Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 1206–1211
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

# Resolution of diastereomeric flavonoid (1*S*)-(−)-camphanic acid esters via reversed-phase HPLC

Casey S. Philbin, Steven J. Schwartz *

*Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Columbus, OH 43210, USA*

Received 18 October 2006; received in revised form 21 December 2006
Available online 23 March 2007

**Abstract**

Prenylflavonoids are an unique class of phytochemicals found in the inflorescences of the hop plant (*Humulus lupulus*). These flavonoids have demonstrated a wide range of biological activities, which may be influenced by their stereochemical configuration. Additionally, recent studies suggest that hop prenylflavonoids are subject to biotransformations which could alter or enrich their stereochemistry. In order to facilitate studies of the stereoisomers of flavanones, a facile method was developed for resolving the diastereomeric esters of flavanones via reversed-phase HPLC. Herein, a method for forming the tri-(1*S*)-(−)-camphanic acid esters of the 4′,5,7-trihydroxy flavanones naringenin, 8-prenylnaringenin and 6-prenylnaringenin, is described. The respective diastereomers were separated using analytical reversed-phase HPLC. Diastereomeric esters were isolated by preparative HPLC to >98% d.e. based on HPLC, with their absolute configurations established by application of CD spectrometry.


*Keywords:* *Humulus lupulus*; Cannabaceae; Hops; High performance liquid chromatography; Chiral resolution; Naringenin; 8-Prenylnaringenin; 6-Prenylnaringenin; Prenylflavonoids; Camphanic acid chloride

## 1. Introduction

Prenylflavonoids represent a unique class of phytochemicals which can be found in the vine *Humulus lupulus*, more commonly known as hops, and have demonstrated a wide range of biological activities (Gerhauser, 2005; Stevens and Page, 2004). Hops are traditionally used in beer brewing, and hop prenylflavonoids are generally found in beer as flavanones (Fig. 1), formed by isomerization of their corresponding chalcones during boiling: the most abundant prenylflavanone in beer is isoxanthohumol (**1**), followed by 6-prenylnaringenin (**2**) and 8-prenylnaringenin (**3**) (Stevens et al., 1999).

Of the most abundant flavanones found in beer, **3** has recently received a great deal of interest as a potent phytoestrogen (Milligan et al., 1999, 2000, 2002; Schaefer et al., 2003). Milligan et al. (2002) found that both enantiomers had comparable binding affinities for the two isoforms of human recombinant estrogen receptors ERα and ERβ, although (*S*)-**3** showed slightly higher affinity for ERβ than (*R*)-**3**. In contrast, Schaefer et al. (2003) found racemic **3** to be selective for human recombinant ERβ, and (*S*)-**3** to show moderately higher affinity for both receptors in a competitive binding assay.

Although **3** consumed directly from hops or beer is likely to be found as a racemic mixture (Stevens and Page, 2004), recent research suggests that other compounds in hops and beer may act as precursors to **3** *in vivo*. The major prenylflavonoid in beer, **1**, may be *O*-demethylated to **3** by endogenous microflora in the gut. This conversion has been observed *in vitro* using fecal cultures (Possemiers et al., 2005), and *in vivo* as demonstrated by the presence of **3** in human urine samples after oral administration of **1** (Possemiers et al., 2006). In addition, *in vitro* digestion studies using liver microsomes have shown that **1** may undergo *O*-demethylation (Nikolic et al., 2005), catalyzed by CYP1A2 enzymes (Guo et al., 2006).

* Corresponding author. Tel.: +1 614 292 2934; fax: +1 614 292 4233.
*E-mail address:* schwartz.177@osu.edu (S.J. Schwartz).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.01.022

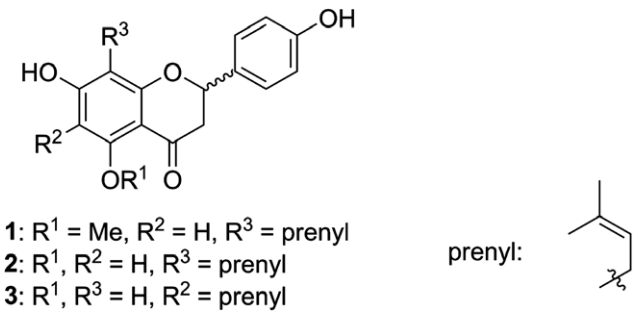
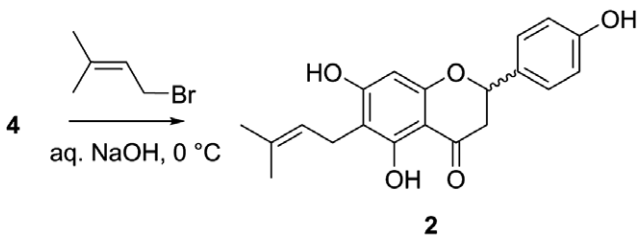



Fig. 1. Structures of the hop flavanones found in hops and beer.

The optical purity of **3** from hops has previously been examined using chiral chromatography, whereby the authors found that approximately equal levels of each enantiomer were present (Milligan et al., 2002). Although chiral chromatography may be used to determine the enantiomeric purity of compounds such as **3** (Kitaoka et al., 1998; Milligan et al., 2002), chiral columns can be costly. In order to overcome this shortcoming, we have developed a facile method for producing the diastereomeric (1*S*)-(−)-camphanic acid esters of racemic naringenin (**4**), a flavanone derived from citrus, **2** and **3**, and subsequently separating them using reversed-phase high performance liquid chromatography (HPLC). Optically pure diastereomers were isolated using preparative chromatography, and the diastereomeric purity and absolute configuration were confirmed by HPLC and CD spectrometry.

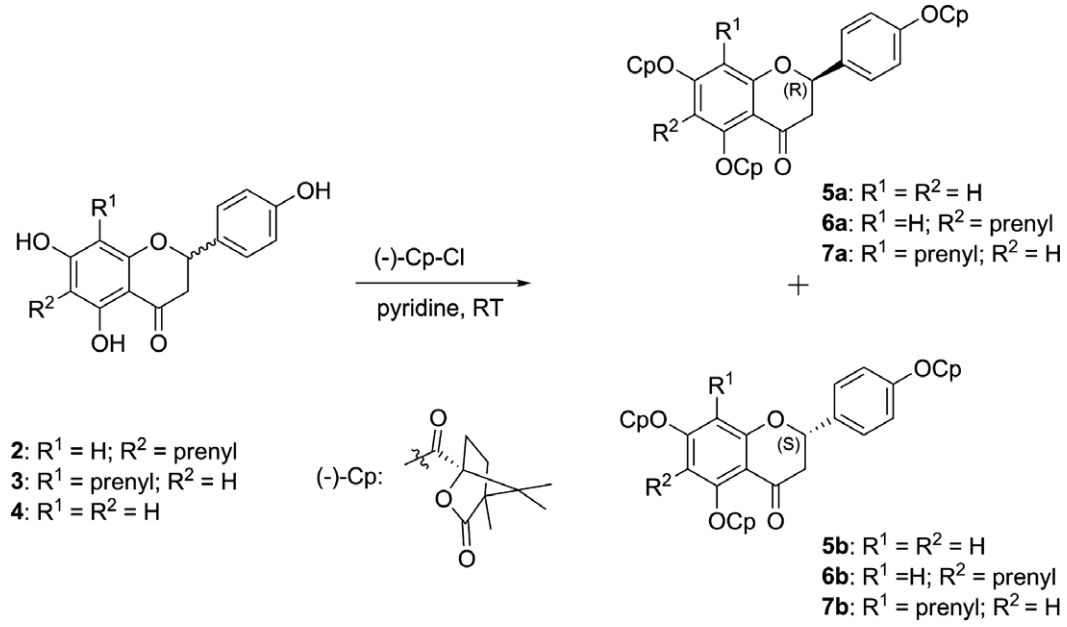



Scheme 1.

## 2. Results and discussion

Racemic **3** was prepared from **4** using previously published methods (Gester et al., 2001). However, in our hands, published procedures for the synthetic preparation of **2** were unsatisfactory. Direct *C*-prenylation of **4** under basic conditions (Mizobuchi and Sato, 1984) and using Lewis acid catalysts (Jain et al., 1978) resulted in poor yields. A total synthesis of **2** has recently been described, but, final yields were still low (Bu et al., 1997). One drawback of direct nuclear prenylation is poor regioselectivity, yielding mixtures of various *C*- and *O*-alkylated products. It has been suggested that strongly hydrogen bonding solvents favor *C*-alkylation of phenoxide ions (Kornblum et al., 1963) due to solvation interactions interfering with the nucleophilic activity of the phenolate anion. By carrying out this reaction under aqueous conditions (Scheme 1), *C*-alkylation was favored, and at low temperatures, **2** was formed as the major product. Although over-alkylation was still apparent under these conditions, yield was satisfactory compared with previously described *C*-prenylation methods (Jain et al., 1978; Mizobuchi and Sato, 1984).

Naringenin (**4**) was used as a model flavonoid in order to optimize the esterification and chromatographic separation of 4′,5,7-trihydroxyflavanones due to its commercial avail-




Scheme 2.

ability and established stereochemical properties (Gaffield, 1970). In order to produce diastereomeric esters that could be separated by column chromatography, (1*S*)-(−)-camphanoyl chloride was chosen as a resolving agent. (1*S*)-(−)-camphanic acid esters have been previously employed in resolving chroman derivatives via crystallization (Esterhuysen et al., 2005; Zehnter and Gerlach, 1995), as well as several different classes of compounds including carotenoids (Vecchi and Muller, 1979), binaphthols (Hua et al., 2001), and helicenols (Thongpanchang et al., 2000) either through crystallization or chromatographic methods. In order to avoid complex mixtures of mono-, di- and tri-esters, **4** was treated with a large excess of (1*S*)-(−)-camphanoyl chloride (Scheme 2), resulting in quantitative conversion to the tri-camphanoate (**5**) based on LCMS analysis and a characteristic hypsochromic shift in the UV spectrum.

Using a tertiary solvent system including aqueous MeOH and MeCN, near-baseline separation of **5a** and **5b** was achieved (Fig. 2a). These methods were applied to the hop prenylflavonoids **2 and 3**, and quantitative conversion to the tri-camphanoyl esters **6** and **7** was observed for all compounds based on HPLC. Due to the non-polar nature of the prenylflavonoids **6** and **7** relative to **5**, the mobile phase was adjusted accordingly to reduce retention time with minimal loss in resolution, and near-baseline separation (Fig. 2b and c).

In order to isolate each diastereomer for further analysis, several iterations of preparative HPLC were employed using chromatographic conditions analogous to analytical methods, eventually yielding diastereomers of >98% d.e., diastereomeric excess (Fig. 2). The isolated diastereomers of **5**, **6** and **7** were analyzed using CD spectrometry, and the observed spectra were similar to those previously observed for **1** (Gaffield, 1970; Prescott et al., 2002) and **3** (Kitaoka et al., 1998). It is known that 2*S* flavanones exhibit a positive Cotton effect in the $n \rightarrow \pi^*$ region, and a negative Cotton effect in the $\pi \rightarrow \pi^*$ region of the CD spectrum (Gaffield, 1970). Based on the signs of the Cotton effects in these regions, absolute configurations were assigned to each diastereomer.

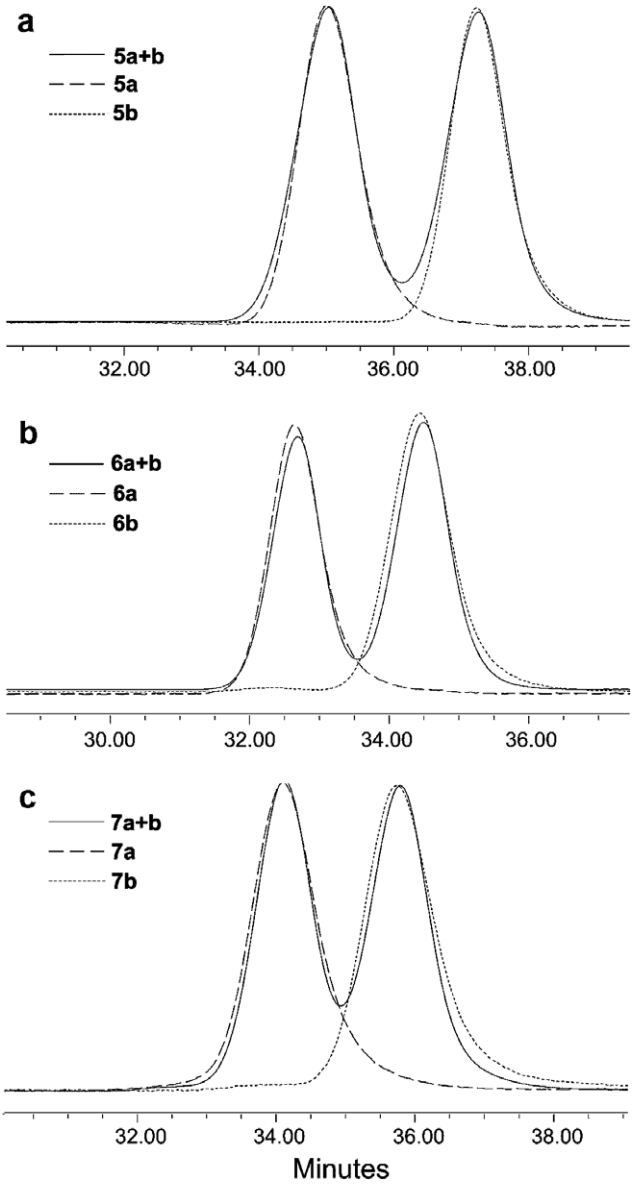



Fig. 2. Chromatographic separations of the (1*S*)-(−)-camphanic acid esters of (a) **5**, (b) **6**, and (c) **7**.

### 2.1. Concluding remarks

Herein, a facile method is thus described for the formation and chromatographic resolution of the diastereomeric (1*S*)-(−)-camphanic acid esters of prenylated and non-prenylated flavanones using RP-HPLC. Despite the advantages in circumventing chiral chromatography via a simple and inexpensive esterification, recovering enantiomerically enriched flavanones using this method would require alkaline hydrolysis of isolated diastereomers. Further investigation is necessary to determine the extent of isomerization and racemization, which could occur via a chalcone intermediate, under various alkaline hydrolysis conditions. However, this method may be used to analyze the enantiomeric purity of various flavanones, and to evaluate the effects of various treatments such as extractions or hydrolysis on the enantiomeric purity of those compounds when access to chiral chromatography is limited.

## 3. Experimental

### 3.1. General

All reagents were purchased from Sigma–Aldrich (St. Louis, MO) and HPLC-grade solvents were purchased from Fisher Scientific (Fairlawn, NJ). UV/Vis spectra were obtained on an HP8453 diode-array spectrophotometer (Agilent, Palo Alto, CA). $^1$H NMR spectra were obtained in $CDCl_3$ using a Bruker 600 MHz spectrometer (Bruker

Biospin, Rheinstetten, Germany) at ambient temperatures. CD spectra were recorded using an AVIV 202 Circular Dichroism Spectrometer (AVIV Biomedical, Lakewood, NJ). ESIMS were recorded on a Micromass Q-TOF (Micromass, Manchester, UK) measured at 30 eV, coupled to an HP1100 HPLC gradient system (Agilent, Palo Alto, CA) equipped with DAD and applying the chromatographic methods described below.

### 3.2. HPLC methods

Analytical separations were carried out on a Waters 2695 System coupled to a 2996 PDA detector, using a 4 μm Waters (Milford, MA) Nova-Pak® C18 column (3.9 × 150 mm) equipped with a Nova-Pak® guard cartridge (4 μm, 3.9 × 20 mm). The following solvent systems were employed at a flow rate of 1 mL/min: (A) 40–80% MeCN in aq. AcOH (1%) at a flow rate of 1 mL/min over 40 min; (B) 40% MeOH, 24% MeCN, 36% aq. AcOH (1%) isocratic; (C) 50% MeOH, 20% MeCN, 30% aq. AcOH (1%) isocratic.

Preparative separations were carried out on an HP1050 quaternary gradient system coupled to a Waters 2996 PDA detector, using a 5 μm Waters Sunfire™ Prep C18 column (19 × 150 mm) equipped with a Sunfire™ Prep guard cartridge (5 μm, 19 × 10 mm). The following isocratic solvent systems were employed at a flow rate of 10 mL/min: (D) 40% MeCN in aq. AcOH (1%); (E) 45% MeOH, 22% MeCN, 33% 1% aq. AcOH; (F) 55% MeOH, 18% MeCN, 27% aq. AcOH (1%).

### 3.3. Prenylflavonoids

#### 3.3.1. 6-Prenylnaringenin (**2**)

To a suspension of **4** (0.5 mmol) in $H_2O$ (0.5 mL), stirred in an ice bath, was added dropwise 5 N aq. NaOH (2.0 mmol). The suspension was removed from the ice bath until the suspension became homogeneous, returned to the ice bath and allowed to cool to 0 °C before adding neat 3,3-dimethylallyl bromide (1.0 mmol). This emulsion became homogeneous, then heterogeneous as an insoluble tar began to form over 15 min. The heterogeneous mixture was washed with hexanes (3 × 2 mL) before acidifying with 5% aq. HCl. The aqueous suspension was extracted with EtOAc (3 × 4 mL), with organics combined and washed with 10% $NaHCO_3$ (4 × 5 mL), dried ($Na_2SO_4$), filtered and the solvent removed under nitrogen flow. Crude product was subjected to preparative HPLC (Method D) and the major product was collected and recovered by lyophilization yielding 27 mg (16%) of desired product as an off-white powder. $R_t$: 11.5 min (Method A). UV $\lambda_{max}^{MeOH}$: 293, 334 sh. $^1$H NMR spectroscopic data were in agreement with previously published spectra (Haensel and Schulz, 1988; Tabata et al., 1997). ESIMS (30 eV) *m/z* (rel. int.): 341.2 $[MH]^+$ (32), 285.2 $[MH-C_4H_8]^+$ (100); ESI-CAD-MS *m/z* (rel. int.): 285.2 $[MH-C_4H_8]^+$ (42), 165.1 $[MH-C_{11}H_{12}O_2]^+$ (100).

#### 3.3.2. 8-Prenylnaringenin (**3**)

*rac*-8-Prenylnaringenin was produced synthetically from **4** (58 mg, 28% overall yield) as previously described by Gester et al. (2001). $R_t$: 7.5 min (Method A). UV $\lambda_{max}^{MeOH}$: 293, 334 sh. $^1$H NMR spectroscopic data were in agreement with previously published spectra (Mizobuchi and Sato, 1984). ESIMS (30 eV) *m/z* (rel. int.): 341.2 $[MH]^+$ (100), 285.2 $[MH-C_4H_8]^+$ (9); ESI-CAD-MS *m/z* (rel. int.): 285.2 $[MH-C_4H_8]^+$ (12), 165.1 $[MH-C_{11}H_{12}O_2]^+$ (100).

### 3.4. Typical (1*S*)-(−)-camphanoylation of flavanones

To a solution of **3** (0.23 mmol) in pyridine (1.2 mL) was added (1*S*)-(−)-camphanoyl chloride (2.06 mmol, 9 equiv.). The resulting solution almost immediately became heterogeneous, and was stirred for 2 h. Quantitative conversion was observed by an HPLC analysis (Method B), and excess pyridine was removed under $N_2$. The resulting paste was dissolved in $CH_2Cl_2$ (5 mL) methylene chloride, washed 10% aq. $NaHCO_3$ (3 × 5 mL), dried ($MgSO_4$) and filtered. Solvent was removed under $N_2$, providing crude **7a+b** (250 mg). The crude diastereomeric mixture was purified by preparative reversed-phase HPLC (Method F). Diastereomerically enriched fractions were combined and re-purified several times to yield each diastereomer >98% d.e. by HPLC (Method B). Fractions were stored at −20 °C to prevent hydrolysis of camphanic acid esters. Solvent was removed under nitrogen flow and lyophilization yielding 12 mg (35% yield) of each diastereomer.

#### 3.4.1. (*R*)-4′,5,7-tris-*O*-[(1*S*)-(−)-camphanoyl]-8-prenylnaringenin (**7a**)

$R_t$: 34.1 min (Method C). CD: $[\theta]_{341}$ −15 000; $[\theta]_{309}$ +17 000; $[\theta]_{238}$ +300; $[\theta]_{258}$ +7900; $[\theta]_{275}$ +1800 (MeCN). UV $\lambda_{max}^{MeCN}$ ($\log\varepsilon$): 258 (3.95), 319. ESIMS (30 eV) *m/z* (rel. int.): 881.6 $[MH]^+$ (100); ESI-CAD-MS *m/z* (rel. int.): 881.7 $[MH]^+$ (30), 825.6 $[MH-C_4H_8]^+$ (16), 727.5 $[MH-\text{camphonolactone}]^+$ (29), 645.5 $[MH-Cp-C_4H_8]^+$ (100), 581.5 $[MH-Cp-C_8H_8O]^+$ (63).

#### 3.4.2. (*S*)-4′,5,7-tris-*O*-[(1*S*)-(−)-camphanoyl]-8-prenylnaringenin (**7b**)

$R_t$: 35.7 min (Method C). CD: $[\theta]_{341}$ +15 000; $[\theta]_{309}$ −17 000; $[\theta]_{238}$ +300; $[\theta]_{254}$ −6500; $[\theta]_{275}$ −800 (MeCN). UV $\lambda_{max}^{MeCN}$ ($\log\varepsilon$): 258, (3.95), 319.0. ESIMS (30 eV) *m/z* (rel. int.): 881.6 $[MH]^+$ (100); ESI-CAD-MS *m/z* (rel. int.): 881.7 $[MH]^+$ (33), 825.6 $[MH-C_4H_8]^+$ (16), 727.5 $[MH-\text{camphonolactone}]^+$ (26), 645.5 $[MH-Cp-C_4H_8]^+$ (100), 581.5 $[MH-Cp-C_8H_8O]^+$ (71).

### 3.5. (1*S*)-(−)-camphanoylation of prenylflavanones

This method was used to separate the diastereomeric *tris*-(1*S*)-(−)-camphanate esters **6a** and **b** providing 7 mg (15% yield) of each diastereomer, and the diastereomeric naringenin *tris*-(1*S*)-(−)-camphanate esters **5a** and **b** were

separated using preparative chromatography (Method E), providing 10 mg (30% yield) of each diastereomer. The configuration of each respective diastereomer was characterized by CD spectrometry.

### 3.5.1. (R)-4′,5,7-tris-O-[(1S)-(−)-camphanoyl]-naringenin (**5a**)

$R_t$: 35.0 min (Method B). CD: $[\theta]_{336}$ −9600; $[\theta]_{307}$ +13 000; $[\theta]_{274}$ 300; $[\theta]_{256}$ +3300 (MeCN). UV $\lambda_{max}^{MeCN}$ ($\log\varepsilon$): 255 (3.90), 311. ESIMS (30 eV) *m/z* (rel. int.): 813.5 $[MH]^+$ (100); ESI-CAD-MS *m/z* (rel. int.): 813.6 $[MH]^+$ (40), 767.6 $[MH-CH_3O_2]^+$ (23), 659.4 [MH−camphonolactone]$^+$ (100), 633.4 $[MH-Cp]^+$ (22).

### 3.5.2. (S)-4′,5,7-tris-O-[(1S)-(−)-camphanoyl]-naringenin (**5b**)

$R_t$: 37.3 min (Method B). CD: $[\theta]_{336}$ +11 000; $[\theta]_{307}$ −14 000; $[\theta]_{272}$ +600; $[\theta]_{256}$ −4720 (MeCN). UV $\lambda_{max}^{MeCN}$ ($\log\varepsilon$): 255 (3.90), 311. ESIMS (30 eV) *m/z* (rel. int.): 813.5 $[MH]^+$ (100); ESI-CAD-MS *m/z* (rel. int.): 813.6 $[MH]^+$ (50), 767.6 $[MH-CH_3O_2]^+$ (20), 659.4 [MH−camphonolactone]$^+$ (100), 633.4 $[MH-Cp]^+$ (23).

### 3.5.3. (R)-4′,5,7-tris-O-[(1S)-(−)-camphanoyl]-6-prenylnaringenin (**6a**)

$R_t$: 32.7 min (Method C). CD: $[\theta]_{345}$ −15 000; $[\theta]_{313}$ +19 000; $[\theta]_{269}$ 0; $[\theta]_{254}$ +3000 (MeCN). UV $\lambda_{max}^{MeCN}$ ($\log\varepsilon$): 256 (3.94), 322. ESIMS (30 eV) *m/z* (rel. int.): 881.6 $[MH]^+$ (100); ESI-CAD-MS *m/z* (rel. int.): 881.7 $[MH]^+$ (25), 825.6 $[MH-C_4H_8]^+$ (75), 727.5 [MH−camphonolactone]$^+$ (15), 645.5 $[MH-Cp-C_4H_8]^+$ (100).

### 3.5.4. (S)-4′,5,7-tris-O-[(1S)-(−)-camphanoyl]-6-prenylnaringenin (**6b**)

$R_t$: 34.5 min (Method C). CD: $[\theta]_{345}$ +15 000; $[\theta]_{313}$ −17 000; $[\theta]_{269}$ 0; $[\theta]_{254}$ −6000 (MeCN). UV $\lambda_{max}^{MeCN}$ ($\log\varepsilon$): 256 (3.94), 322. ESIMS (30 eV) *m/z* (rel. int.): 881.6 $[MH]^+$ (100); ESI-CAD-MS *m/z* (rel. int.): 881.7 $[MH]^+$ (30), 825.6 $[MH-C_4H_8]^+$ (76), 727.5 [MH−camphonolactone]$^+$ (15), 645.5 $[MH-Cp-C_4H_8]^+$ (100).

## References


Bu, X., Zhao, L., Li, Y., 1997. A facile synthesis of 6-*C*-prenylflavanones. Synthesis 1997, 1246–1248.

Esterhuysen, C., Bredenkamp, M.W., Lloyd, G.O., 2005. Resolution of (*S*,*S*)-4-(2,2,4-trimethyl-chroman-4-yl)phenyl camphanate and its 4-chromanyl epimer by crystallization. Acta Crystallogr., Sect. C: Cryst. Struct. Commun. C61, o32–o34.

Gaffield, W., 1970. Circular dichroism, optical rotatory dispersion and absolute configuration of flavanones, 3-hydroxyflavanones and their glycosides. Tetrahedron 26, 4093–4108.

Gerhauser, C., 2005. Beer constituents as potential cancer chemopreventive agents. Eur. J. Cancer. 41, 1941–1954.

Gester, S., Metz, P., Zierau, O., Vollmer, G., 2001. An efficient synthesis of the potent phytoestrogens 8-prenylnaringenin and 6-(1,1-dimethylallyl)naringenin by europium(III)-catalyzed Claisen rearrangement. Tetrahedron 57, 1015–1018.

Guo, J., Nikolic, D., Chadwick, L.R., Pauli, G.F., van Breemen, R.B., 2006. Identification of human hepatic cytochrome P450 enzymes involved in the metabolism of 8-prenylnaringenin and isoxanthohumol from hops (*Humulus lupulus* L.). Drug Metab. Dispos. 34, 1152–1159.

Haensel, R., Schulz, J., 1988. Demethylxanthohumol: isolation from hops and cyclization to flavanones. Arch. Pharm. 321, 37–40.

Hua, D.H., Nguyen, T.X.C., McGill, J.W., Chen, Y., Robinson, P.D., 2001. Resolution of 8′-alkoxy- and hydroxy-1,1′-binaphthalen-8-ols. Tetrahedron: Asymmetr. 12, 1999–2004.

Jain, A.C., Gupta, R.C., Sarpal, P.D., 1978. Synthesis of (±)-lupinifolin, di-*O*-methylxanthohumol and isoxanthohumol and related compounds. Tetrahedron 34, 3563–3567.

Kitaoka, M., Kadokawa, H., Sugano, M., Ichikawa, K., Taki, M., Takaishi, S., Iijima, Y., Tsutsumi, S., Boriboon, M., Akiyama, T., 1998. Prenylflavonoids: a new class of non-steroidal phytoestrogen (Part 1). Isolation of 8-isopentenylnaringenin and an initial study on its structure–activity relationship. Planta Med. 64, 511–515.

Kornblum, N., Berrigan, P.J., Noble, W.J.L., 1963. Solvation as a factor in the alkylation of ambident anions: the importance of the hydrogen bonding capacity of the solvent. J. Am. Chem. Soc. 85, 1141–1147.

Milligan, S.R., Kalita, J.C., Heyerick, A., Rong, H., De Cooman, L., De Keukeleire, D., 1999. Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. J. Clin. Endocrinol. Metab. 84, 2249–2252.

Milligan, S.R., Kalita, J.C., Pocock, V., Van De Kauter, V., Stevens, J.F., Deinzer, M.L., Rong, H., De Keukeleire, D., 2000. The endocrine activities of 8-prenylnaringenin and related hop (*Humulus lupulus* L.) flavonoids. J. Clin. Endocrinol. Metab. 85, 4912–4915.

Milligan, S., Kalita, J., Pocock, V., Heyerick, A., De Cooman, L., Rong, H., De Keukeleire, D., 2002. Oestrogenic activity of the hop phyto-oestrogen, 8-prenylnaringenin. Reproduction 123, 235–242.

Mizobuchi, S., Sato, Y., 1984. A new flavanone with antifungal activity isolated from hops. Agric. Biol. Chem. 48, 2771–2775.

Nikolic, D., Li, Y., Chadwick, L.R., Pauli, G.F., van Breemen, R.B., 2005. Metabolism of xanthohumol and isoxanthohumol, prenylated flavonoids from hops (*Humulus lupulus* L.), by human liver microsomes. J. Mass Spectrom. 40, 289–299.

Possemiers, S., Heyerick, A., Robbens, V., De Keukeleire, D., Verstraete, W., 2005. Activation of proestrogens from hops (*Humulus lupulus* L.) by intestinal microbiota; conversion of isoxanthohumol into 8-prenylnaringenin. J. Agric. Food Chem. 53, 6281–6288.

Possemiers, S., Bolca, S., Grootaert, C., Heyerick, A., Decroos, K., Dhooge, W., De Keukeleire, D., Rabot, S., Verstraete, W., Van de Wiele, T., 2006. The prenylflavonoid isoxanthohumol from hops (*Humulus lupulus* L.) is activated into the potent phytoestrogen 8-prenylnaringenin in vitro and in the human intestine. J. Nutr. 136, 1862–1867.

Prescott, A.G., Stamford, N.P.J., Wheeler, G., Firmin, J.L., 2002. In vitro properties of a recombinant flavonol synthase from *Arabidopsis thaliana*. Phytochemistry 60, 589–593.

Schaefer, O., Huempel, M., Fritzemeier, K.-H., Bohlmann, R., Schleuning, W.-D., 2003. 8-Prenylnaringenin is a potent ER a selective phytoestrogen present in hops and beer. J. Steroid Biochem. Mol. Biol. 84, 359–360.

Stevens, J.F., Page, J.E., 2004. Xanthohumol and related prenylflavonoids from hops and beer: to your good health! Phytochemistry 65, 1317–1330.

Stevens, J.F., Taylor, A.W., Clawson, J.E., Deinzer, M.L., 1999. Fate of xanthohumol and related prenylflavonoids from hops to beer. J. Agric. Food Chem. 47, 2421–2428.

Tabata, N., Ito, M., Tomoda, H., Omura, S., 1997. Xanthohumols, diacylglycerol acyltransferase inhibitors, from *Humulus lupulus*. Phytochemistry 46, 683–687.

Thongpanchang, T., Paruch, K., Katz, T.J., Rheingold, A.L., Lam, K.-C., Liable-Sands, L., 2000. Why (1*S*)-camphanates are excellent resolving agents for helicen-1-ols and why they can be used to analyze absolute configurations. J. Org. Chem. 65, 1850–1856.

Vecchi, M., Muller, R.K., 1979. Separation of (3*S*, 3′*S*)-, (3*R*,3′*R*)- and (3*S*, 3′*R*)-astaxanthin via (−)-camphanic acid esters. J. High Resolut. Chromatogr. 2, 195–496.

Zehnter, R., Gerlach, H., 1995. Enantiodifferentiation in taste perception of the phyllodulcins. Tetrahedron: Asymmetr. 6, 2779–2786.