d, J = 7.4, H-1′′), 6.20 (1H, d, J = 2.0, H-6), 6.44 (1H, d, J = 2.0, H-8), 6.90 (2H, m, H-3′, H-5′), 8.10 (2H, m, H-2′, H-6′).

^{13}C NMR (100 MHz, DMSO-d_6, δ): 156.3 (C-2), 132.9 (C-3), 177.5 (C-4), 161.3 (C-5), 98.6 (C-6), 164.0 (C-7), 93.6 (C-8), 155.3 (C-9), 104.6 (C-10), 120.9 (C-1′), 130.9 (C-2′, 6′), 115.2 (C-3′, 5′), 156.0 (C-4′), 97.9 (C-1′′), 73.9 (C-2′′), 76.2 (C-3′′), 69.4 (C-4′′), 76.9 (C-5′′), 65.8 (C-6′′), 104.0 (C-1′′′), 73.9 (C-2′′′), 77.6 (C-3′′′), 69.6 (C-4′′′), 77.6 (C-5′′′), 60.5 (C-6′′′) [19].

ACKNOWLEDGMENT

This work was supported by the "Hundred Talents Program" of the Chinese Academy of Sciences and the National Natural Sciences Foundation of China (NSFC No. 20775083) as well as the Science and Technology Key Project of Gansu Province (2GS64-A43-019-06).

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