JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



Article

# Analysis of the Components of Hard Resin in Hops (*Humulus lupulus* L.) and Structural Elucidation of Their Transformation Products Formed During the Brewing Process

Yoshimasa Taniguchi, Harumi Taniguchi, Makiko Yamada, Yasuko Matsukura, Hideki Koizumi, Kazuo Furihata, and Kazutoshi Shindo

*J. Agric. Food Chem.*, **Just Accepted Manuscript** • DOI: 10.1021/jf504394h • Publication Date (Web): 29 Oct 2014



## Just Accepted



# Analysis of the Components of Hard Resin in Hops (*Humulus lupulus* L.) and Structural Elucidation of Their Transformation Products Formed During the Brewing Process

## AUTHORSHIP

Yoshimasa Taniguchi,[*,†] Harumi Taniguchi,[†] Makiko Yamada,[†] Yasuko Matsukura,[†] Hideki Koizumi,[†] Kazuo Furihata, [‡] and Kazutoshi Shindo[§]

[†]Central Laboratories for Key Technologies, Research & Development Division, KIRIN Company, Ltd., 1-13-5, Fukuura Kanazawa-ku, Yokohama-shi, Kanagawa 236-0004, Japan.

[‡]Division of Agriculture and Agricultural Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.

[§]Department of Food and Nutrition, Japan Women's University, 2-8-1, Mejirodai Bunkyo-ku, Tokyo 112-8681, Japan.

[*]; Author to whom correspondence should be addressed (telephone +81-45-330-9007; fax +81-45-782-3657; e-mail Yoshimasa_Taniguchi@kirin.co.jp).

**ABSTRACT:**

The resins from hops (*Humulus lupulus* L.), which add the bitter taste to beer, are classified into two main sub-fractions; namely soft and hard resins. α-Acids and β-acids in soft resin and their transformation during the wort boiling process are well-studied, however, other constituents in resins, especially hard resin, have been unidentified. In this study, we identified humulinones and hulupones as soft resin components, in addition to 4′-hydroxyallohumulinones and tricyclooxyisohumulones A and B as hard resin components. These compounds are all oxidation products derived from α-acids or β-acids. We also investigated compositional changes in the hard resin during the wort boiling process, which has a significant effect on the taste of the beer, by utilizing model boiling experiments. The major changes were identified to be isomerization of 4′-hydroxyallohumulinones into 4′-hydroxyallo-*cis*-humulinones followed by decomposition into *cis*-oxyhumulinic acids. These findings will be helpful in systematically evaluating and optimizing the effect of the hard resin on beer quality.

**KEYWORDS:** Hop, *Humulus lupulus* L., soft resin, hard resin, humulinone, hulupone, 4′-hydroxyallohumulinone, 4′-hydroxyallo-*cis*-humulinone, *cis*-oxyhumulinic acid, wort boiling, beer

## INTRODUCTION

Hops (*Humulus lupulus* L.) are widely used in the brewing industry to add characteristic bitterness and aroma to beer. The lupulin glands from female inflorescences of hops are known to accumulate resins and essential oils. The resins are composed of many different substances and are responsible for the fine bitter taste of beer. According to the European Brewery Convention and the American Society of Brewing Chemists, resins in hops can be divided into two main sub-fractions; namely soft resin (i.e., the fraction soluble in low-boiling paraffin hydrocarbons such as hexane) and hard resin (i.e., the fraction insoluble in hexane but soluble in ether and cold MeOH).[1-4]

α-Acids, one of the best studied group of compounds in hops, are found in the soft resin fraction. These α-acids comprise three main congeners, cohumulone (**1a**), *n*-humulone (**1b**) and adhumulone (**1c**), which differ in their acyl side chains. During the wort boiling process, α-acids thermally isomerize into iso-α-acids *via* an acyloin-type ring contraction, resulting in the generation of two epimeric isomers: *cis*- (**3a-c**) and *trans*-iso-α-acids (**4a-c**) (Figure 1).[5] Iso-α-acids are known to be the major contributors to the bitter taste of beer,[6, 7] and to the stalility of beer foam.[8] In addition to α-acids, β-acids, which consist of three main congeners, colupulone (**2a**), *n*-lupulone (**2b**) and adlupulone (**2c**), are also found in the soft resin fraction. The oxidative transformation products of β-acids generated during the wort boiling process contribute to the bitter taste of beer.[9, 10] There have been numerous published studies that have focused on the soft resin components and their impact on the properties of beer.

By contrast, however, there is a lack of information concerning the hard resin components and their effect on beer quality. Xanthohumol (**10**), a prenyl chalcone, is a well-known constituent of hard resin.[4, 11] However, other components of hard resin have not, until now, been identified.[4, 11] It has been reported that the α- and β-acids undergo rapid oxidation during the storage of hops, and the amount of hard resin fraction increases.[12, 13] Thus, the hard resin is considered to be mainly composed of the oxidation

products derived from α-acids and/or β-acids.[4, 11]

The organoleptic properties and functionality of the hard resin have received considerable attention over the years. However, opinion concerning the effect of the hard resin accumulated in stored hops on the bitterness quality of beer remains contentious.[6, 14-18] Previous studies using hard resin enriched extracts for brewing proved inconclusive and failed to explain the effect of the hard resin on the quality of the beer.[13, 19] The apparent inconsistency in the results of these studies could be due to the lack of chemical analysis of materials used in the experiments. Recently, Alamaguer et al. reported that the hard resin positively contributed to the foam stability of beer and produced a more pleasant bitter taste as opposed to the sharper bitterness of iso-α-acids. However, the hard resin enriched extracts used in the study were not subjected to rigorous chemical compositional analysis.[11] Thus, a suitable method to fully analyze the chemical composition of the hard resin was needed to properly examine its effect on the properties of beer. Furthermore, transformation of the hard resin components during the wort boiling process, which is known to have a significant effect on beer taste, was not understood, except for the conversion of xanthohumol (**10**) into isoxanthohumol (**11**).[20] Knowledge of the transformation of hard resin components during the wort boiling process is also essential to evaluate and optimize the effect of the hard resin on beer quality.

In our previous study, we developed an HPLC method suitable for investigation of the oxidation products in stored hops, which showed that α-acids are oxidized into humulinones (**5a-c**), 4′-hydroxyallohumulinones (**6a-c**) and tricyclooxyisohumulones A (**7a-c**) and B (**8a-c**), and that β-acids are oxidized into hulupones (**9a-c**) (Figure 1).[21, 22] These oxidation products are thought to be components in the soft or hard resins.

In this study, we analyzed the soft and hard resins derived from stored hops using our newly developed analytical method and clarified the existence of these oxidation compounds for the first time. We also investigated the transformation of the hard resin

components through wort boiling by utilizing model boiling experiments, and successively isolated and determined the structures of the transformation products. The concentration changes of these products and their precursors during the boiling process were also investigated.

## MATERIALS AND METHODS

**Chemicals and Materials.** The following chemicals were obtained commercially: ethylenediaminetetraacetic acid (EDTA), $H_3PO_4$, MeCN, EtOH, hexane, diethyl ether, $CH_2Cl_2$ (Wako Pure Chemicals, Osaka, Japan); xanthohumol, isoxanthohumol (Funakoshi, Tokyo, Japan). Deionized water for chromatography was purified by means of a Milli-Q Gradient A10 system (Millipore, Billerica, MA). Hop pellets, cultivar Hallertau Perle (HPE), were purchased from Hopsteiner (Mainburg, Germany).

**Preparation of Humulinones (5a-c), Hulupones (9a-c), and Tricyclooxyiso-*n*-humulones A (7b) and B (8b).** Humulinones (**5a-c**) and hulupones (**9a-c**) were prepared according to a protocol reported previously.[21] Tricyclooxyiso-*n*-humulones A (**7b**) and B (**8b**) were isolated from the autoxidation products of *n*-humulone.[22]

**Preparation of 4′-Hydroxyallohumulinones (6a-c and 6c′) from Stored Hops.** Hop pellets (850 g) were stored at 60 °C for 120 h and extracted with $H_2O$ (8.5 L) at 50 °C for 1 h. The extract was filtered and then lyophilized to yield a brown powder (176 g). A portion of this powder (120 g) was dissolved in $H_2O$ (8.1 L) and partitioned with $CH_2Cl_2$ (16.2 L) after adding 1 N HCl (810 mL) to the solution. The $CH_2Cl_2$ layer containing 4′-hydroxyallohumulinones was collected and dried over anhydrous $Na_2SO_4$ and concentrated to dryness *in vacuo* to give a yellowish brown oil (22 g). This oil was dissolved in EtOH and repeatedly subjected to preparative HPLC (column: 150 × 22 mm id, 5 μm, Alltima $C_{18}$ column (Systech, Tokyo, Japan); solvent: $H_2O/H_3PO_4$ (85%), 100/1, (v/v) (solvent A) and MeCN (solvent B), a linear gradient from 30 to 90% B in 0 → 40 min and 90% B for 40.1 → 45 min; flow rate: 18.8 mL/min; detector: 270 nm; column temperature: RT), and divided into 11 fractions depending on their elution times. Compounds **6a**, **6b**, and **6c/c′** were contained in fractions 3 ($t_R$ 8.6 – 9.5 min), 6 ($t_R$ 11.0 – 12.1 min) and 7 ($t_R$ 12.1 – 12.9 min), respectively. Compound **6a** in fraction 3 and compound **6b** in fraction 6 were further purified by repeatedly subjecting them to a

second preparative HPLC step (column: 50 × 20 mm id, 5 μm, L-column 2 ODS (Chemicals Evaluation and Research Institute, Tokyo, Japan); solvent: 10 mM $NH_4HCO_3$ (solvent A) and MeCN (solvent B), isocratic elution at 20% B; flow rate: 9.5 mL/min; detector: 270 nm; column temperature: 40 ˚C). Compounds **6a** and **6b** were eluted at 3.8 – 4.7 min and at 5.5 – 7.5 min, respectively. Each eluate was immediately diluted two times with $H_2O$, adjusted to pH 2.0 with 1 N HCl, and partitioned with $CH_2Cl_2$ (1/2 volume of the diluted eluate × 2 times). The respective $CH_2Cl_2$ layer was dried over $Na_2SO_4$ and concentrated to dryness to yield pure **6a** (814 mg) and **6b** (1.2 g). Similarly, compounds **6c/c′** in fraction 7 were further purified by repeatedly subjecting them to a second preparative HPLC (column: 150 × 20 mm id, 5 μm, L-column 2 ODS (Chemicals Evaluation and Research Institute, Tokyo, Japan); solvent: 100 mM $NH_4HCO_3$ (solvent A) and MeCN (solvent B), isocratic elution at 20% B; flow rate: 18.8 mL/min; detector: 270 nm; column temperature: RT). Compounds **6c** and **6c′** were eluted separately at 9.8 – 10.8 min and at 10.8 – 11.8 min, respectively. Pure **6c** (40.3 mg) and **6c′** (37.2 mg) were recovered from each eluate in the same way as that described for **6a** and **6b**.

***4′-Hydroxyalloadhumulinone A (6c):*** pale yellow oil; $[\alpha]^{20}_{D}$ +4.01 (*c* 0.4, MeOH); $^{1}H$ and $^{13}C$ NMR data, see Table 2; HRESIMS (negative) *m/z* 393.1916 $[M - H]^-$ (calcd for $C_{21}H_{29}O_7$, 393.1919).

***4′-Hydroxyalloadhumulinone B (6c′):*** pale yellow oil; $[\alpha]^{20}_{D}$ +12.1 (*c* 0.3, MeOH); $^{1}H$ and $^{13}C$ NMR data, see Table 2; HRESIMS (negative) *m/z* 393.1914 $[M - H]^-$ (calcd for $C_{21}H_{29}O_7$, 393.1919).

**Preparation of Soft and Hard Resins.** Soft and hard resins from hops were prepared according to Analytica EBC[3] with some modification.

Hop pellets (100 g) were stored at 60 °C for 48 h, and a portion (10 g) was extracted with MeOH (100 mL) by stirring at room temperature for 1 h and then filtered. The filtrate was kept at 4 °C for 1 day, and a waxy precipitate that formed was removed by

filtration. To a portion of the filtrate (20 mL), MeOH (10 mL), hexane (80 mL) and 1 N HCl (20 mL) were added, and the two layer solution (hexane/acidic MeOH-$H_2O$) was partitioned in a separating funnel. The hexane layer, prepared by repeating the above partition four times (320 mL), was evaporated to dryness to give a yellowish brown oil (250 mg, soft resin).

The residual acidic MeOH- $H_2O$ layer was partitioned with diethyl ether (80 mL × 2 times). The diethyl ether layer (160 mL) was washed with sat. NaCl solution (160 mL), dried over anhydrous $Na_2SO_4$, and concentrated to dryness to give a brown solid (200 mg, hard resin).

**Boiling Test for the Hard Resin.** To 500 μL of 10 mM citrate buffer (pH 5.5) pre-heated to 100 °C was added a 25 μL solution of the hard resin in EtOH (5.0 mg/mL). The combined solution was kept at 100 °C and analyzed by HPLC at 30, 60, 90 and 120 min.

**Boiling Test of 4′-Hydroxyallohumulinones (6a-c, c′).** To a 500 μL aliquot of 10 mM citrate buffer (pH 5.5) pre-heated to 100 °C was added 25 μL solution of either 4′-hydroxyallocohumulinone (**6a**), 4′-hydroxyallo-*n*-humulinone (**6b**), 4′-hydroxyalloadhumulinones A (**6c**) or B (**6c′**) (1.0 mg/mL in EtOH). Each combined solution was kept at 100 °C, and analyzed by HPLC at 30, 60, 90, and 120 min.

**Isolation of the Transformation Products Produced by Boiling of 4′-Hydroxyallocohumulinone (6a) and 4′-Hydroxyallo-*n*-humulinone (6b).** 4′-Hydroxyallocohumulinone (**6a**) (200 mg) was dissolved in EtOH (50 mL) and added to 10 mM citrate buffer (pH 5.5, 1 L) pre-heated to 100 °C and maintained at this temperature for 90 min. After cooling to room temperature, the solution was acidified with 1 N HCl (100 mL) and partitioned with $CH_2Cl_2$ (250 mL × 3 times). The $CH_2Cl_2$ layer (750 mL) was washed with sat. NaCl solution (400 mL), dried over anhydrous $Na_2SO_4$, and concentrated to dryness to give a yellowish brown oil (150 mg).

The oil was dissolved in EtOH and repeatedly subjected to preparative HPLC

(column: 150 × 10 mm id, 5 μm, Alltima $C_{18}$ column (Systech, Tokyo, Japan); solvent: $H_2O/H_3PO_4$ (85%), 100/1, (v/v) (solvent A) and MeCN (solvent B), a linear gradient from 30% B for 0 → 15 min, 30 to 90% B in 15 → 18 min, and 90% B for 18 → 22 min; flow rate: 4.7 mL/min; detector: 270 nm; column temperature: 40 °C). In this preparative HPLC, 4′-hydroxyallo-*cis*-cohumulinone (**12a**) was eluted at 8.6 – 10.0 min and *cis*-oxycohumulinic acid (**13a**) was eluted at 10.0 – 10.6 min. Each eluate was diluted with $H_2O$ to lower the concentration of MeCN to less than 10%, and extracted with $CH_2Cl_2$ (1/4 volume of the diluted eluate × 3 times). The $CH_2Cl_2$ layer was dried over anhydrous $Na_2SO_4$ and concentrated to dryness to yield **12a** (33.4 mg) and **13a** (4.0 mg).

The transformation products (**12b** and **13b**) of 4′-hydroxyallo-*n*-humulinone (**6b**) were prepared and purified in almost the same way as described for **12a** and **13a**. In summary, 4′-hydroxyallo-*n*-humulinone (**6b**) (200 mg) was boiled and partitioned between $CH_2Cl_2$ and acidic $H_2O$. The $CH_2Cl_2$ layer was concentrated to give an oil (167 mg). The oil was repeatedly subjected to preparative HPLC (column, flow rate, detector, and column temperature are identical to those used for the isolation of **12a** and **13a**; solvent: $H_2O/H_3PO_4$ (85%), 100/1, (v/v) (solvent A) and MeCN (solvent B), a linear gradient from 30 to 48% B in 0 → 12 min, 48 to 90% B in 12 → 18 min, and 90% B for 18 → 21 min). In this preparative HPLC, 4′-hydroxyallo-*cis*-*n*-humulinone (**12b**) and *cis*-oxy-*n*-humulinic acid (**13b**) were eluted at 9.0 – 9.6 min and at 10.0 – 10.3 min, respectively. Pure **12b** (41.3 mg) and **13b** (5.9 mg) were recovered from each eluate in the same way as described for **12a** and **13a**.

***4′-Hydroxyallo-cis-cohumulinone (12a):*** pale yellow oil; $[\alpha]^{20}_D$ ±0 (*c* 0.7, MeOH); $^1H$ and $^{13}C$ NMR data, see Table 1; HRESIMS (negative) *m/z* 379.1753 $[M - H]^-$ (calcd for $C_{20}H_{27}O_7$, 379.1762).

***4′-Hydroxyallo-cis-n-humulinone (12b):*** pale yellow oil; $[\alpha]^{20}_D$ ±0 (*c* 0.4, MeOH); $^1H$ and $^{13}C$ NMR data, see Table 1; HRESIMS (negative) *m/z* 393.1911 $[M - H]^-$ (calcd for $C_{21}H_{29}O_7$, 393.1919).

***Cis-oxycohumulinic acid (13a):*** white, amorphous solid; $[\alpha]^{20}_{D}$ ±0 (*c* 0.1, MeOH); $^{1}$H and $^{13}$C NMR data, see Table 1; HRESIMS (negative) *m/z* 267.1230 $[M - H]^-$ (calcd for $C_{14}H_{19}O_5$, 267.1238).

***Cis-oxy-n-humulinic acid (13b):*** pale yellow oil; $[\alpha]^{20}_{D}$ ±0 (*c* 0.1, MeOH); $^{1}$H and $^{13}$C NMR data, see Table 1; HRESIMS (negative) *m/z* 281.1390 $[M - H]^-$ (calcd for $C_{15}H_{21}O_5$, 281.1395).

**Preparation of *Cis*- (13b) and *Trans*-oxy-*n*-humulinic acids (14b) by hydrolysis of *n*-humulinone (5b).** Both *cis*- and *trans*-oxy-*n*-humulinic acids were prepared by alkaline hydrolysis of *n*-humulinone (**5b**) according to the previous report[23] with some modification.

*N*-humulinone was prepared according to a protocol reported previously.[21] To 2 M NaOH*aq* (300 mL) pre-heated at 100 ˚C, 30 mL solution of *n*-humulinone in MeOH (10 mg/mL) was added and mixed well. The temperature was maintained at 100 ˚C for 10 min before cooling the solution to room temperature, acidifying with 6 N HCl (120 mL) and partitioning with $CH_2Cl_2$ (200 mL × 2 times). The $CH_2Cl_2$ layer was dried over anhydrous $Na_2SO_4$ and concentrated to dryness to give a yellow oil (270 mg). The oil was dissolved in EtOH and repeatedly subjected to preparative HPLC (column: 150 × 22 mm id, 5 μm, Alltima $C_{18}$ column (Systech, Tokyo, Japan); solvent: $H_2O/H_3PO_4$ (85%), 100/1, (v/v) (solvent A) and MeCN (solvent B), isocratic elution at 45% B; flow rate: 22.8 mL/min; detector: 270 nm; column temperature: RT). In this preparative HPLC, *cis*- (**13b**) and *trans*-oxy-*n*-humulinic acids (**14b**) were eluted at 5.1 – 5.5 min and at 6.7 – 7.2 min, respectively. Pure **13b** (74.6 mg) and **14b** (94.6 mg) were recovered from each eluate in the same way as described for **12a** and **13a**.

***Trans-oxy-n-humulinic acid (14b):*** white, amorphous solid; $[\alpha]^{20}_{D}$ ±0 (*c* 0.3, MeOH); $^{1}$H and $^{13}$C NMR data, see Table 1; HRESIMS (negative) *m/z* 281.1389 $[M - H]^-$ (calcd for $C_{15}H_{21}O_5$, 281.1395).

**High-Performance Liquid Chromatography (HPLC).** A Shimadzu Prominence

UFLC™ system (Shimadzu, Kyoto, Japan) was used for all HPLC analysis. Data were processed with LCsolution software (Shimadzu, Kyoto, Japan).

HPLC analyses were performed to investigate the constituents in the soft and hard resins of hops and to analyze compositional changes in the hard resin components in the boiling tests using the following conditions[21, 22]: column: 100 × 2.1 mm id, 3 μm, Alltima $C_{18}$ column (Systech, Tokyo, Japan); solvent: $H_2O/H_3PO_4$ (85%), 1000/0.2, (v/v) containing EDTA (0.02% w/v) (solvent A) and MeCN (solvent B), a linear gradient from 10 to 52% B in 0 → 26.7 min, 52% B for 26.7 → 30 min, 52 to 75% B in 30 → 32.7 min, 75 to 85% B in 32.7 → 36.7 min, and 85% B for 36.7 → 37.7 min; flow rate: 0.6 mL/min; detector: 270 nm; column temperature: 40 ˚C. The injection volume was 3.0 μL. In the boiling tests, transformation products were quantified using isolated reference compounds. 4′-Hydroxyallo-*cis*-adhumulinones A (**12c**) and B (**12c′**) were quantified using the calibration curve of 4′-hydroxyallo-*cis*-*n*-humulinone (**12b**) and *cis*-oxyadhumulinic acids A (**13c**) and B (**13c′**) were quantified using the calibration curve of *cis*-oxy-*n*-humulinic acid (**13b**).

To separate and check the purity of 4′-hydroxyalloadhumulinones A (**6c**) and B (**6c′**) by HPLC (Figure 7), the following LC conditions were used: column: 100 × 2.1 mm id, 3 μm, L-column 2 ODS (Chemicals Evaluation and Research Institute, Tokyo, Japan); solvent: 5 mM $HCOONH_4$ (pH 8.5) (solvent A) and MeCN (solvent B), a linear gradient from 10 to 36% B in 0 → 39 min, 36 to 80% B in 39 → 44 min, and 80% B for 44 → 52 min; flow rate: 0.25 mL/min; column temperature: 40 ˚C. The injection volume was 3.0 μL.

**High-Resolution Electrospray Ionization Mass Spectrometry (HRESIMS).** HRESIMS of the purified compounds was measured using a Thermo Scientific LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Before measuring the samples, accurate mass calibration was carried out using polytyrosine-1,3,6 as a mass standard (CS Bio Company, Menlo Park, CA).

**Nuclear Magnetic Resonance Spectroscopy (NMR).** $^{1}H$, $^{13}C$, and 2D NMR spectra were measured with a Bruker AVANCE400 spectrometer (Bruker BioSpin, Rheinstetten, Germany). Samples were dissolved in methanol-$d_4$. Chemical shifts were referenced to the solvent signals ($\delta_H/\delta_C$ 3.30/49.0). Data processing was performed using TopSpin-NMR software (version 3.0) (Bruker BioSpin). *J*-Resolved HMBC experiments were conducted on a Varian INOVA-500 NMR spectrometer (Agilent, Santa Clara, CA) using the previously reported method.[24]

## RESULTS AND DISCUSSION

**Identification of the Components in the Soft and Hard Resins Derived from Stored Hops.** Recently, we reported the transformation of α-acids to humulinones (humulinones were further transformed to 4′-hydroxyallohumulinones) and to tricyclooxyisohumulones A and B due to oxidation.[21, 22] During these studies, we also developed a HPLC method that is suitable for the analysis of α- and β-acid-derived oxidation compounds.[21, 22] Using this HPLC method, we analyzed a MeOH extract of the stored hops, and soft and hard resins prepared from the MeOH extract. The results are shown in Figure 2 (2A: MeOH extract of the stored hop, 2B: the soft resin, 2C: the hard resin).

As shown in Figure 2B, the presence of humulinones (**5a-c**, **c′**), hulupones (**9a-c**) in addition to α-acids (**1a-c**) in the soft resin can clearly be observed. This is the first study reporting the presence of humulinones (**5a-c**, **c′**) and hulupones (**9a-c**) in soft resin.

The compounds contained in the soft resin were barely detectable in the hard resin (Figure 2C). Indeed, xanthohumol (**10**) and the hydrophilic α-acid-derived oxidation products such as tricyclooxyisohumulones A (**7a**, **b**) and B (**8a**, **b**), and 4′-hydroxyallohumulinones (**6a-c**, **c′**) were found only in the hard resin extract. This is the first study to report the existence of these α-acid-derived oxidation products in hard resin.

These findings also verify changes in the composition of the soft and hard resin constituents of hops caused by oxidation, because the concentration changes in α- and β-acid-derived oxidation products in soft and hard resins detected in this study during oxidation of hops have been clarified in our previous study.[21, 22]

**Transformation of the Constituents of Hard Resin during the Wort Boiling Process**. There has been little reported data on the compositional changes in hard resin during the wort boiling process. Thus, we investigated the constituents in the hard resin after wort boiling. Changes in the chemical composition of hard resin constituents were

determined by using a model boiling experiment conducted in an aqueous buffer (pH 5.5). HPLC analysis of the hard resin after 90 min of boiling (Figure 3B) clearly showed the transformation of xathohumol (**10**) into isoxanthohumol (**11**), which was reported previously.[20] In addition, the HPLC analysis also detected a decrease in the concentration of 4′-hydroxyallohumulinones (4′-hydroxyallocohumulinone (**6a**), 4′-hydroxyallo-*n*-humulinone (**6b**) and 4′-hydroxyalloadhumulinone (**6c/c′**)), and the appearance of some new compounds (Figure 3B). These findings suggested, for the first time, further transformation of 4′-hydroxyallohumulinones in the hard resin during the wort boiling process.

**Isolation and Structural Elucidation of the Transformation Products of 4′-hydroxyallocohumulinone (6a) and 4′-hydroxyallo-*n*-humulinone (6b).** To verify the transformation of 4′-hydroxyallohumulinones into new compounds, isolated 4′-hydroxyallocohumulinone (**6a**) and 4′-hydroxyallo-*n*-humulinone (**6b**) were boiled in the same buffer. The HPLC analyses clearly showed the transformation of **6a** into **12a** and **13a** (Figure 4A1 and A2) and of **6b** into **12b** and **13b** (Figure 4B1 and B2). We also boiled tricyclooxyiso-*n*-humulones A (**7b**) and B (**8b**), but the compounds were stable (i.e., unchanged) under these conditions (data not shown).

To identify the transformation products of 4′-hydroxyallocohumulinone (**6a**) and 4′-hydroxyallo-*n*-humulinone (**6b**), each 200 mg sample of isolated **6a** and **6b** was subjected to the boiling test. The compounds in the reaction products were purified using two phase solvent partition and preparative ODS HPLC, affording **12a** (33.4 mg) and **13a** (4.0 mg) from **6a**, and **12b** (41.3 mg) and **13b** (5.9 mg) from **6b**.

Compound **12b** was obtained as a pale yellow oil. The HRESIMS data showed an $[M-H]^-$ ion at *m/z* 393.1911, and together with the $^{13}C$ NMR data, indicated a molecular formula of $C_{21}H_{30}O_7$, which is identical to that of **6b**. Analysis of the $^1H$, $^{13}C$ NMR, $^1H$-$^1H$ COSY, HMQC and HMBC spectra of **12b** in methanol-$d_4$ established that **12b** and **6b** possessed the same planar structure, whereas the $^{13}C$ NMR chemical shifts of C-5 and

C-1″ were different between **12b** (δ 85.1 (C-5) and δ 36.7 (C-1″)) and **6b** (δ 81.6 (C-5) and δ 31.3 (C-1″))[21] (Table 1). Furthermore, the NOE observed between H-2′ and H-1″ in **6b**[21] was not observed in **12b**. From these results, **12b** was determined to be the C-5 epimer of **6b**, and named 4′-hydroxyallo-*cis*-*n*-humulinone, which has not been previously reported (Figure 5).

Compound **13b** was obtained as a pale yellow oil. The HRESIMS data showed an $[M - H]^-$ ion at *m/z* 281.1390, and together with the $^{13}C$ NMR data, indicated a molecular formula of $C_{15}H_{22}O_5$ (**12b** − $C_6H_8O_2$). Analysis of the $^1H$, $^{13}C$ NMR, $^1H$-$^1H$ COSY, HMQC, and HMBC spectra of **13b** in methanol-$d_4$ established that the *trans*-4-hydroxy-4-methyl-2-pentenosyl side chain attached at C-4 in **12b** was absent in **13b** (Table 1). Thus, the planar structure of **13b** was determined as oxy-*n*-humulinic acid (Figure 5). To determine relative configurations at C-4 and C-5 in **13b**, we prepared *cis*- and *trans*-oxy-*n*-humulinic acids (epimers at C-5) by alkaline hydrolysis of *n*-humulinone (**5b**)[23] as a mixture, and then isolated each isomer using ODS preparative HPLC. By $^1H$ and $^{13}C$ NMR analysis (Table 1), one of the two isomeric compounds was identified to be **13b** and thus the other (**14b**) was determined to be the C-5 epimer of **13b**. In the NOESY experiments on **13b** and **14b**, the NOE between H-4 and H-1″ (the prenyl $CH_2$ attached at C-5) was clearly observed in **14b**, but was barely detected in **13b** (Figure S1). Furthermore, *J*-resolved HMBC experiments[24] on **13b** and **14b** showed that the absolute value of the two-bond $^{13}C$-$^1H$ coupling constant between H-4 and C-5 was greater in **13b** than in **14b** ($^2J$(C5, 4H) = 5.9 Hz in **13b**, $^2J$(C5, 4H) ≦ 3.0 Hz in **14b**) (Figure S2), indicating a larger dihedral angle between H-4 and 5-OH in **14b** than **13b**.[25] These observations clearly indicated that H-4 and 5-OH were in the α-orientation in **13b**, and that H-4 and the prenyl group attached at C-5 were in the α-orientation in **14b**. Thus, **13b** was determined as *cis*-oxy-*n*-humulinic acid and **14b** was determined as *trans*-oxy-*n*-humulinic acid (Figure 5). Although the structures of **13b** and **14b** were reported about 50 years ago,[23] we present their NMR data for the first time (Table 1).

Compound **12a** was obtained as a pale yellow oil. The HRESIMS data showed an $[M - H]^-$ ion at *m/z* 379.1753, and together with the $^{13}C$ NMR data, indicated a molecular formula of $C_{20}H_{28}O_7$, which was the same as for **6a**. Analysis of the $^1H$, $^{13}C$ NMR, $^1H$-$^1H$ COSY, HMQC, and HMBC spectra established that **12a** possessed an isobutyryl side chain at C-2 instead of an isovaleryl side chain in **12b** (Table 1). Thus, **12a** was identified as 4′-hydroxyallo-*cis*-cohumulinone, which has not been previously reported (Figure 5).

Compound **13a** was obtained as a white, amorphous solid. The HRESIMS data showed an $[M - H]^-$ ion at *m/z* 267.1230, and together with the $^{13}C$ NMR data, indicated a molecular formula of $C_{14}H_{20}O_5$ (**12a** − $C_6H_8O_2$). Analysis of the $^1H$, $^{13}C$ NMR, $^1H$-$^1H$ COSY, HMQC, and HMBC spectra established that **13a** also possessed an isobutyryl side chain at C-2 instead of an isovaleryl side chain in **13b** (Table 1). Thus, **13a** was identified as *cis*-oxycohumulinic acid, which has not been previously reported (Figure 5).

Compounds **12a**, **12b**, **13a** and **13b** were determined to be racemates because none of these compounds showed optical activity. Moreover **12a**, **12b**, **13a** and **13b** were formed *via* a simple thermal reaction in aqueous solution from the precursors **6a** and **6b,** which do exist as racemates.[21]

**Analyses of the Transformation Products of 4′-hydroxyalloadhumulinone (6c/c′).** Adhumulinone, a precursor of 4′-hydroxyalloadhumulinone, is a mixture of two diastereomers (**5c** and **5c′**) (Figure 6).[21] Thus, 4′-hydroxyalloadhumulinone in hard resin is also a mixture of two diastereomers (**6c** and **6c′**) (Figure 6). Although ODS HPLC analysis using an acidic buffer could not separate the diastereomers (**6c** and **6c′**) (Figure 4C1 and C2), we successfully separated them through ODS HPLC analysis using a basic buffer (Figure 7). By employing a mobile phase containing the basic buffer, we isolated each diastereomer successively, and named the former eluate in analytical HPLC ($t_R$ 16.6 min) as 4′-hydroxyalloadhumulinone A (**6c**) and the latter eluate ($t_R$ 17.5 min) as 4′-hydroxyalloadhumulinone B (**6c′**) (Figure 7). The $^1H$ and $^{13}C$ NMR data of **6c** and **6c′** were almost identical (Table 2), and we could not analyze the respective

stereochemistries of **6c** and **6c′**.

The yield of isolated **6c** and **6c′** was too low to enable the separation of their transformation products in the boiling tests and perform structural determination by NMR analyses. Thus, we tentatively identified **12c** (main transformed product of **6c**) as 4′-hydroxyallo-*cis*-adhumulinone A and **13c** (minor transformed product of **6c**) as *cis*-oxyadhumulinic acid A, by comparison with the transformation products from 4′-hydroxyallocohumulinone (**6a**) and 4′-hydroxyallo-*n*-humulinone (**6b**) (Figure 4C3). Similarly, **12c′** and **13c′** (transformed products of **6c′**) were tentatively identified as 4′-hydroxyallo-*cis*-adhumulinone B and *cis*-oxyadhumulinic acid B, respectively (Figure 4C4).

The HPLC analysis of isolated **6c** demonstrated that it did not contain **6c′** (Figure 7B). Moreover, HPLC analysis of isolated **6c′** also showed that it did not contain **6c** (Figure 7C). However, the boiling test on **6c** afforded a small amount of **12c′** and **13c′** in the HPLC analysis (Figure 4C3). A small quantity of **12c** and **13c** was also obtained in the boiling test of **6c′** (Figure 4C4). These results suggested that the configuration at C-2‴ in **6c**, **6c′**, **12c**, **12c′**, **13c** and **13c′** may convert through proton exchange of H-2‴ during the boiling process. Figure 8 shows a plausible thermally induced epimerization of **12c** into the enantiomer of **12c′** and of **12c′** into the enantiomer of **12c**.

**Quantitative Analyses of 4′-Hydroxyallohumulinones and Their Transformation Products during the Boiling Process.** We aimed to quantitatively evaluate the changes in 4′-hydroxyallohumulinones and their transformation products during boiling. Thus, boiling solutions of **6a**, **6b**, and **6c**/**c′** were sampled at 30, 60, 90 and 120 min and analyzed by HPLC. Figure 9 shows the time dependent decrease in 4′-hydroxyallohumulinones, and compositional changes in the transformation products. The degree of conversion of 4′-hydroxyallo-*cis*-humulinones (**12a, 12b,** and **12c/c′**) increased until around 60 min and then slowly decreased thereafter. By contrast, the amount of *cis*-oxyhumulinic acids (**13a, 13b, and 13c/c′**) increased in inverse proportion

to the decrease in the amount of 4′-hydroxyallo-*cis*-humulinones.

These results suggested that during boiling, 4′-hydroxyallohumulinones (**6a-c**, **c′**) primarily isomerized into 4′-hydroxyallo-*cis*-humulinones (**12a-c, c′**), which then decomposed to *cis*-oxyhumulinic acids (**13a-c, c′**). The total amount of generated 4′-hydroxyallo-*cis*-humulinones and *cis*-oxyhumulinic acids accounted for 40 to 50% of the decomposed 4′-hydroxyallohumulinones. Our analysis indicates that under these experimental conditions about 10-15% of ad-congener substrates (**6c** and **6c′**) are epimerized through proton exchange at C-2‴ (calculated from the ratio of **12c** and **12c′** generated from **6c** (Figure 9C) and of **12c′** and **12c** generated from **6c′** (Figure 9D)). This is the first study to report time-dependent changes of α-acid-derived oxidation products in the hard resin during boiling. We believe these findings will be extremely valuable when evaluating effects of the hard resin generated through the oxidation of hops on beer properties.

In conclusion, we have clarified the main constituents, other than α-acids and β-acids, of the soft and hard resins from stored hops. Furthermore, we investigated the compositional changes in the hard resin through wort boiling using model experiments, and found 4′-hydroxyallohumulinones (main constituents of the hard resin) were transformed to 4′-hydroxyallo-*cis*-humulinones and *cis*-oxyhumulinic acids. We believe our results will help to systematically evaluate the hard resin in order to optimize the effects of the hard resin on beer quality.

**ACKNOWLEDGEMENT**

We thank members of our research groups for valuable discussions, especially Mikio Katayama and Yasuji Kawachi.

Supporting Information Available: The NOESY spectra and *J*-HMBC spectra of compounds **13b** and **14b**. This material is available free of charge via the Internet at http://pubs.acs.org.

## REFERENCES

(1) CURRENT REVIEW. *J. Inst. Brew.* **1957**, *63*, 281-293.

(2) Krauss, G.; Cook, A. H.; Verzele, M. Recommendations concerning nomenclature of hop resin components. *J. Inst. Brew., London* **1969**, *75*, 340-342.

(3) Methods: 7.5 Bitter substances in hops and hop products: Lead conductance value and total resin, soft resin and hard resin, In Analytica-EBC, European Brewery Convention, Carl Getränke-Fachverlag: Nürnberg, Germany, 2000.

(4) Palamand, S. R.; Aldenhoff, J. M. Bitter tasting compounds of beer. Chemistry and taste properties of some hop resin compounds. *J. Agr. Food Chem.* **1973**, *21*, 535-543.

(5) Urban, J.; Dahlberg, C. J.; Carroll, B. J.; Kaminsky, W. Absolute Configuration of Beer's Bitter Compounds. *Angew. Chem., Int. Ed.* **2013**, *52*, 1553-1555.

(6) Kowaka, M.; Kokubo, E. Composition of bitter substances of hops and characteristics of beer bitterness. *J. Am. Soc. Brew. Chem.* **1977**, *35*, 16-21.

(7) De Keukeleire, D. Fundamentals of beer and hop chemistry. *Quim. Nova* **2000**, *23*, 108-112.

(8) Bishop, L. R.; Whitear, A. L.; Inman, W. R. Scientific basis for beer foam formation and cling. *J. Inst. Brew., London* **1974**, *80*, 68-80.

(9) Haseleu, G.; Intelmann, D.; Hofmann, T. Structure determination and sensory evaluation of novel bitter compounds formed from β-acids of hop (*Humulus lupulus* L.) upon wort boiling. *Food Chem.* **2009**, *116*, 71-81.

(10) Haseleu, G.; Intelmann, D.; Hofmann, T. Identification and RP-HPLC-ESI-MS/MS Quantitation of Bitter-Tasting β-Acid Transformation Products in Beer. *J. Agric. Food Chem.* **2009**, *57*, 7480-7489.

(11) Almaguer, C.; Gastl, M.; Arendt, E. K.; Becker, T. Contributions of hop hard resins to beer quality. *BrewingSci. - Monatsschr. Brauwiss.* **2012**, *65*, 118-129.

(12) Burton, J. S.; Stevens, R. Evaluation of hops. XI. The hard resin and presence of hulupinic acid. *J. Inst. Brew.* **1965**, *71*, 51-56.

(13) Laws, D. R. J. Hops resins and beer flavor. V. The significance of oxidized hop resins in brewing. *J. Inst. Brew.* **1968**, *74*, 178-182.

(14) Ono, M.; Kakudo, Y.; Yamamoto, R.; Nagami, K.; Kumada, J. Simultaneous analysis of hop bittering components by high-performance liquid chromatography. II. Evaluation of hop deterioration. *J. Am. Soc. Brew. Chem.* **1987**, *45*, 61-69.

(15) Foster, R. T., II; Weber, K.; Jangaard, N. O. The effect of hop acids transformation on kettle utilization and finished beer flavor and aroma. *Tech. Q. - Master Brew. Assoc. Am.* **1981**, *18*, 109-115.

(16) Aitken, R. A.; Bruce, A.; Harris, J. O.; Seaton, J. C. The bitterness of hop-derived materials in beer. *J. Inst. Brew., London* **1970**, *76*, 29-36.

(17) Whitear, A. L. Changes in resin composition and brewing behavior of hops during storage. *J. Inst. Brew.* **1966**, *72*, 177-183.

(18) Howard, G. A.; Martin, P. A. Bittering power of stored hops. *J. Inst. Brew.* **1964**, *70*, 424-439.

(19) Rigby, F. L. The practical significance of recent developments in the chemical analysis of hops. *The Brewers Digest* **1958**, 50-59.

(20) Stevens, J. F.; Taylor, A. W.; Clawson, J. E.; Deinzer, M. L. Fate of Xanthohumol and Related Prenylflavonoids from Hops to Beer. *J. Agric. Food Chem.* **1999**, *47*, 2421-2428.

(21) Taniguchi, Y.; Matsukura, Y.; Ozaki, H.; Nishimura, K.; Shindo, K. Identification and quantification of the oxidation products derived from α-acids and β-acids during storage of hops (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2013**, *61*, 3121-3130.

(22) Taniguchi, Y.; Taniguchi, H.; Matsukura, Y.; Kawachi, Y.; Shindo, K. Structural Elucidation of Humulone Autoxidation Products and Analysis of Their Occurrence in Stored Hops. *J. Nat. Prod.* **2014**, *77*, 1252-1261.

(23) Dierckens, J.; Verzele, M. Oxidation products of humulone and their stereoisomerism. *J. Inst. Brew., London* **1969**, *75*, 453-456.

(24) Furihata, K.; Seto, H. J-Resolved HMBC, a new NMR technique for measuring

494 heteronuclear long-range coupling constants. *Tetrahedron Lett.* **1999**, *40*, 6271-6275.
495 (25) Morvai, M.; Nagy, T.; Kocsis, A.; Szabo, L. F.; Podanyi, B. Effect of oxygen
496 substituents on two- and three-bond carbon-proton spin-spin coupling constants. *Magn.*
497 *Reson. Chem.* **2000**, *38*, 343-359.
498
499

## FIGURE CAPTIONS

**Figure 1.** Structures of α-acids: cohumulone (**1a**), *n*-humulone (**1b**), adhumulone (**1c**) and β-acids: colupulone (**2a**), *n*-lupulone (**2b**), adlupulone (**2c**) and *cis*-iso-α-acids: *cis*-isocohumulone (**3a**), *cis*-iso-*n*-humulone (**3b**), *cis*-isoadhumulone (**3c**) and *trans*-iso-α-acids: *trans*-isocohumulone (**4a**), *trans*-iso-*n*-humulone (**4b**), *trans*-isoadhumulone (**4c**) and humulinones: cohumulinone (**5a**), *n*-humulinone (**5b**), adhumulinone (**5c**) and 4′-hydroxyallohumulinones: 4′-hydroxyallocohumulinone (**6a**), 4′-hydroxyallo-*n*-humulinone (**6b**), 4′-hydroxyalloadhumulinone (**6c**) and tricyclooxyisohumulones A: tricyclooxyisocohumulone A (**7a**), tricyclooxyiso-*n*-humulone A (**7b**), tricyclooxyisoadhumulone A (**7c**) and tricyclooxyisohumulones B: tricyclooxyisocohumulone B (**8a**), tricyclooxyiso-*n*-humulone B (**8b**), tricyclooxyisoadhumulone B (**8c**) and hulupones: cohulupone (**9a**), *n*-hulupone (**9b**), adhulupone (**9c**) and xanthohumol (**10**) and isoxanthohumol (**11**).

**Figure 2.** HPLC chromatograms of the MeOH extract of hops stored at 60 °C for 48 h (A), the soft resin fraction prepared from the MeOH extract (B), and the hard resin fraction prepared from the MeOH extract (C). Structures of the compounds are given in Figures 1 and 6.

**Figure 3.** HPLC chromatograms of the hard resin (A) before and (B) after boiling in an aqueous buffer (pH 5.5) for 90 min. Structures of the compounds are given in Figures 1, 5, and 6.

**Figure 4.** HPLC chromatograms of the isolated compounds before and after boiling in an aqueous buffer (pH 5.5) for 90 min. 4′-Hydroxyallocohumulinone (**6a**) (A1) before and

(A2) after boiling; 4′-hydroxyallo-*n*-humulinone (**6b**) (B1) before and (B2) after boiling; 4′-hydroxyalloadhumulinones A (**6c**) and B (**6c**′) (C1 and C2, respectively) before and (C3 and C4, respectively) after boiling.

**Figure 5.** Chemical structures of 4′-hydroxyallo-*cis*-cohumulinone (**12a**), 4′-hydroxyallo-*cis*-*n*-humulinone (**12b**), *cis*-oxycohumulinic acid (**13a**), *cis*-oxy-*n*-humulinic acid (**13b**), and *trans*-oxy-*n*-humulinic acid (**14b**).

**Figure 6.** Chemical structures of adhumulinone diastereomers (**5c** and **5c′**) and 4′-hydroxyalloadhumulinones A and B (**6c** and **6c′**).

**Figure 7.** HPLC chromatograms of 4′-hydroxyalloadhumulinones A and B (**6c** and **6c′**, respectively) in the mobile phase using a basic aqueous buffer before (A) and after (B and C) their isolation.

**Figure 8.** Proposed epimerization of 4′-hydroxyallo-*cis*-adhumulinones A and B (**12c** and **12c′**) through the proton exchange at C-2‴ as a result of boiling the sample.

**Figure 9.** Concentration changes in 4′-hydroxyallocohumulinone (**6a**), 4′-hydroxyallo-*cis*-cohumulinone (**12a**), and *cis*-oxycohumulinic acid (**13a**) (A) and in 4′-hydroxyallo-*n*-humulinone (**6b**), 4′-hydroxyallo-*cis*-*n*-humulinone (**12b**), and *cis*-oxy-*n*-humulinic acid (**13b**) (B) and in 4′-hydroxyalloadhumulinone A (**6c**), 4′-hydroxyallo-*cis*-adhumulinones A and B (**12c** and **c**′), and *cis*-oxyadhumulinic acids A and B (**13c** and **c**′) (C) and in 4′-hydroxyalloadhumulinone B (**6c**′), 4′-hydroxyallo-*cis*-adhumulinones A and B (**12c** and **c**′), and *cis*-oxyadhumulinic acids A and B (**13c** and **c**′) (D) during boiling in an aqueous buffer (pH 5.5).

**Table 1. NMR Spectroscopic Data (400 MHz, Methanol-$d_4$) for Compounds 12a, 12b, 13a, 13b, and 14b[a]**

| | 12a | | 12b | | 13a | | 13b | | 14b | |
|---|---|---|---|---|---|---|---|---|---|---|
| pos. | $\delta_C$, type | $\delta_H$, mult. (*J* in Hz) | $\delta_C$, type | $\delta_H$, mult. (*J* in Hz) | $\delta_C$, type | $\delta_H$, mult. (*J* in Hz) | $\delta_C$, type | $\delta_H$, mult. (*J* in Hz) | $\delta_C$, type | $\delta_H$, mult. (*J* in Hz) |
| 1 | 200.9, C | - | 201.6, C | - | 199.6, C | - | 200.2, C | - | 200.5, C | - |
| 2 | 113.3, C | - | 114.1, C | - | 111.6, C | - | 112.6, C | - | 112.8, C | - |
| 3 | 195.4, C | - | 196.0, C | - | 197.8, C | - | 198.3, C | - | 199.8, C | - |
| 4 | 92.0, C | - | 92.3, C | - | 80.6, CH | 4.35, s | 80.9, CH | 4.35, s | 74.2, CH | 4.14, s |
| 5 | 84.9, C | - | 85.1, C | - | 82.2, C | - | 82.4, C | - | 77.7, C | - |
| 1' | 198.2, C | - | 198.3, C | - | - | - | - | - | - | - |
| 2' | 121.9, CH | 6.78, d (15.5) | 121.9, CH | 6.79, d (15.5) | - | - | - | - | - | - |
| 3' | 155.9, CH | 6.92, d (15.5) | 156.0, CH | 6.92, d (15.5) | - | - | - | - | - | - |
| 4' | 71.4, C | - | 71.4, C | - | - | - | - | - | - | - |
| 5' | 29.1, $CH_3$ | 1.27, s | 29.1, $CH_3$ | 1.28, s | - | - | - | - | - | - |
| 6' | 29.1, $CH_3$ | 1.29, s | 29.1, $CH_3$ | 1.29, s | - | - | - | - | - | - |
| 1" | 36.79, $CH_2$ | 2.63, dd (13.9, 8.7)<br>2.48, dd (13.9, 6.6) | 36.7, $CH_2$ | 2.62, dd (14.0, 8.5)<br>2.45, dd (14.0, 6.7) | 34.8, $CH_2$ | 2.61, dd (13.5, 9.2)<br>2.45, dd (13.5, 6.4) | 34.8, $CH_2$ | 2.60, dd (13.6, 9.1)<br>2.43, dd (13.6, 6.4) | 34.6, $CH_2$ | 2.54, dd (14.0, 7.7)<br>2.48, dd (14.0, 6.9) |
| 2" | 119.1, CH | 5.10, m | 119.0, CH | 5.10, m | 119.3, CH | 5.03, m | 119.2, CH | 5.02, m | 118.3, CH | 5.03, m |
| 3" | 136.6, C | - | 136.6, C | - | 136.5, C | - | 136.4, C | - | 137.6, C | - |
| 4" | 17.9, $CH_3$ | 1.52, s | 17.9, $CH_3$ | 1.53, s | 17.8, $CH_3$ | 1.52, s | 17.9, $CH_3$ | 1.52, s | 18.1, $CH_3$ | 1.65, s |
| 5" | 26.2, $CH_3$ | 1.60, s | 26.2, $CH_3$ | 1.61, s | 26.1, $CH_3$ | 1.58, s | 26.1, $CH_3$ | 1.58, s | 26.1, $CH_3$ | 1.68, s |
| 1"' | 203.5, C | - | 197.8, C | - | 205.2, C | - | 199.9, C | - | 201.9, C | - |
| 2"' | 36.75, CH | 3.50, sep (6.9) | 46.6, $CH_2$ | 2.74, dd (13.6, 7.2)<br>2.69, dd (13.6, 7.2) | 37.3, CH | 3.49, sep (6.9) | 47.7, $CH_2$ | 2.77, dd (13.8, 7.0)<br>2.64, dd (13.8, 7.1) | 48.4, $CH_2$ | 2.78, dd (14.5, 7.0)<br>2.73, dd (14.5, 6.9) |
| 3"' | 18.0, $CH_3$ | 1.10, d (6.9) | 27.6, CH | 2.11, m | 17.8, $CH_3$ | 1.09, d (6.9) | 27.1, CH | 2.11, m | 26.7, CH | 2.13, m |
| 4"' | 18.6, $CH_3$ | 1.14, d (6.9) | 22.86, $CH_3$ | 0.95, d (6.7) | 18.7, $CH_3$ | 1.12, d (6.9) | 22.8, $CH_3$ | 0.94, d (6.2) | 22.8, $CH_3$ | 0.96, d (6.7) |
| 5"' | - | - | 22.94, $CH_3$ | 0.97, d (6.7) | - | - | 22.9, $CH_3$ | 0.96, d (6.2) | 22.9, $CH_3$ | 0.96, d (6.7) |

[a] Arbitrary numbering according to structures **12a**, **12b**, **13a**, **13b** and **14b** shown in Figure 5.

**Table 2. NMR Spectroscopic Data (400 MHz, Methanol-$d_4$) for Compounds 6c and 6c′[a]**

| | 6c | | 6c′ | |
|---|---|---|---|---|
| pos. | $\delta_C$, type | $\delta_H$, mult. (*J* in Hz) | $\delta_C$, type | $\delta_H$, mult. (*J* in Hz) |
| 1 | 201.8, C | - | 201.6, C | - |
| 2 | 111.3, C | - | 111.3, C | - |
| 3 | 198.9, C | - | 199.0, C | - |
| 4 | 90.1, C | - | 89.9, C | - |
| 5 | 81.6, C | - | 81.6, C | - |
| 1' | 200.0, C | - | 200.0, C | - |
| 2' | 121.4, CH | 7.03, d (15.7) | 121.4, CH | 7.04, d (15.7) |
| 3' | 155.8, CH | 6.87, d (15.7) | 155.8, CH | 6.87, d (15.7) |
| 4' | 71.4, C | - | 71.4, C | - |
| 5' | 29.3, $CH_3$ | 1.31, s | 29.3, $CH_3$ | 1.31, s |
| 6' | 29.4, $CH_3$ | 1.31, s | 29.4, $CH_3$ | 1.31, s |
| 1" | 31.0, $CH_2$ | 2.47, dd (14.8, 4.7)<br>2.42, dd (14.8, 8.9) | 31.1, $CH_2$ | 2.47, dd (15.1, 5.5)<br>2.41, dd (15.1, 8.5) |
| 2" | 118.9, CH | 5.30, m | 118.9, CH | 5.30, m |
| 3" | 135.5, C | - | 135.5, C | - |
| 4" | 18.1, $CH_3$ | 1.41, s | 18.1, $CH_3$ | 1.41, s |
| 5" | 26.1, $CH_3$ | 1.62, s | 26.1, $CH_3$ | 1.62, s |
| 1"' | 204.1, C | - | 204.2, C | - |
| 2"' | 42.6, CH | 3.46, ddq (6.8, 6.8, 6.8) | 42.7, CH | 3.44, ddq (6.8, 6.8, 6.8) |
| 3"' | 16.0, $CH_3$ | 1.13, d (6.8) | 15.9, $CH_3$ | 1.09, d (6.8) |
| 4"' | 27.3, $CH_2$ | 1.64-1.74, m<br>1.36-1.46, m | 27.3, $CH_2$ | 1.70-1.80, m<br>1.40-1.50, m |
| 5"' | 11.9, $CH_3$ | 0.87, dd (7.4, 7.4) | 12.0, $CH_3$ | 0.92, dd (7.4, 7.4) |

[a] Arbitrary numbering according to structures **6c** and **6c′** shown in Figure 6.

**Figure 1**

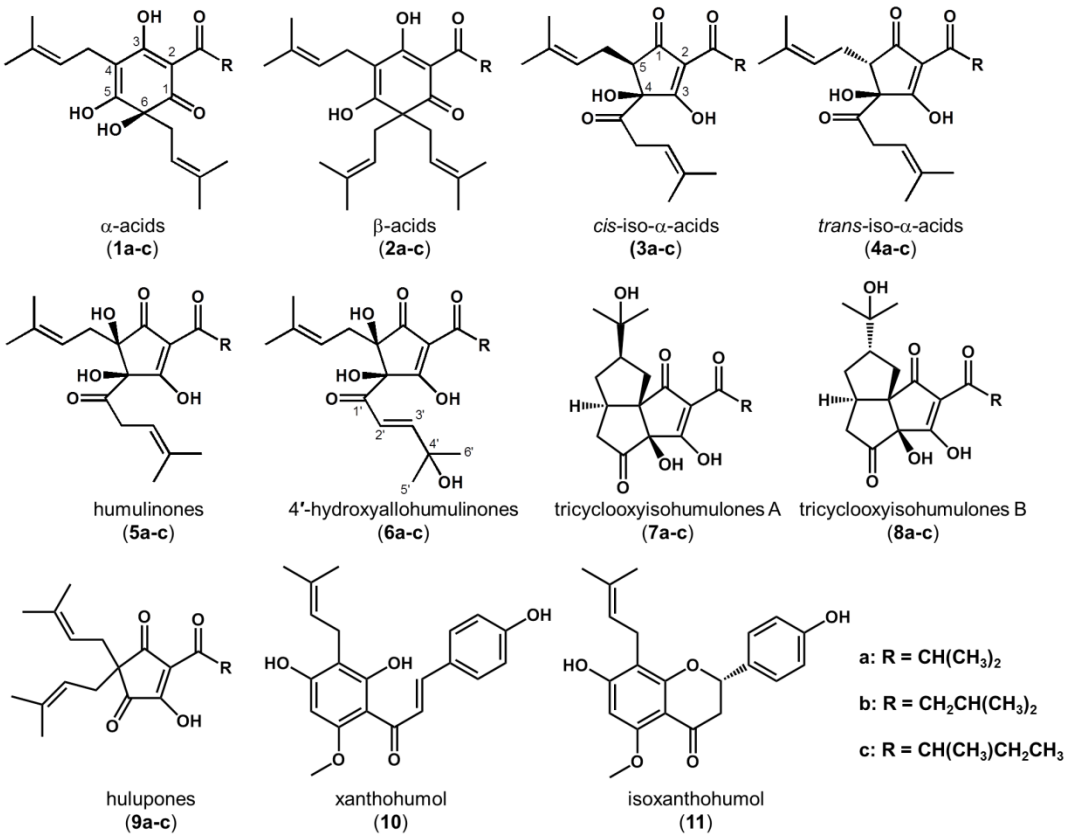

α-acids
(1a-c)
β-acids
(2a-c)
cis-iso-α-acids
(3a-c)
trans-iso-α-acids
(4a-c)
humulinones
(5a-c)
4′-hydroxyallohumulinones
(6a-c)
tricyclooxyisohumulones A
(7a-c)
tricyclooxyisohumulones B
(8a-c)
hulupones
(9a-c)
xanthohumol
(10)
isoxanthohumol
(11)
a: R = CH(CH3)2
b: R = CH2CH(CH3)2
c: R = CH(CH3)CH2CH3

**Figure 2**

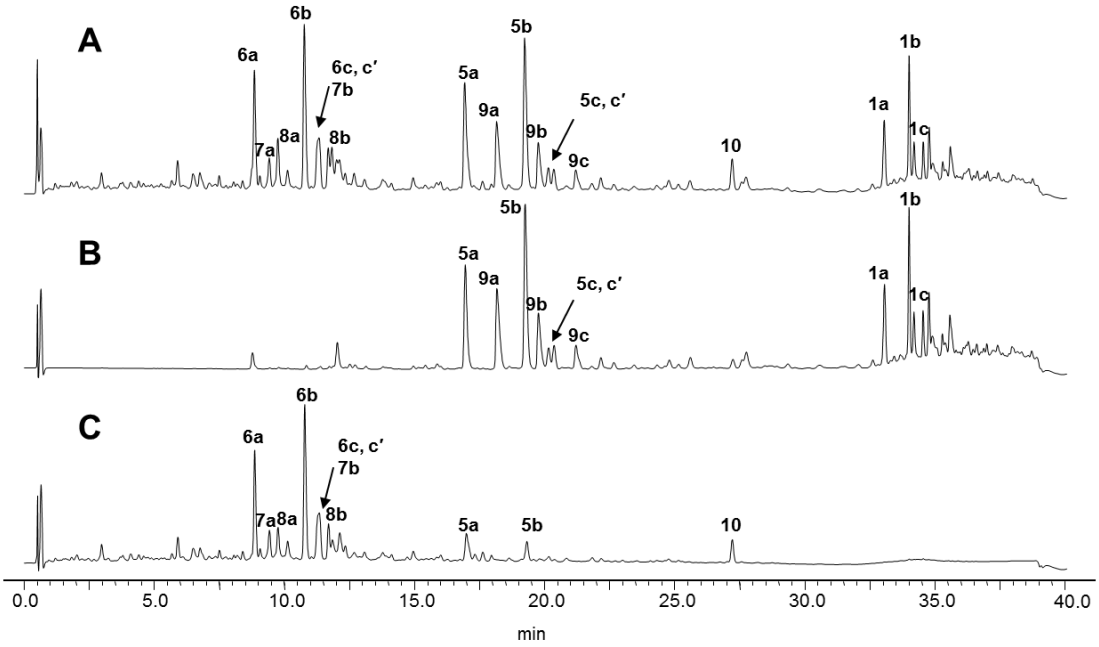

A
6a
6b
6c, c′
7b
7a
8a
8b
5a
9a
5b
9b
5c, c′
9c
10
1a
1b
1c
B
5a
9a
5b
9b
5c, c′
9c
1a
1b
1c
C
6a
6b
6c, c′
7b
7a
8a
8b
5a
5b
10
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

**Figure 3**

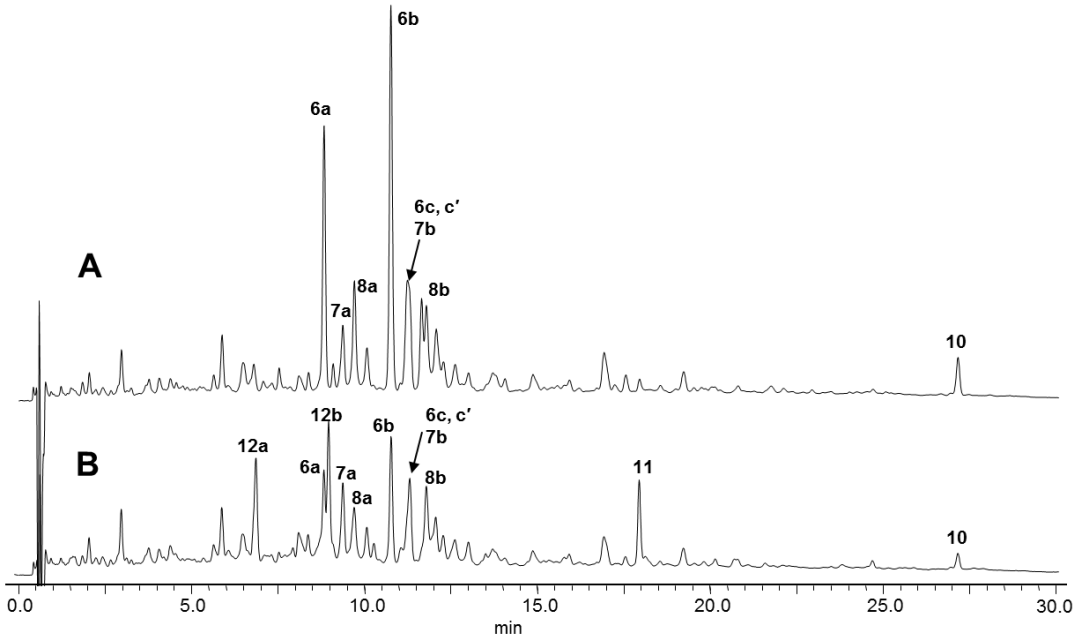

A
6b
6a
6c, c′
7b
8a
8b
7a
10
B
12b
6b
6c, c′
7b
12a
6a
7a
8a
8b
11
10
0.0
5.0
10.0
15.0
20.0
25.0
30.0
min

**Figure 4**

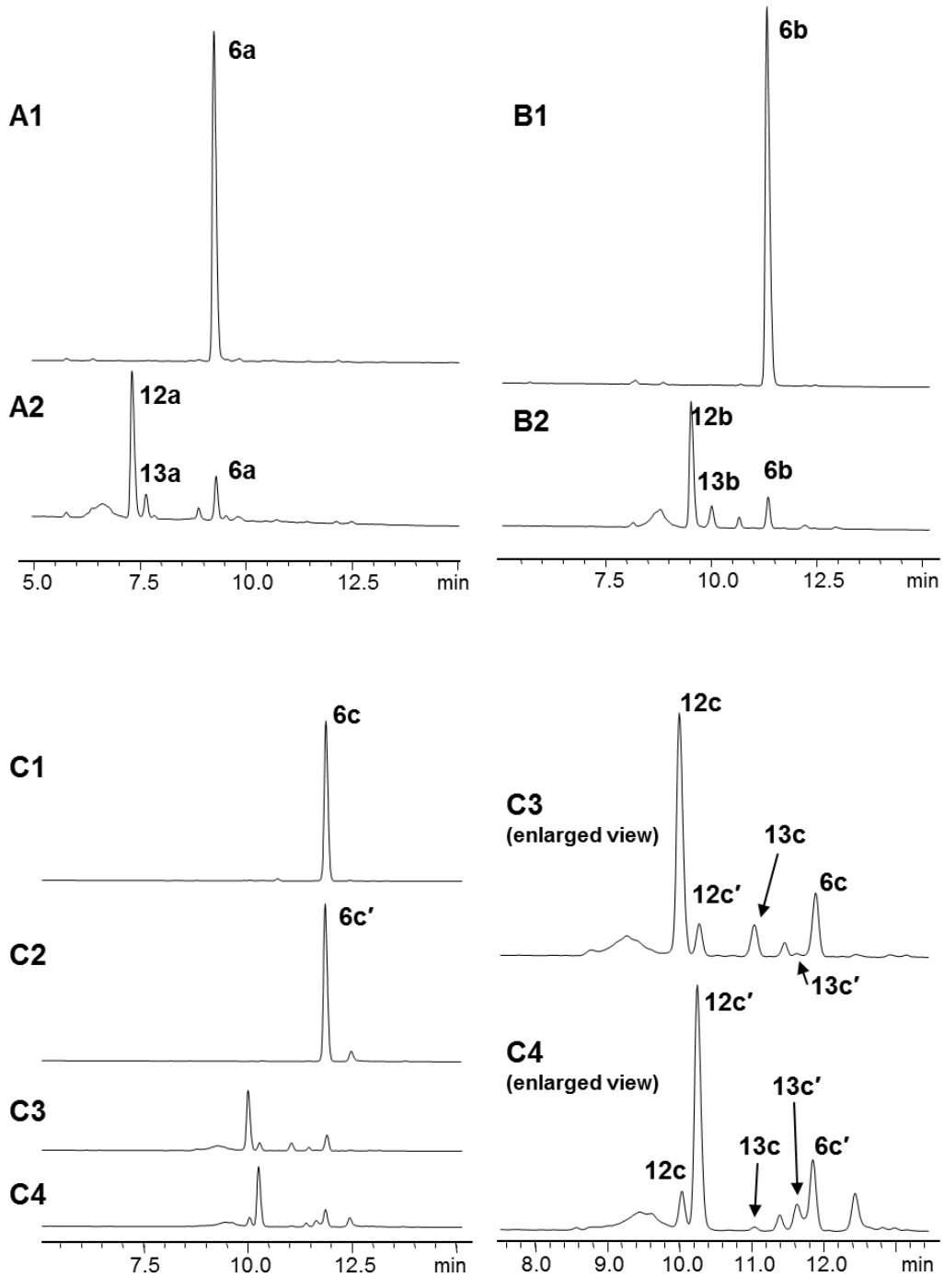

A1
6a
A2
12a
13a
6a
5.0
7.5
10.0
12.5
min
B1
6b
B2
12b
13b
6b
7.5
10.0
12.5
min
C1
6c
C2
6c′
C3
C4
7.5
10.0
12.5
min
C3
(enlarged view)
12c
12c′
13c
6c
13c′
C4
(enlarged view)
12c′
13c′
13c
6c′
12c
8.0
9.0
10.0
11.0
12.0
min

**Figure 5**

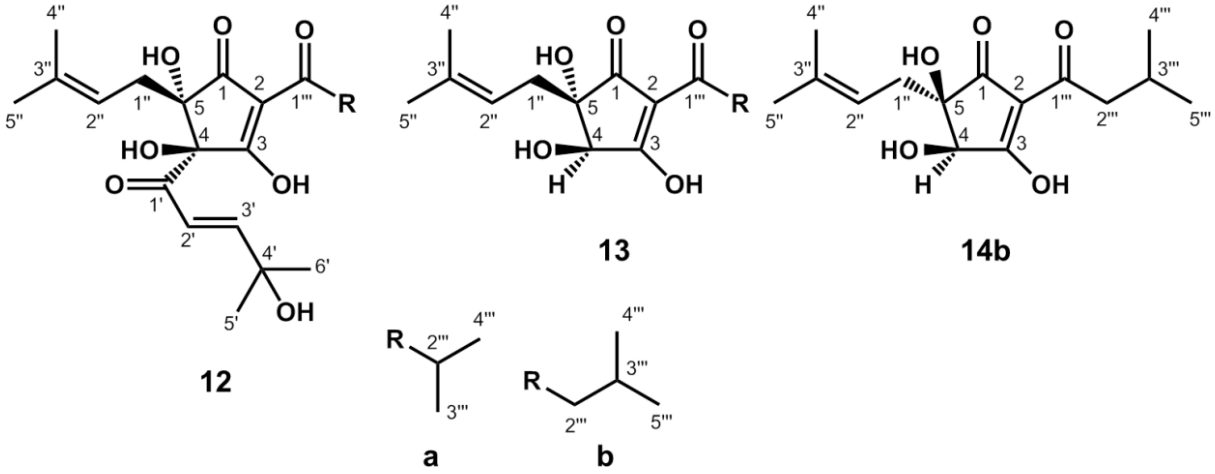
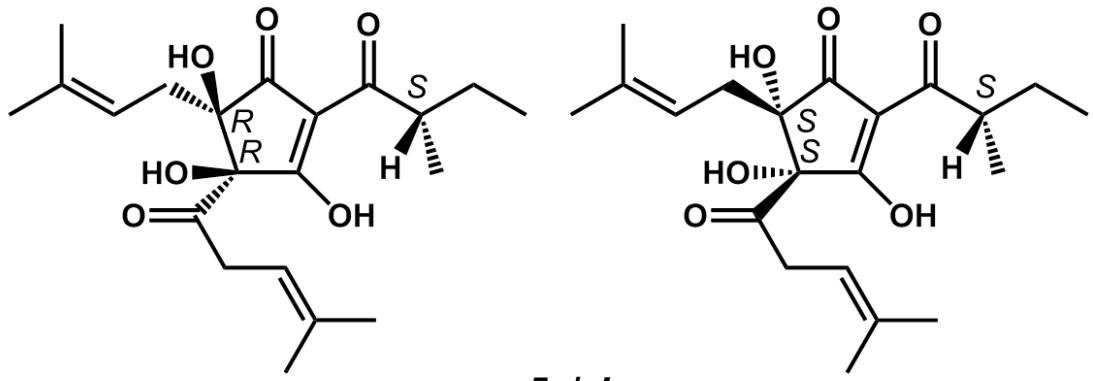
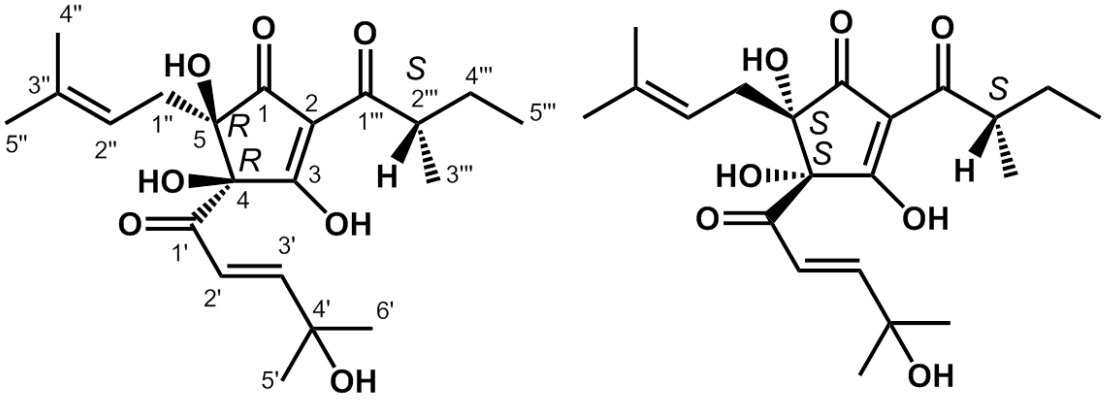


**Figure 6**




5c/c′




6c/c′

**Figure 7**

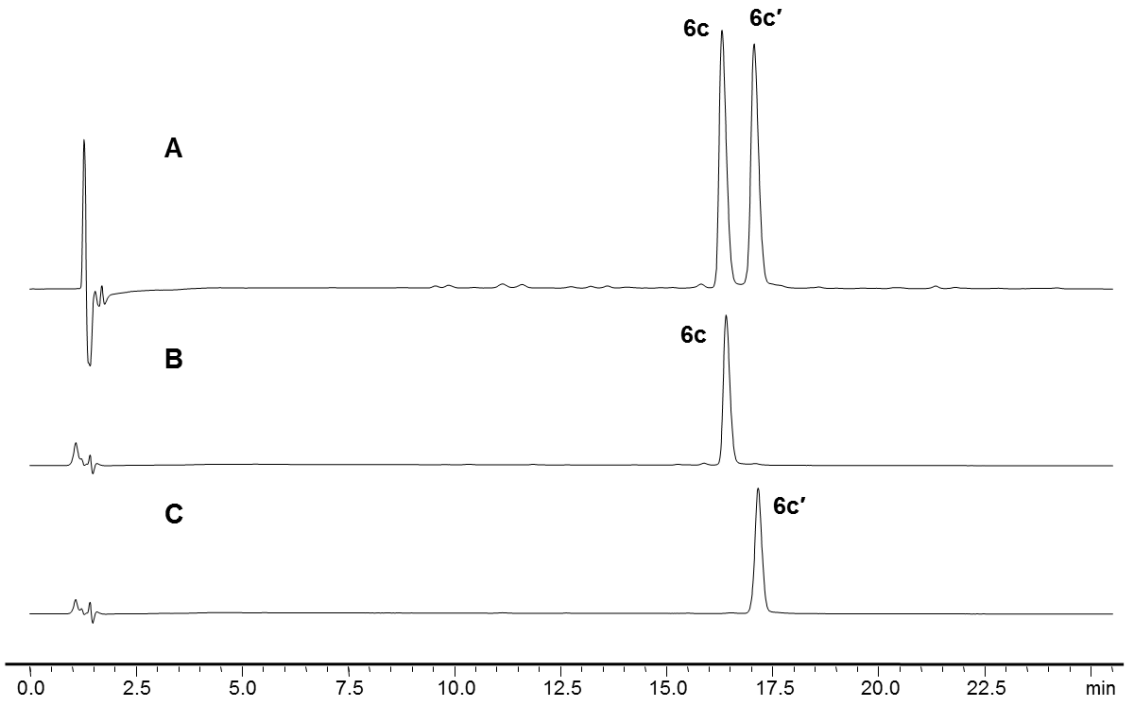


**Figure 8**

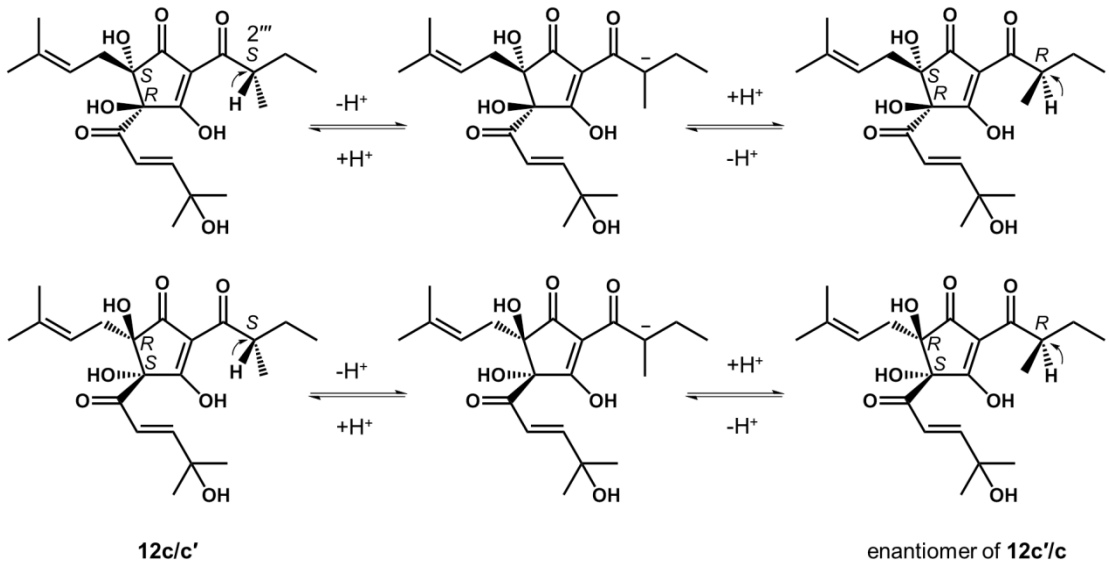
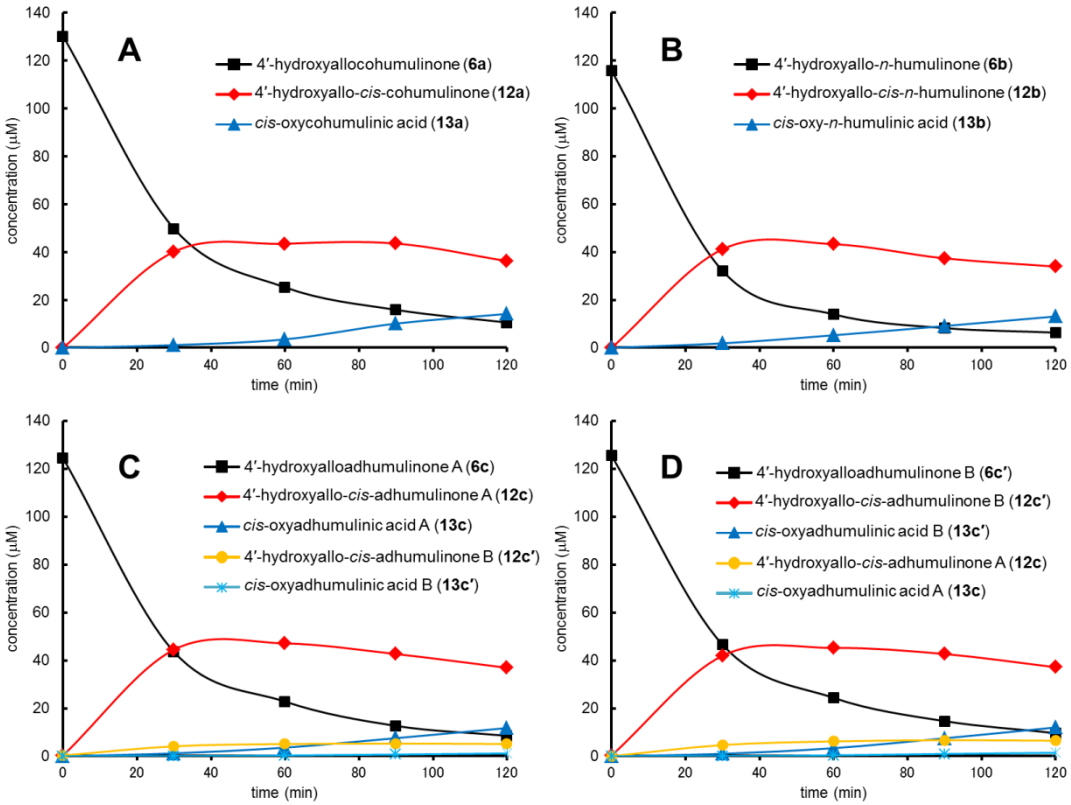
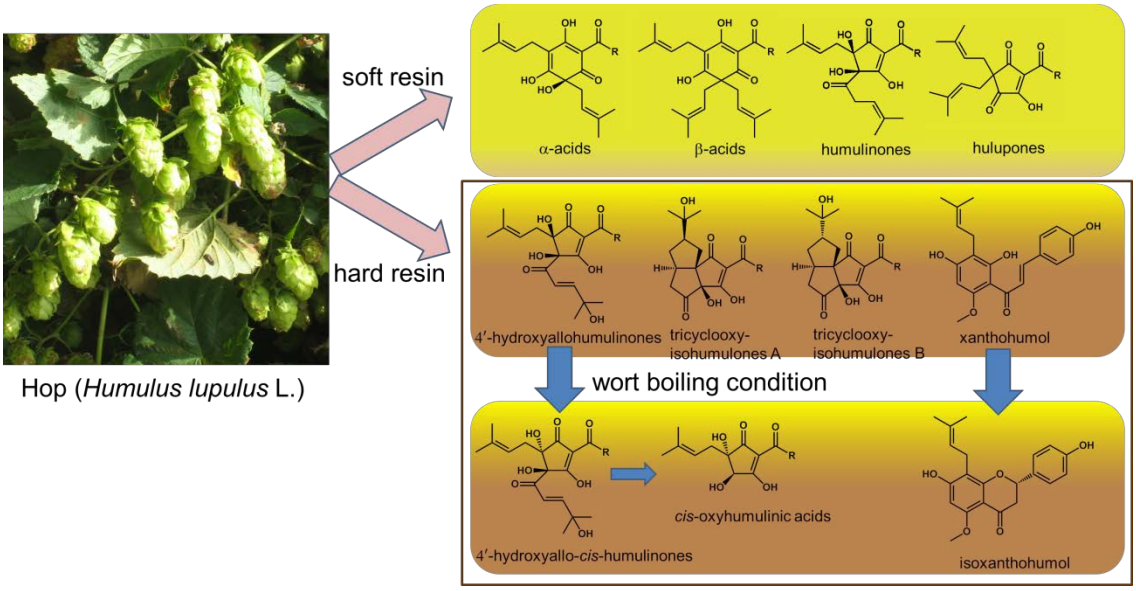


**Figure 9**


A
4′-hydroxyallocohumulinone (6a)
4′-hydroxyallo-cis-cohumulinone (12a)
cis-oxycohumulinic acid (13a)
B
4′-hydroxyallo-n-humulinone (6b)
4′-hydroxyallo-cis-n-humulinone (12b)
cis-oxy-n-humulinic acid (13b)
C
4′-hydroxyalloadhumulinone A (6c)
4′-hydroxyallo-cis-adhumulinone A (12c)
cis-oxyadhumulinic acid A (13c)
4′-hydroxyallo-cis-adhumulinone B (12c′)
cis-oxyadhumulinic acid B (13c′)
D
4′-hydroxyalloadhumulinone B (6c′)
4′-hydroxyallo-cis-adhumulinone B (12c′)
cis-oxyadhumulinic acid B (13c′)
4′-hydroxyallo-cis-adhumulinone A (12c)
cis-oxyadhumulinic acid A (13c)
concentration (μM)
time (min)

## TABLE OF CONTENTS GRAPHICS




Hop (*Humulus lupulus* L.)