New Chalcones from Hop *Humulus lupulus* L.

Nicole Etteldorf, Nadine Etteldorf and Hans Becker*

Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, D-66041 Saarbrücken, Germany. Fax: +49-681.302-2476. E-mail: pb13hb@rz.uni-sb.de

* Author for correspondence and reprint requests

Z. Naturforsch. **54c,** 610–612 (1999); received March 29/April 27, 1999

Hop, *Humulus lupulus*, Chalcones

Eight known flavonoids were isolated from an ethanol extract of hop cones (*Humulus lupulus* L., Cannabaceae) together with two new hop chalcones. The new compounds were identified as α,β-dihydroxanthohumol and *iso*-dehydrocycloxanthohumol hydrate.

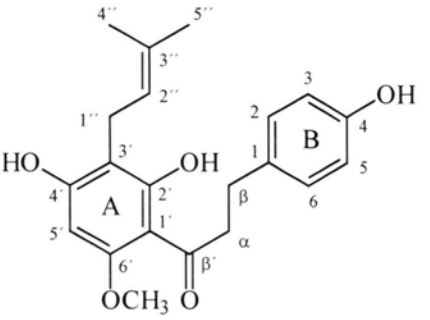
Introduction

Large amounts of hops are used in the brewing industry (Forster, 1992). To a lesser amount hop extracts are used as sedative drugs (Fintelmann, 1992). The main chemical compounds of the extracts are the so called bitter acids and flavonoid derivatives including chalcones (Hölzl 1992, Verzeele and DeKeukeleere, 1991). The flavonoids have recently been described in detail by Stevens *et al.* (1997). Reports on positive health effects of beer and hops (e.g. Arimoto-Kobayashi *et al.*, 1999, Gorinstein *et al.*, 1997, Tabata *et al.*, 1997, Tobe *et al.*, 1997) prompted us to isolate hop constituents for biological tests. In the course of this procedure we isolated two new prenylated chalcones.

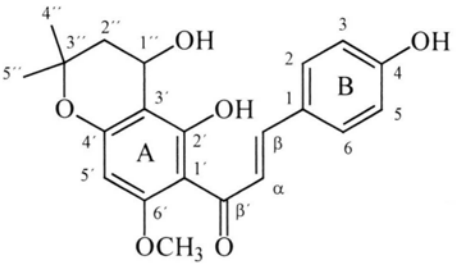
Results and Discussion

A combination of size exclusion chromatography on Sephadex LH 20, vacuum liquid chromatography and HPLC upon silicagel afforded 5.6 mg of **1** and 0.6 mg of **2**.

The ion peak of **1** at m/z=357 $[M+H]^+$ in the CI-mass spectrum corresponded to the molecular formula $C_{21}H_{24}O_5$. The ^1H and ^{13}C NMR (Tables I and II) showed the presence of a prenylated dihydrochalcone with an additional methoxyl group. The doublets at δ_H 7.02 (H-2 and H-6) and δ_H 6.68 (H-3 and H-5) displayed a *para* substitution for the B-ring with a hydroxyl group at C-4 (δ_C 156.5).



α,β-dihydroxanthohumol (**1**)



iso-dehydrocycloxanthohumol hydrate (**2**)

Table I. ^1H-NMR data for compound **1** (CD_3OD) and **2** ($CDCl_3$) [β_H ppm, mult. (J in Hz)].

H	**1**	**2**
H-β	2.82 *t* (8.0)	7.73 *s*
H-α	3.20 *m* (8.0)	7.73 *s*
2,6	7.02 *d* (8.4)	7.49 *d* (8.5)
3,5	6.68 *d* (8.4)	6.84 *d* (8.5)
5′	5.97 *s*	5.99 *s*
1″	3.20 *d* (7.1)	4.73 *dd* (9.5; 8.0)
2″	5.17 *t* (7.1)	3.11 *dd* (15.0; 9.5)
		3.01 *dd* (15.0; 8.0)
4″	1.64 *s*	1.32 *s*
5″	1.74 *s*	1.21 *s*
6′-OCH_3	3.82 *s*	3.90 *s*

The A-ring, which beared only one proton (δ_H 5.97, H-5′), was identified as a phloroglucin moiety by the signals at δ_C 165.7, 163.7 and 162.7 (C-

0939-5075/99/0700-0610 $ 06.00

Table II. ^{13}C NMR data of compound **1** in CD_3OD.

C	β_C
C-β	31.6
C-α	47.3
C-β′	206.2
C-1	134.0
C-2.6	130.2
C-3.5	116.2
C-4	156.5
C-1′	106.0
C-2′	165.7
C-3′	109.4
C-4′	163.7
C-5′	91.5
C-6′	162.7
C-1″	22.2
C-2″	124.3
C-3″	131.4
C-4″	25.9
C-5″	17.8
6′-OCH_3	55.9

Chemical shifts were assigned from HSQC and HMBC.

2′, C-4′and C-6′) in the ^{13}C NMR. The prenyl side chain could be deduced by the signals for a trisubstituted double bond (δ_H 5.17, H-2″; δ_C 124.3 and 131.4; C-2″ and C-3″) two methyl groups attached to the double bond (δ_H 1.64 and 1.74, 3H each, H-4″ and H-5″) and a methylene group (δ_H 3.20, 2H, H-1″). There remained signals for a methoxyl group (δ_H 3.82) and the C-3 moiety (δ_C 206.2, C-β′; δ_H 3.20, H-α; δ_H 2.82, H-β) between the two aromatic rings of the dihydrochalcone.

The 3′-C position of the prenyl group was concluded from the correlation between H-1″ and C-2′, C-3′and C-4′ in the HMBC spectrum. The methoxyl group showed a correlation with C-6′ in the HMBC spectrum and with H-5′ in the NOESY spectrum. Therefore the methoxyl substituent was located at C-6′. Because of the close structural relationship of **1** to xanthohumol we named **1** α,β-dihydroxanthohumol.

Verzele *et al.* (1957) reported a semi-synthetic product called dihydroxanthohumol B. Dihydroxyxanthohumol B is obtained when xanthohumol is reduced with zinc in acetic acid. This product is identical to the natural chalcone **1.**

Compound **2**, *iso*-dehydrocycloxanthohumol hydrate, was only obtained in small amounts (0.6 mg). Its structure could be elucidated on the basis of the ^1H-NMR data (tab. I), which were close to those of dehydrocycloxanthohumol hydrate (Stevens *et al.*1997). Differences exist only for the resonances of H-1″ (δ_H 4.73) and H-2″(δ_H 3.11 and 3.01) of the former prenyl side chain indicating that in **2** the hydroxyl function is located at C-1″ and not at C-2″ as found for dehydrocycloxanthohumol hydrate.

Experimental

A commercial ethanolic hop extract (Hallertauer Northern Brewer) was obtained from Karlsberg Brauerei, Homburg. 30 g of the extract were dissolved in $MeOH/CH_2Cl_2$ (1:1 v/v) and chromatographed on Sephadex LH 20 with $MeOH/CH_2Cl_2$ (1:1 v/v) and (4:1 v/v) as eluent. Fractions of 15 ml were collected and monitored by silica gel TLC. The flavonoid containing fractions were combined and evaporated. The residue was fractionated on silica gel via vacuum liquid chromatography with a hexane/EtOAc gradient. The 18% EtOAc fraction contained compound **1**, the 35% EtOAc fraction contained compound **2**. The respective fractions were further purified by HPLC on LiChrospher silicagel with n-hexane-EtOAc-HCOOH (70:3:2 v/v) for **1**, and 60:40:1 for **2**. *NMR:* ^1H-(400 or 500 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AM 400 NMR-spectrometer or on a Bruker DRX 500 NMR-spectrometer in $CDCl_3$ or CD_3OD. The chemical shifts are given in δ (ppm) with the solvent ($CDCl_3$ or CD_3OD) as internal standard.

Arimoto-Kobayashi S., Sugiyama C., Harada N., Takeuchi M., Takemura M., and Hayatsu H. (1999), Inhibitory effects of beer and other alcoholic beverages on mutagenesis and DNA adduct formation induced by several carcinogens. J. Agric. Food Chem. **47**, 221–230.

Fintelmann V. (1992), Klinisch-ärztliche Bedeutung des Hopfens. Z. Phytotherapie **13**, 165–168.

Forster A. (1992), Großtechnische Verfahren zur Extraktion von Hopfen. Z. Phytotherapie **13**, 162–164.

Gorinstein S., Zemser M., Lichman I., Berebi A., Kleipfish A., Libman I., Trakhtenberg S., and Caspi A. (1997), Moderate beer consumption and the blood coagulation in patients with coronary artery disease. J. Intern. Med. **241**, 47–51.

Hölzl J. (1992) Inhaltsstoffe des Hopfens (*Humulus lupulus* L.). Z. Phytotherapie **13**, 155–161.

Stevens J. F., Ivancic M., Hsu V. L., and Deinzer M. L. (1997) Prenylflavonoids from *Humulus lupulus*. Phytochemistry **44**, 1575–1585.

Tabata N., Ito M., Tomoda H., and Omura S. (1997), Xanthohumols, diacyclglycerol acetyltransferase inhibitors, from *Humulus lupulus*. Phytochemistry **46**, 683–687.

Tobe H., Muraki Y., Kitamura K., Komiyama O., Sato Y., Sugioka T., Maruyama H. B., Matsuda E., and Nagai M. (1997), Bone resorption inhibitors from hop extract. Biosci. Biotech. Biochem. **61**, 158–159.

Verzele D., and DeKeueleire D. (1991), Chemistry, and Analysis of Hop and Beer Bitter Acids. Elsevier, Amsterdam.

Verzele D., Stockx J., Fontijn F., and Anteunis M., (1957), Xanthomumol, a new natural chalcone. Bull. Soc. Chim. Belg. **66**, 462–464.