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PRENYLATED CHALCONES FROM *Humulus lupulus*

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Common hops (*Humulus lupulus*) is an important industrial culture that is grown in practically all countries primarily as a raw material for beer production. Hops cones impart to beer bitterness and flavor and possess antiseptic properties. Its fruit is used as an alalgesic, anti-ulcer, sedative, and soporific agent [1, 2].

Hops contain a large number of biologically active compounds such as bitter and polyphenolic compounds and hops essential oil. Much attention has been focused in the last decade on polyphenols that are prenylated chalcones, which exhibit antioxidant, anticancer, antiviral, and antimicrobial properties [3-5]. Twelve prenylchalcones have been isolated from hops. Of these, xanthohumol, which makes up 80-90% of the total mass of prenylchalcones in hops, is the most valuable for brewing [3].

Our goal was to estimate the quantitative content of the dominant prenylchalcone, xanthohumol, in domestic hops varieties and to determine the effect of industrial processing on its content in hops products.

A standard sample of xanthohumol was produced by preparative separation of the prenylchalcone fraction. The isolated compounds were identified by spectral methods. The prenylchalcone fraction was obtained from granulated hops of the Magnum variety (Germany), which is widely used in Russian breweries.

Magnum hops were extracted successively by solvents with increasing polarity, i.e., hexane:EtOAc:$CHCl_3$:MeOH, in a Soxhlet apparatus. Chromatography of the EtOAc fraction over a column of SiO_2 (grade L, particle size 0.063-0.1 mm) isolated pure compounds xanthohumol (**1**) and xanthohumol D (**2**) in addition to a mixture of xanthohumol C (**3**) and 1″,2″-dihydroxanthohumol C (**4**). The required resolution of the peaks for **3** and **4** was not achieved upon separation of the mixture by HPLC. Compounds **3** and **4** were identified in the isolated mixture of these compounds using HPLC-MS (Fig. 1) and PMR spectra.

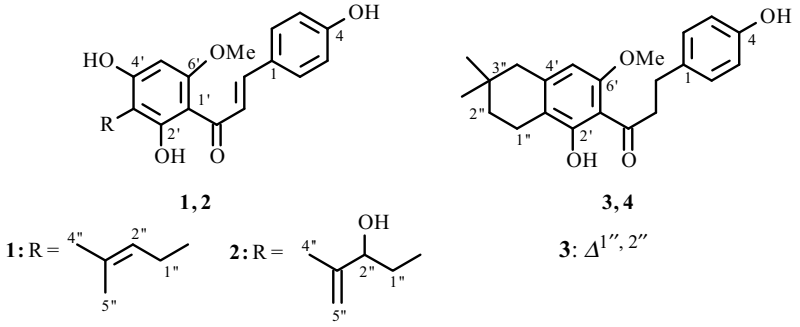

Table 1 lists results from quantitative determination of xanthohumol in cultivated and wild samples. The xanthohumol content in ground pelletized hops from varieties Podvyaznyi and Krylatskii was significantly greater than in pressed cone samples. The amount of xanthohumol in products from variety Sumer′ did not depend on the industrial processing of raw material and was comparable with its concentration in wild plants collected in Irkutsk Oblast′.

Xanthohumol in hops samples was determined quantitatively by extraction in a Soxhlet apparatus successively with hexane and then EtOH. The EtOH fraction was analyzed in a Milikhrom A-02 liquid chromatograph using gradient elution (eluent A, aqueous KH_2PO_4, 0.05 M, pH 3.0; eluent B, MeOH) over a column (2 × 72 mm) packed with ProntoSIL-120-5-C18 sorbent (3,500 theor. plates for chrysene). The flow rate was 200 μL/min; column temperature, 35°C. An external standard was used for the quantitative determination.

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TABLE 1. Xanthohumol Content in Russian Hops Varieties

Sample No.	Hops variety	Xanthohumol content, % of air-dried mass
	Pressed hops cones	
1	Podvyaz	0.06
2	Krylat	0.70
3	Sumer	0.51
4	Wild, Irkutsk Obl.	0.55
	Ground pelletized hops	
5	Podvyaz	0.25
6	Krylat	1.09
7	Sumer	0.57

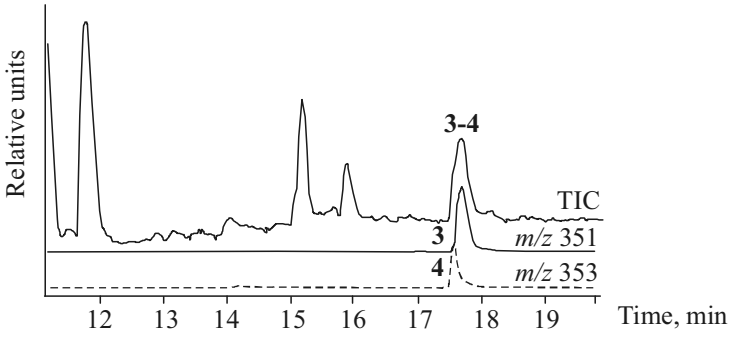

Fig. 1. HPLC-MS chromatogram of fraction enriched in xanthohumol C (**3**) and 1″,2″-dihydroxanthohumol (**4**).

High-resolution mass spectra of **1** and **2** were recorded on a JEOL SX 102A instrument with double focusing reversed geometry.

Mass spectra of fractions enriched in **3** and **4** were obtained in an Agilent GC—MS (HPLC 1200, MS-TOF) by separation of samples over a column (2 × 72 mm) packed with ProntoSIL-120-5-C18 sorbent using gradient elution (eluent A, aqueous NH_4HCO_3, 0.1 M; eluent B, CH_3CN). Detection used negative-ion mode.

NMR spectra were recorded on a Bruker-250 spectrometer (250 MHz) equipped with a computer and XWIN-NMR software. The isolated compounds were dissolved in $CDCl_3$.

Xanthohumol (1): $C_{21}H_{22}O_5$; MS-TOF, *m*/*z*: $[MH]^+$ 355.1549; calcd for $C_{21}H_{22}O_5$, 355.1545.

PMR spectrum (250 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.68 (1H, s, OH-2′), 7.78 (1H, s, H-*β*), 7.77 (1H, s, H-*α*), 7.52 (2H, d, J = 8.07, H-2,6), 6.87 (2H, d, J = 8.07, H-3,5), 6.3 (1H, s, OH-4), 5.96 (1H, s, H-5′), 5.0 (s, OH-4′), 3.91 (3H, s, OCH_3-6′), 3.41 (2H, d, J = 7.01, H-1″*α*,1″*β*), 5.31 (1H, br.t, J = 7.61, H-2″), 1.78 (3H, s, H-4″), 1.84 (3H, s, H-5″).

^{13}C NMR spectrum: 192.94 (C=O), 165.23 (C-2′), 161.93 (C-4′), 161.28 (C-6′), 157.64 (C-4), 142.17 (C-*β*), 135.92 (C-3″), 130.36 (C-2,6), 128.64 (C-1), 125.59 (C-*α*), 121.89 (C-2″), 115.99 (C-3,5), 106.42 (C-3″), 106.29 (C-1′), 91.28 (C-5′), 55.88 (OCH_3-6′), 25.91 (C-5″), 21.70 (C-1″), 18.00 (C-4″).

Xanthohumol D (2): $C_{21}H_{22}O_6$; MS-TOF, *m*/*z*: $[MH]^+$ 371.1494; calcd for $C_{21}H_{22}O_6$, 371.1495.

PMR spectrum (250 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.85 (1H, s, OH-4), 7.65 (1H, d, J = 15.5, H-*β*), 7.63 (1H, d, J = 15.6, H-*α*), 7.37 (2H, d, J = 8.07, H-2,6), 6.73 (2H, d, J = 8.07, H-3,5), 5.91 (1H, s, H-5′), 4.87 (1H, br.s, H-5″*α*), 4.74 (1H, br.s, H-5″*β*), 4.23 (1H, m, H-2″), 3.78 (3H, s, OCH_3-6′), 3.00 (1H, m, H-1″*α*), 2.65 (1H, m, H-1″*β*), 1.87 (3H, s, CH_3-4″).

^{13}C NMR spectrum (from HSQC spectrum): 192.7 (C=O), 146.9 (C-5″*α*), 142.1 (C-*β*), 130.2 (C-2,6), 125.5 (C-*α*), 115.8 (C-3,5), 110.3 (C-5″*β*), 92.7 (C-5′), 77.7 (C-2″), 55.5 (OCH_3-6′), 28.2 (C-1″*β*), 18.2 (C-4″CH_3).

Xanthohumol C (3): $C_{21}H_{20}O_5$; MS-TOF, *m*/*z*: 351 $[M - H]^+$.

PMR spectrum (250 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.59 (1H, s, OH-2′), 7.73 (2H, s, H-*α*,*β*), 7.47 (2H, d, J = 8.6, H-2,6), 6.82 (2H, d, J = 8.6, H-3,5), 6.64 (1H, d, J = 10.0, H-1″), 5.89 (1H, s, H-5′), 5.42 (1H, d, J = 10.0, H-2″), 3.88 (3H, s, OCH_3-6′), 1.21–1.54 (6H, s, $2CH_3$-4″,5″).

1″,2″-Dihydroxanthohumol C (4): $C_{21}H_{22}O_5$; MS-TOF, *m/z*: 353 $[M - H]^+$.

PMR spectrum (250 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.76 (1H, s, OH-2′), 7.73 (2H, s, H-α,β), 7.47 (2H, d, J = 8.6, H-2,6), 6.82 (2H, d, J = 8.6, H-3,5), 5.84 (1H, s, H-5′), 3.85 (3H, s, OCH_3-6′), 2.59 (2H, t, J = 6.8, H-1″), 1.77 (2H, t, J = 6.8, H-2″), 1.21–1.42 (6H, s, $2CH_3$-4″,5″).

Chemical shifts in PMR spectra of all four compounds agreed with the literature values [6]. ^{13}C NMR spectra of xanthohumol D have not been reported.

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