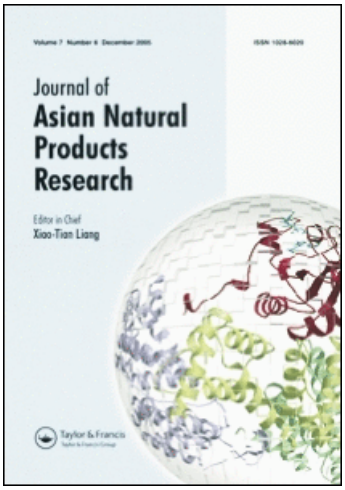

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New prenylchalcones from the hops of *Humulus lupulus*

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Two new prenylchalcones, xanthohumol C and D, together with two known prenylchalcone derivatives, xanthohumol (**1**) and 5″-(2″-hydroxyisopropyl)-dihydrofurano-[2″,3″-b]-4,4′-dihydroxy-6′-methoxy-chalcone (**4**) were isolated and identified from the hops of *Humulus lupulus* L. The structures of xanhohumol C and D were elucidated as 5″-hydroxy-6″,6″-dimethyl-dihydropyrano-[2″,3″-b]-4,4′-dihydroxy-6′-methoxy-chalcone (**2**) and 5″-hydroxy-6″,6″-dimethyl-dihydropyrano-[2″,3″-b]-4′,6′-dimethoxy-4-hydroxy-chalcone (**3**) on the basis of HRFAB–MS, 1D and 2D NMR (HMQC, HMBC) spectroscopic data. Among the new prenylchalcones, compound **2** showed marginal cytotoxic activity against human stomach carcinoma BGC-823 and human hepatic carcinoma HepG2 cells.

Keywords: *Humulus lupulus*; cannabinaceae; hops; prenylchalcones; cytotoxicity

1. Introduction

Humulus lupulus L. is cultivated widely throughout the world. Since the 14th century, hops, which comprise the female influorescences of *H. lupulus*, has been added into beers to give its flavour, aroma, bitterness and stability.[1] Numerous flavonoid glycosides were found in the water-soluble part of the hops,[2] while the weakly polar part of the hops contains prenylated flavonoids, such as prenyl dihydroflavonoid and prenylchalcones,[3] among which 6-prenylnaringenin, 8-prenylnaringenin and xanthohumol are potent phytoestrogens.[4,5] It has also been reported that xanthohumol, the principal prenylchalcone in hops,[6,7] showed cytotoxic effects on some human cancer cell lines.[8] Thus, one can drink prenylflavonoids through the consumption of beer.[9] In China, the hops are also a traditional herbal medicine for the treatment of anorexia, inflammation, pulmonary tuberculosis and insomnia.[10] The hops studied by us were collected in the primary producing area of hops in China. In this paper we report the isolation and identification of two new prenylchalcones, xanthohumol C (**2**) and xanthohumol D (**3**), along with known compounds xanthohumol (**1**) and 5″-(2″-hydroxyisopropyl)-dihydrofurano-[2″,3″-b]-4,4′-dihydroxy-6′-methoxy-chalcone (**4**) [11]. Cytotoxic activities of the new compounds against human stomach carcinoma BGC-823 and human hepatic carcinoma HepG2 cells were studied.

2. Results and discussion

From the petroleum ether (60–90°C) part of the ethanolic extract of the hops, two new chalcones, together with two known chalcones, were isolated and identified.

HRFAB–MS measurement of **2** gave a $[M + H]^+$ at *m/z* 371.1529. The molecular formula was determined as $C_{21}H_{22}O_6$, showing

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Table 1. ^{1}H NMR spectral data of compounds **2**, **3** and **4**[†] (400 MHz, DMSO-d_6, δ in ppm, TMS).

H	2	3	4
α	6.64 (d, 16.0)	6.69 (d, 16.0)	6.97 (d, 16.0)
β	7.02 (d, 16.0)	7.07 (d, 16.0)	7.26 (d, 16.0)
2, 6	7.39 (d, 8.0)	7.44 (d, 8.0)	7.45 (d, 8.8)
3, 5	6.74 (d, 8.0)	6.78 (d, 8.0)	6.75 (d, 8.8)
5′	6.13 (s)	6.29 (s)	5.95 (s)
4″	2.30 (dd, 15.8, 7.8)	2.35 (dd, 16.2, 8.8)/	2.90 (dd, 16.0, 9.0)/
	2.75 (dd, 15.8, 5.6)	2.72 (dd, 16.2, 5.6)	2.93 (dd, 16.0, 9.0)
5″	3.56 (dd, 7.8, 5.6)	3.57 (dd, 8.8, 5.6)	4.52 (t, 9.0)
6″-Me	1.14 (s)	1.14 (s)	1.07 (s)
6″-Me	1.04 (s)	1.04 (s)	1.06 (s)
4′-OMe	–	3.70 (s)	–
6′-OMe	3.57 (s)	3.84 (s)	3.62
2′-OH	–	–	–

[†] Signal multiplicity and coupling constants, *J* value (Hz) in parentheses.

11 degrees of unsaturation, which was in good agreement with the information (9 × C, 8 × CH, 1 × CH_2 and 3 × CH_3) obtained from ^{13}C NMR and DEPT spectra. The ^{1}H NMR spectrum of **1** showed the *trans* double bond protons at δ 7.78 (d, $J = 16.0$ Hz, 1H) and 7.80 (d, $J = 16.0$ Hz, 1H), the aromatic protons at δ 6.83 (d, $J = 8.6$ Hz, 2H), 7.55 (d, $J = 8.6$ Hz, 2H) and 6.09 (s, 1H), and the prenyl protons at δ 3.13 (d, $J = 6.8$ Hz, 1H), 3.37 (brs, 1H), 5.13 (t, $J = 6.0$ Hz, 1H), 1.69 (s, 3H) and 1.60 (s, 3H). Compared with the above data, ^{1}H NMR spectrum of compound **2** (Tables 1 and 2) indicated that it has a similar skeleton to **1**.[6,12] However, in ^{13}C NMR spectrum of **2**, instead of two prenyl double bond carbons in xanthohumol, a quaternary carbon at δ 77.1 and an oxygenated CH at δ 67.9 appeared. Moreover, ^{1}H NMR spectrum of **2** also showed a corresponding oxygenated proton at δ 3.56 (dd, $J = 7.8$, 5.6 Hz, 1H), instead of an olefinic proton at δ 5.13 (t, $J = 6.0$ Hz, 1H) in the prenyl of xanthohumol. Furthermore, two methyl signals of **2** appeared at a higher field than those of xanthohumol in the ^{1}H NMR spectrum. Thus, compared with xanthohumol, compound **2** has no double bond. On the basis of the unchanged unsaturated degree, a ring adjacent to ring A of **2** should exist. Actually, ^{1}H NMR and ^{13}C NMR spectra of this compound have almost the same externality as those of xanthohumol B.[12] (Figure 1), except that some proton and carbon signals in **2** differed from the corresponding signals in xanthohumol B. Among the three oxygenated benzene carbons in ring A of **2**, the bridged one appeared in the highest field δ 151.4 (C-2′) (Table 2), and HMBC spectrum showed cross peaks between the proton at δ

Table 2. ^{13}C NMR and DEPT spectral data of compounds **2**, **3** and **4** (100 MHz, DMSO-d_6, δ, ppm).

	2	3	4
C=O	193.3 s	189.5 s	189.2 s
α	125.2 d	125.4 d	125.4 d
β	143.7 d	143.0 d	141.9 d
1	126.0 s	126.3 s	125.6 s
2, 6	130.2 d	130.1 d	130.1 d
3, 5	116.1 d	115.9 d	115.9 d
4	160.4 s	160.0 s	160.0 s
1′	109.3 s	109.0 s	105.2 s
2′	151.4 s	151.1 s	156.2 s
3′	100.4 s	100.2 s	105.2 s
4′	157.1 s	158.3 s	160.3 s
5′	91.5 d	92.5 d	92.1 d
6′	156.0 s	156.3 s	158.8 s
4″	26.1 t	26.5 t	26.2 t
5″	67.9 d	67.3 d	90.1 d
6″	77.1 s	77.5 s	70.1 s
6″-Me	25.6 q	25.4 q	26.2 q
6″-Me	19.9 q	20.7 q	24.5 q
4′-OMe	–	55.8 q	–
6′-OMe	55.4 q	55.8 q	55.7 q

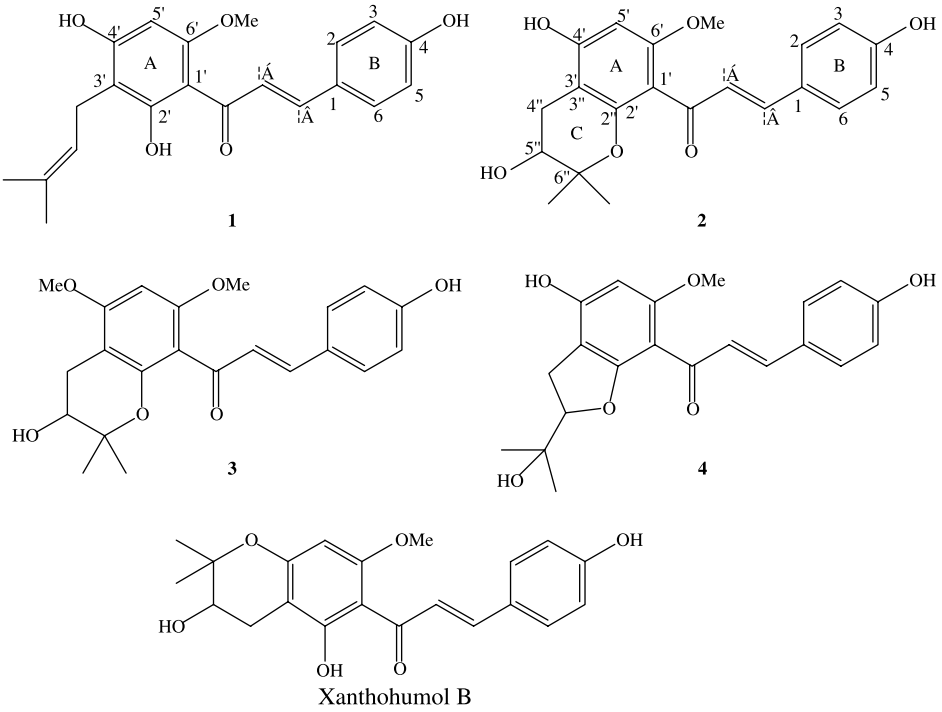

Figure 1. Structures of compounds **1–4**.

6.13 (H-5′) and the two lower field oxygenated benzene carbons at δ 157.1 (C-4′) and 156.0 (C-6′), respectively (Figure 2). Only in the structure of **2** could such correlation peaks be observed, suggesting a different location of the pyrano ring in compound **2**. Finally, the relative configuration of **2** was analysed. The coupling constant values of 5″-proton at δ 3.56 (dd, 2H) were 7.8 and 5.6 Hz, showing a/a and a/e couplings between H-4″ and H-5″, thus, 5″-OH of this compound was assumed to be in an equatorial position on a half chair ring, which is the preferred conformation of benzopyrans.[13,14] Conclusively, **2** was elucidated as 5″-hydroxy-6″,6″-dimethyl-dihydropyrano-[2″,3″-b]-4,4′-dihydroxy-6′-methoxy-chalcone. Due to structural similarities between xanthohumol B and **2**, compound **2** was called xanthohumol C.

Compound **3** has almost the same NMR spectra as those of **2**. The only difference between them was the presence of a second methoxy in both ^{1}H NMR and ^{13}C NMR spectra of **3** (Tables 1 and 2). $[M + H]^{+}$ at *m/z*

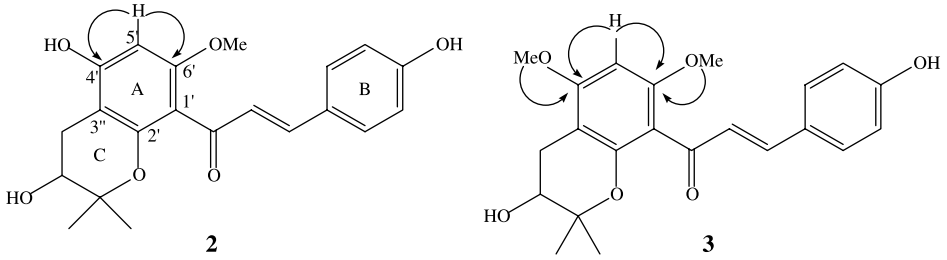

Figure 2. Key HMBC correlations of compounds **2** and **3**.

Table 3. The cytotoxic activities of compounds **2**, **3**, and **4** against BGC-823 and HepG2[†] (IC_{50}, μg/ml).

Cells	**2**	**3**	**4**	Cisplatin
BGC-823	134 ± 28.2	691 ± 110.2	673 ± 108.5	2.2 ± 0.1
HepG2	155 ± 27.3	–[‡]	301 ± 58.6	3.9 ± 0.1

[†] BGC-823 cell = human stomach carcinoma, HepG2 cell = human hepatic carcinoma.
[‡] In the test, all the chosen concentration of **3** showed no inhibition, except for the highest concentration, so IC_{50} could not be calculated.

385.1646 in HRFAB–MS of **3** indicated the corresponding molecular formula $C_{22}H_{24}O_6$, with 11 unsaturated degrees. Careful comparison with **2** led to the conclusion that the second methoxy in **3** should be attached on C-4′ of A ring. The conclusion was supported by cross peaks of both protons at δ 3.70 (s, 3H) and 6.29 (s, 1H) with the carbon at δ 158.3 (C-4′), and the cross peak between the proton at δ 3.84 (s, 3H) and the carbon at δ 156.3 (C-6′) in HMBC spectrum of **3** (Figure 2). The relative stereochemistry of 5″-OH was also assumed to be in an equatorial position in a half chair conformation of benzopyran ring, according to the coupling constants of 5″ and 4″-protons [13,14] (Table 1). Therefore, this prenylchalcone was determined as 5″-hydroxy-6″,6″-dimethyl-dihydropyrano-[2″,3″-b]-4′,6′-dimethoxy-4-hydroxy-chalcone, which was called xanthohumol D.

Cytotoxic activities of compounds **2**, **3** and **4** against human stomach carcinoma BGC-823 and human hepatic carcinoma HepG2 cells were evaluated. IC_{50} (μg/ml) values of each compound and positive control against both cancer cells are listed in Table 3, showing that compound **2**, compared with compounds **3** and **4**, inhibited two cancer cells weakly.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yanaco MP-500 micro melting point apparatus and given without correction. Optical rotations were measured with a Perkin–Elmer 241 polarimeter in methanol solution. UV spectra were measured on a Shimadzu UV-240 instrument. IR spectra were recorded on a Nicolet MAGNA-IR 750 spectrophotometer in methanol. ^{1}H NMR, ^{13}C NMR and 2D NMR (HMQC, HMBC) were recorded in DMSO-d_6 on a Bruker AM 400 FT-NMR spectrometer with TMS as internal standard. HRFAB–MS data were obtained using an Autospec-Utima ETOF Spec mass spectrometer. Silica gel GF_{254} for TLC and silica gel (200–300 mesh) for column chromatography were purchased from Qingdao Marine Chemical Co., Qingdao, China. Solvents and chemicals were of analytical grade and purchased from Beijing Chemical Co., Beijing, China. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.2 Plant material

Hops of *Humulus lupulus* L. were collected in Bayingguoleng, Mongolia Autonomous, Xinjiang Uighur Autonomous Region of China in August 2002. It was identified by Dr Reile Pan, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (PJH020816) has been preserved in Bescholar Research Centre, Peking University, Beijing, China.

3.3 Extraction and isolation

Dried hops (5000 g) were percolated with 85% ethanol (40,000 ml) for 24 h. The percolate was concentrated under reduced pressure to give an ethanolic solution (10,000 ml), which was extracted by petroleum ether (10,000 ml). Evaporation of petroleum ether afforded a deep green residue (240 g). Half of the residue (120 g) was

subjected to column chromatography (CC) on silica gel (6.0 × 120 cm, 1600 g, 200–300 mesh) and eluted with a mixture of petroleum ether (PE 60–90°C) and acetone (petroleum ether/acetone (v/v) 30:1, 25:1, 20:1, 15:1, 10:1, 8:1, 5:1, 3:1, 2:1, 1:1, and 0:1), 500 ml each eluant. According to TLC detection, 12 crude fractions A–L were collected. Fraction I (2.0 g) was subjected to CC on silica gel (2.6 × 60 cm, 60 g) and eluted with petroleum ether (60–90°C)/EtOAc (6:1) (450 ml) to yield crude **3**, which was then purified by preparative TLC twice [GF_{254}, petroleum ether/EtOAc (2:1), 60 ml; then $CHCl_3$/acetone (3:1), 60 ml] to obtain compound **3** (6.5 mg, $R_f = 0.70$). Fraction J (5.2 g) was purified by silica gel column (3.0 × 60 cm, 120 g) and eluted with petroleum ether/EtOAc (3:1, 1000 ml) to afford **1** (200 mg, $R_f = 0.50$). Fraction K (7.0 g) was separated on silica gel column (3.8 × 80 cm, 160 g), eluted with $CHCl_3$/acetone (4:1) repeatedly to afford crude **2** (750 ml) and **4** (1200 ml), respectively. Both of them were then purified on preparative TLC (GF_{254}) by $CHCl_3$/acetone (3:1), respectively, three times, (60 ml for each time) to afford **2** (12.0 mg, $R_f = 0.33$) and **4** (10.0 mg, $R_f = 0.31$).

*3.3.1 5″-Hydroxy-6″,6″-dimethyl-dihydropyrano-[2″,3″-b]-4,4′-dihydroxy-6′-methoxychalcone (**2**)*

Yellow oil. $[\alpha]_D^{25} + 11.76$ (*c* 0.225, MeOH); UV (MeOH) λ_{max}(log ε): 215 (3.40), 238 (3.30), 290 (3.01), 340 (3.26), 360 (3.51) nm; IR (MeOH) ν_{max}: 3361 (OH), 1655, 1600, 1513, 1447, 1370, 979, 833 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data: see tables 1 and 2, respectively. HRFAB–MS *m/z* 371.1529 $[M + H]^+$ (calcd for $C_{21}H_{23}O_6$, 371.1494).

*3.3.2 5″-Hydroxy-6″,6″-dimethyl-dihydropyrano-[2″,3″-b]-4′,6′-dimethoxy-4-hydroxychalcone (**3**)*

Yellow oil. $[\alpha]_D^{25} - 9.4$ (*c* 0.149, MeOH); UV (MeOH) λ_{max}(log ε): 215 (3.38), 239 (3.20), 288 (3.11), 338 (3.37), 360 (3.49) nm; IR (MeOH) ν_{max}: 3360 (OH), 1657, 1632, 1600, 1513, 1468, 1355, 918 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data: see tables 1 and 2, respectively. HRFAB–MS *m/z* 385.1646 $[M + H]^+$ (calcd for $C_{22}H_{25}O_6$, 385.1651).

3.4 Cytotoxicity assays

The cytotoxic assays of prenylchalcones **2**, **3** and **4** were carried out at Bescholar Research Centre, Peking University. The results were evaluated according to MTT method.[15] Human stomach carcinoma BGC-823 and human hepatic carcinoma HepG2 cell lines were used for experiments, compounds **2**, **3**, and **4** were used as test samples, and cisplatin as a positive control.

Cells were seeded into 96 well plates at a density of 10^4 cells per well in growth medium. The plates were incubated at 37°C under the condition of humidified atmosphere containing 5% CO_2. After 24 h, the medium was discarded and test solutions were added. Five wells were used for each concentration and cell controls. After 72 h incubation at 37°C, the medium was removed and 200 μl of MTT solution (0.5 mg MTT dissolved into 1 ml DMEM) were added to each well. After 4 h at 37°C, the supernatant was removed and the formazan product was solubilised by the addition of 200 μl DMSO. The optical density of each well was measured using an automatic plate reader (Multiscan MK3) with the test wavelength of 570 nm. The absorbance was directly proportional to the number of living cells. The cytotoxicity of each compound was expressed as an IC_{50} value, i.e., the concentration in μg/ml that inhibits cell growth by 50% compared with cell controls, and was calculated by linear regression analysis.

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