Pollenopyrroside A and B, Novel Pyrrole Ketohexoside Derivatives from Bee-Collected *Brassica campestris* Pollen

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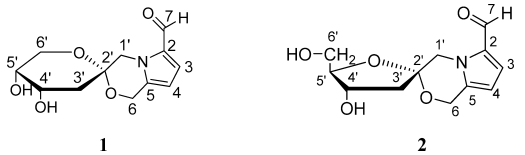
Pollenopyrroside A and pollenopyrroside B, novel pyrrole ketohexoside derivatives, were isolated from the extract of Bee-collected *Brassica campestris* pollen. Their structures were elucidated by spectroscopic analysis (UV, IR, MS, NMR and X-ray) and chemical evidence. Pollenopyrrosides A and B represent a novel carbon skeleton with a six–six and five–six member dioxaspirocycle bearing nitrogen atom, respectively.

Key words *Brassica campestris* pollen; pyrrole ketohexoside derivative; pollenopyrroside A; pollenopyrroside B

Bee-collected pollen is a hive product that bees pack pollen grains from the flower into pollen pellets on their hind legs with the help of combs and hairs, to feed their larvae in the early stages of development. The constituents of pollen are different according to the floral species or cultivars,[1] while flavonols are commonly encountered in the pollen of flowering plants and perform an essential physiological function in pollen germination and pollen tube growth. *Brassica campestris* L., also called rape, belongs to *Brassica* genus (Crucifer) and is widely grown in south of China as an economic crop. Bee-collected *Brassica campestris* pollen is often used in China as a healthy food and an herbal medicine in strengthening the body's resistance against disease. It has already been found to possess a wide range of biological activities, including antioxidant,[2] antitumor,[3] regulating serum lipids,[4] and treatment of prostatitis.[5] Up to date, bee-collected *Brassica campestris* pollen are known as a rich source of protein, polysaccharide, fatty acid and flavonoids.[6] In our study on the active components of bee-collected *Brassica campestris* pollen, two novel pyrrole ketohexoside derivatives, named pollenopyrroside A (**1**) and pollenopyrroside B (**2**), were isolated from it. Their structures were identified on basis of spectroscopic data (UV, IR, MS, NMR and X-ray) and chemical evidence. Compounds **1** and **2** are two novel pyrrole ketohexoside derivatives with five–six and six–six member dioxaspirocycle, respectively (Fig. 1). Their cytotoxicities were evaluated against A549, Bel7420, BGC-823, HCT-8, and A2780.

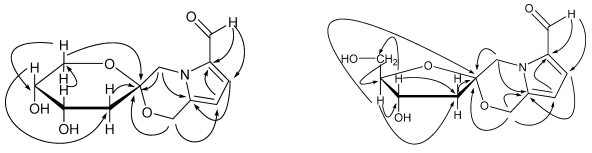
Restults and Discussion

Compound **1** was obtained as colorless crystal. Its molecular formula $C_{12}H_{15}NO_5$, was deduced from its electrospray ionization (ESI)-MS ($[M+H]^+$ ion at *m*/*z* 254.1) and NMR data, corresponding to six degrees of unsaturation. The ^{1}H-NMR spectrum of **1** showed two mutually-coupling protons at δ_H 6.99 (1H, d, J=4.0 Hz) and δ_H 6.08 (1H, d, J=4.0 Hz), which were characteristic of H-3 and H-4 protons of a pyrrole, respectively, one oxymetheneyl protons at δ_H 4.93 (1H, d, J=16.0 Hz) and 4.86 (1H, d, J=16.0 Hz), and an aldehyde proton at δ_H 9.48 (1H, s). The ^{13}C-NMR spectrum of **1** exhibited two olefinic carbons at δ_C 105.3 and δ_C 124.1, two sp^2 quaternary carbons at δ_C 135.1 and δ_C 132.2, a carbonyl carbon at δ_C 179.1, and an oxymethyleneyl group at δ_C 58.1, suggested that 5-oxymethy1-2-formyl-pyrrole skeleton existed in **1**.[7] These were supported by heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 2) of H-7 with C-2 and C-3, of H-6 with C-4 and C-5, of H-3 with C-2, C-4, and C-5, and of H-4 with C-5 and C-2. In addition, the ^{1}H- and ^{13}C-NMR spectra showed the resonances of two methylene carbons at δ_C 52.3 [δ_H 4.49 (1H, d, J=14.0 Hz), 4.03 (1H, d, J=14.0 Hz)] and δ_C 38.4 [δ_H 2.24 (1H, dd, J=14.5, 3.5 Hz), 2.09 (1H, dd, J=14.5, 3.5 Hz)], two methine carbons at δ_C 67.6 [δ_H 4.05 (1H, m)] and δ_C 66.9 [δ_H 3.71 (1H, m)], one oxymethlene carbon at δ_C 60.9 [δ_H 3.76 (1H, dd, J=10.5, 10.5), 3.53 (1H, m)], one quaternary carbon at δ_C 95.0. These NMR data suggested that a deoxyketohexose moiety existed in **1**. The connectivity of aglycone and sugar moiety was established on the basis of HMBC experiment. In the HMBC spectrum (Fig. 2), correlations of H-1′ with C-5 and C-2′, and H-6 protons resonating at δ_H 4.93 (1H, d, J=16.0 Hz) and 4.86 (1H, d, J=16.0 Hz) with the quaternary carbon C-2′ were observed. This quaternary carbon was also correlated with some protons of sugar. The above evidence confirmed that the deoxyketohexose moiety fused to the 5-oxymethy1-2-formyl-pyrrole fragment through oxygen and nitrogen atoms and an oxazine ring was formed. The addi-



1 **2**

Fig. 1. Structures of Pyrrole Ketohexoside Derivatives Pollenopyrroside A (**1**) and Pollenopyrroside B (**2**)



1 **2**

Fig. 2. Key HMBC Correlations for Compounds **1** and **2**

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tional correlations of H-5′ with C-3′, C-4′, and C-6′, of H-3′ with C-1′, C-2′, and C-4′, and of H-4′ with C-6′ revealed that the deoxyketohexose moiety existed in **1** as a rare 3′-deoxyfructopyranose pattern. This means that a six–six member dioxaspirocycle moiety bear nitrogen was formed. Hereto, although the planar structure of **1** was once mentioned,[8] the relative and absolute configuration of it was not indicated. So, in our study, the nuclear Overhauser effect spectroscopy (NOESY) experiment showed the correlations of H-1′a at δ_H 4.49 (d, 14.0) with H-3′a at δ_H 2.09 (dd, 14.5, 3.5), of H-4′ with H-5′ and H-3′a, of H-6′b at δ_H 3.76 (1H, dd, 10.5, 10.5) with H-6b at δ_H 4.86 (d, 16.0) (Fig. 3), which confirmed that the relative configuration of the sugar moiety is α-D-3′-deoxyfructopyranose pattern. In order to clearly indicate the correlation of NOESY experiment, a 3D structure of **1** was generated by computer modeling using the program Chem 3D pro 11.0 (Fig. 3). Furthermore, absolute configuration of **1** was established by single-crystal X-ray analysis using Flack parameter method[9] (Fig. 4). Thus, compound **1** was elucidated as depicted and named pollenopyrroside A (Fig. 1).

Compound **2** was obtained as colorless crystal. It possessed the molecular formula $C_{12}H_{15}NO_5$, identical with **1**, as revealed by positive HR-ESI-MS at *m/z* 276.0842 ($[M+Na]^+$). The same degrees of unsaturation as **1** suggested the presence of a tircyclic carbon skeleton in **2**. Comparing the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1**, the primary difference was for the proton and carbon resonances of the deoxyhexose moiety. Similar NMR data to **1** also suggested the existence of an oxazine ring fused by 5-oxymethy1-2-formyl-pyrrole fragment and deoxyhexose in **2**. These were confirmed by HMBC correlations (Fig. 2) of H-1′ with C-2′ and C-5, H-6 with C-5, C-4, and C-2′. On the other hand, the downfield shift of C-2′ at δ_C 104.0 ($\Delta\delta$ 9 ppm), C-3′ at δ_C 45.8 ($\Delta\delta$ 7.4 ppm), C-4′ at δ_C 72.1 ($\Delta\delta$ 4.5 ppm), C-5′ at δ_C 89.5 ($\Delta\delta$ 22.6 ppm), C-6′ at δ_C 62.7 ($\Delta\delta$ 1.8 ppm) along with the upfield shift of C-1′ at δ_C 51.7 ($\Delta\delta$ −0.6 ppm) in **2** implied that sugar moiety was a five-six member dioxaspirocycle in **2**, instead of six–six member dioxaspirocycle in **1**. This assignment was in accordance with the observation of HMBC spectrum. In the HMBC spectrum, correlations of H-3′ with C-2′, C-1′,C-4′ and C-5′, H-4′ with C-2′, C-5′ and C-6′, H-5′ with C-6′, H-6′ with C-2′, suggested that deoxyhexose in **2** existed in a 3′-deoxyfructofuranose form. In the NOESY experiment, the correlations of H-1′b at δ_H 4.20 with H-3′a at δ_H 2.42 and H-3′b at δ_H 2.15, of H-1′a at δ_H 4.54 with H-3′a at δ_H 2.42, of H-4′ with H-6′, of H-5′ with H-6b at δ_H 5.01. These suggested that the sugar moiety existed in **2** as α-D-3′-deoxyfructofuranose form. Thus, compound **2** was elucidated as showed and named pollenopyrroside B (Fig. 1).

Pollenopyrroside A and pollenopyrroside B represent a novel carbon skeleton with a five–six and six–six member dioxaspirocycle, respectively. To our knowledge, 3-deoxy-D-fructose is not available from natural sources, only a few reports can be found for the synthesis of 3-deoxyhexose, such as 3-deoxy-D-fructose.[10,11] So, the structures of two com-

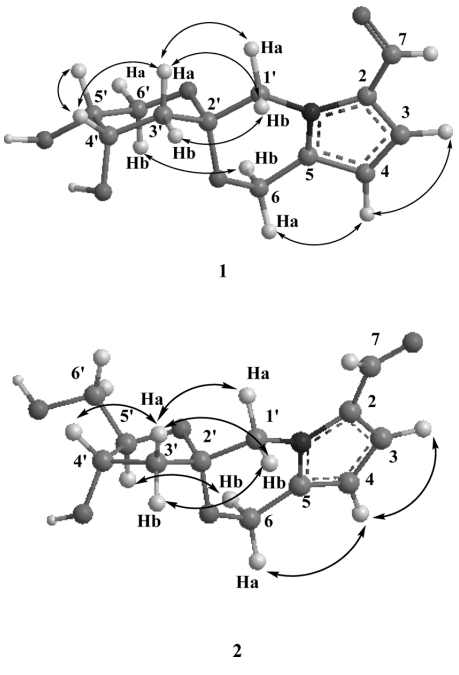

Fig. 3. Key NOESY Correlations for Compounds **1** and **2**

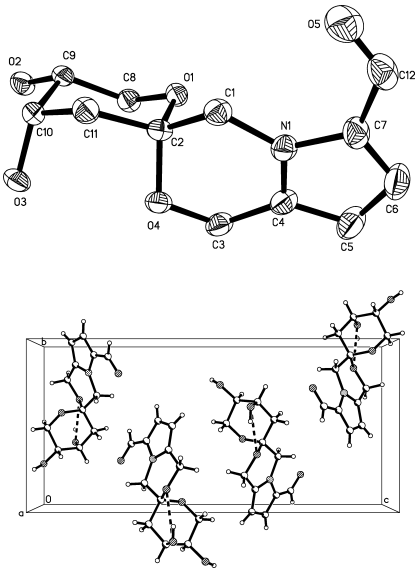

Fig. 4. X-Ray Crystallographic Structure of Compound **1**

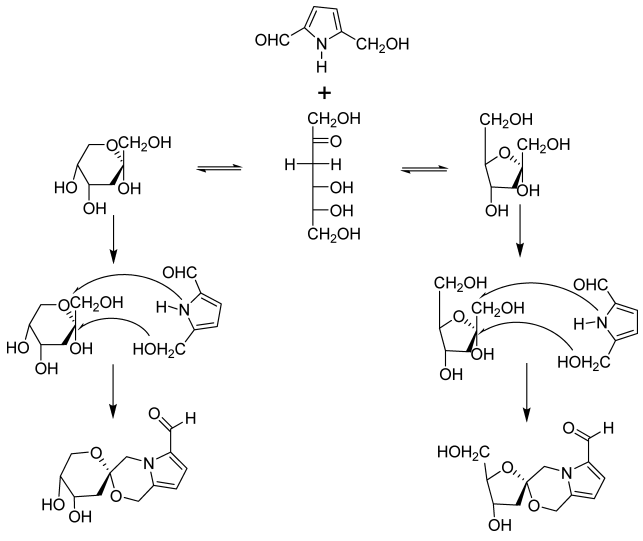

Chart 1. Proposed Biogenesis of Compound **1** and Compound **2**

pounds will enrich the structural diversity of nature product. In addition, from a biosynthetic pathway perspective, it was possible that pollenopyrrosides A and B were formed by a series of reaction of 3-deoxy-D-fructose and 5-oxymethy1-2-formyl-pyrrole as showed in Chart 1.

Bioassay experiments using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[12] revealed that compounds **1** and **2** exhibited no cytotoxicity against A549, Bel7420, BGC-823, HCT-8, A2780 at 10 μg/ml.

Experimental

Plant Material Bee-collected *Brassica campestris* pollen was collected in Zhao county of Hebei province, China, in September 2007 and identified by Dr. Zhang Zhiwu, College of Food Science and Engineering, Inner Mongolia Agricultural University. A voucher specimen has been deposited in the Department of Nature Product Chemistry, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, P. R. China.

General Experimental Procedures Melting points were determined on a Reichert Nr-229 micromelting point apparatus and uncorrected. The optical rotations were measured on a Perkin-Elmer 241 digital polarmeter in CH_3OH. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), heteronuclear single quantum coherence (HSQC), HMBC, NOESY spectra were obtained on a INOVA-500 spectrometers with TMS as internal standard and values were given in ppm (δ). HR-mass spectra were performed on VG-Autospec-300 mass spectrometer. A single crystal was mounted on a Japan MAC DIP-2030K area detector diffraction meter (Tokyo, Japan). Silica gel (160—200, 200—300 mesh) (Qingdao, China) was utilized for column chromatography, and precoated Silica gel plates were used for preparative TLC. Sephadex LH-20 was used for compound purification.

Extraction and Isolation Bee-collected *Brassica campestris* pollen (15 kg) extracted under reflux conditions with 95% EtOH (8 l×3×2 h each). The combined ethanolic extracts were evaporated to near dryness under vacuum and the resulting mixture 8.48 kg was suspended in H_2O and participated successively with petroleum ether (1.5 l×3), EtOAc (1.5 l×3) and *n*-BuOH (1.5 l×3), giving petroleum ether (2 kg), EtOAc (180 g) and *n*-BuOH (540 g). The EtOAc fraction was column chromatographed on a silica gel column, eluting with petroleum ether–$(CH_3)_2CO$ (100 : 1, 50 : 1, 30 : 1, 20 : 1, 10 : 1, 5 : 1, 3 : 1, 1 : 1, 1 : 2, 1 : 3) and $(CH_3)_2CO$ to yield 1—6 fraction, which were combined on the basis of TLC. Fraction 4 was rechromatographed over silica gel column, eluted with petroleum ether–EtOHAc, to afford six subfractions 4a—f. Subfraction 4d was chromatographed on sephadex LH-20, to afford compound **1** (6 mg). Subfraction 4c was chromatographed on sephadex LH-20, to afford compound **2** (5 mg).

Pollenopyrroside A (**1**): Colorless crystal. mp 188—189 °C. $[\alpha]_D^{20}$ +125.9 (c=0.08, MeOH). UV λ_{max}: 300 nm. ESI-MS *m/z*: 505 $[2M-H]^+$, 529 $[2M+Na]^+$. ESI-MS, *m/z*: $[M+H]^+$ 254.1. ^{1}H- and ^{13}C-NMR: see Table 1.

Single Crystal X-Ray Diffraction of 1 Colourless crystal of dimensions 0.10×0.10×0.3 mm was used for X-ray diffraction on a MAC DIP-2030K diffractometer with Cu$K\alpha$ radiation and graphite monochromator by maximum 2θ value of 114.0°. The total number of independent reflections was 1576, of which 1559 were observed ($|F|^2 \geq 2\sigma|F|^2$). Crystal data: molecular formula ($C_{12}H_{15}NO_5$, MW=253.25), orthorhombic system, space group: $P2_12_12_1$ a=5.3566 (1), b=10.1039 (2), c=21.6822 (5) Å, V=1173.50 (4) Å^3, Z=4, d=1.433 g/cm^3. Flack parameter was 0.06 (2). The structure was solved by the direct method (Shelxs 97) and expanded using difference Fourier techniques, refined by the full-matrix least-squares method (NOMCSDP14). Hydrogen atoms were fixed at calculated positions. The final indices were R_1=0.0231, wR_2=0.0590, S=1.061.

Table 1. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR Data for Compounds **1** and **2** (CD_3COCD_3)[a]

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2		132.2		132.2
3	6.99 (d, 4.0)	124.1	6.99 (d, 4.0)	124.2
4	6.08 (d, 4.0)	105.3	6.07 (d, 4.0)	105.3
5		135.1		135.9
6a	4.93 (d, 16.0)	58.1	4.85 (d, 15.5)	58.2
6b	4.86 (d, 16.0)		5.01 (d, 15.5)	
7	9.48 (s)	179.1	9.49 (s)	179.0
1′a	4.49 (d, 14.0)	52.3	4.54 (d, 14.0)	51.7
1′b	4.03 (d, 14.0)		4.20 (d, 14.0)	
2′		95.0		104.0
3′a	2.09 (dd, 14.5, 3.5)	38.4	2.42 (dd, 13.5, 8.5)	45.8
3′b	2.24 (dd, 14.5, 3.5)		2.15 (dd, 13.5, 2.5)	
4′	4.05 (m)	67.6	4.36 (m)	72.1
5′	3.71 (m)	66.9	4.10 (m)	89.5
6′a	3.53 (m)	60.9	3.71 (m)	62.7
6′b	3.76 (dd, 10.5, 10.5)		3.64 (m)	

a) Chemical shifts (δ) are in ppm, and J is Hz.

Pollenopyrroside B (**2**): Colorless crystal. mp 159—160 °C. $[\alpha]_D^{20}$ +242.7 (c=0.08, MeOH). UV λ_{max}: 300 nm. ESI-MS *m/z*: 254 $[M+H]^+$, 276 $[M+Na]^+$. HR-ESI-MS *m/z*: 276.0842 $[M+Na]^+$ (Calcd for $C_{12}H_{15}NO_5Na$, 276.0842). ^{1}H- and ^{13}C-NMR: see Table 1.

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