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Sensomics Analysis of Key Bitter Compounds in the Hard Resin of Hops (*Humulus lupulus* L.) and Their Contribution to the Bitter Profile of Pilsner-Type Beer

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Supporting Information

ABSTRACT: Recent brewing trials indicated the occurrence of valuable bitter compounds in the hard resin fraction of hop. Aiming at the discovery of these compounds, hop's ε-resin was separated by means of a sensory guided fractionation approach and the key taste molecules were identified by means of UV/vis, LC-TOF-MS, and 1D/2D-NMR studies as well as synthetic experiments. Besides a series of literature known xanthohumol derivatives, multifidol glucosides, flavon-3-on glycosides, and *p*-coumaric acid esters, a total of 11 bitter tastants are reported for the first time, namely, 1″,2″-dihydroxanthohumol F, 4′-hydroxytunicatachalcone, isoxantholupon, 1-methoxy-4-prenylphloroglucinol, dihydrocyclohumulohydrochinone, xanthohumols M, N, and P, and isoxanthohumols M, N, and P, respectively. Human sensory analysis revealed low bitter recognition threshold concentrations ranging from 5 (co-multifidol glucopyranoside) to 198 μmol/L (*trans*-*p*-coumaric acid ethyl ester) depending on their chemical structure. For the first time, LC-MS/MS quantitation of these taste compounds in Pilsner-type beer, followed by taste re-engineering experiments, revealed the additive contribution of iso-α-acids and the identified hard resin components to be truly necessary and sufficient for constructing the authentic bitter percept of beer. Finally, brewing trails using the ε-resin as the only hop source impressively demonstrated the possibility to produce beverages strongly enriched with prenylated hop flavonoids.

KEYWORDS: *hops, Humulus lupulus L., xanthohumol, bitter, taste dilution analysis, sensomics*

INTRODUCTION

Due to its characteristic aroma and taste, beer is widely appreciated by consumers all over the world. A vast number of studies have been focused in the past on the volatile aroma components in hops and beers,[1−8] as well as on the nonvolatile terpenoids contributing to bitter taste, foam stability, and antimicrobial activity of the final beer.[9−11]

The bitter principles of hops can be divided into two classes: first, the α-acids (**1**, Figure 1) and β-acids (**2**), both present in the so-called soft resin, are prone to isomerization into *cis*- (**3**) and *trans*-iso-α-acids (**4**) during wort boiling and are considered the major contributors to the beers' bitter taste,[12−14] followed by a series of β-acid and *trans*-iso-α-acid transformation products adding to bitterness perception only to a minor extent.[15−17] In addition to these soft resin derived compounds, the major hard resin component xanthohumol (**5**) and its isomerization product isoxanthohumol (**6**) as well as desmethylxanthohumol (**7**) and its isomerization products 8-prenylnaringenin (**8**) and 6-prenylnaringenin (**9**),[18−20] respectively, have been proposed to contribute to the bitter taste of beer, primarily upon coactivation of the human bitter taste receptors hTAS2R1, hTAS2R14, and hTAS2R40.[21] Very recently, a series of α-acid derived degradation products were isolated from the hard resin fraction of stored hops.[22] It is, however, not clear whether and, if so, which additional hop components are required to fully construct the unique perception of a beer's bitter profile.[23−25] Interestingly, brewing of beer using the hop's hard resin fraction was found to afford beverages with a strong and pleasant bitter character,[26] thus indicating the occurrence of additional valuable bitter compounds.

The objective of the present investigation was, therefore, to locate bitter compounds in hop's hard resin fraction using an activity-guided fractionation approach, to determine their chemical structure by means of LC-MS and NMR experiments, to characterize their sensory activity on the basis of human threshold concentrations, and to verify their taste contribution by means of taste re-engineering experiments. Furthermore, an LC-MS/MS method should be developed for the quantitation of the comprehensive set of hop bitter compounds and applied to the analysis of hop products/extracts and beer samples produced by using hard resin extract.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: formic acid, sulfuric acid, ethanol, hexane (Merck KGaA, Darmstadt, Germany); hydrochloric acid, 0.1 M potassium hydroxide (Riedel-de-Haen, Seelze, Germany); acetonitrile, methanol, hydrobromic acid solution (33% in acetic acid), Amberlyst 15, scandium trifluoromethanesulfonate, *trans*-*p*-coumaric acid (Sigma-Aldrich, Steinheim, Germany); D_2O, CD_3OD, and DMSO-d_6 (Euriso-Top, Saarbrücken,

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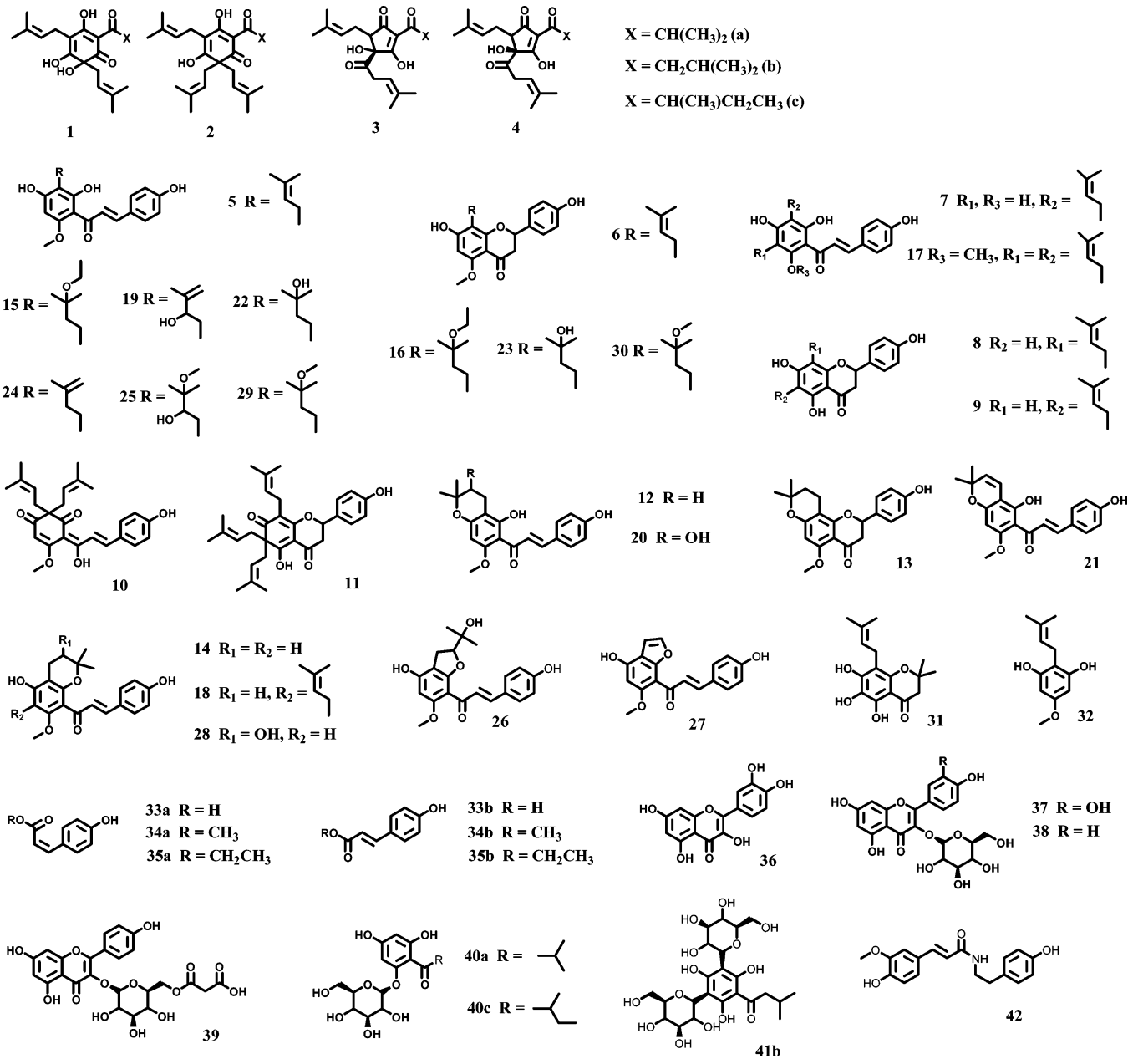

Figure 1. Chemical structures of key taste compounds identified in the hop hard resin fraction: cohumulon (**1a**), *n*-humulon (**1b**), adhumulon (**1c**), colupulon (**2a**), *n*-lupulon (**2b**), adlupulon (**2c**), *cis*-isocohumulon (**3a**), *cis*-isohumulon (**3b**), *cis*-isoadhumulon (**3c**), *trans*-isocohumulon (**4a**), *trans*-isohumulon (**4b**), *trans*-isoadhumulon (**4c**), xanthohumol (**5**), isoxanthohumol (**6**), desmethylxanthohumol (**7**), 8-prenylnaringenin (**8**), 6-prenylnaringenin (**9**), 4′-hydroxytunicatachalcone (**10**), isoxantholupon (**11**), 1″,2″-dihydroxanthohumol C (**12**), 1″,2″-dihydroisoxanthohumol C (**13**), 1″,2″-dihydroxanthohumol K (**14**), xanthohumol P (**15**), isoxanthohumol P (**16**), 5′-prenylxanthohumol (**17**), 1″,2″-dihydroxanthohumol F (**18**), xanthohumol D (**19**), xanthohumol B (**20**), xanthohumol C (**21**), xanthohumol H (**22**), isoxanthohumol H (**23**), xanthohumol N (**24**), 2″-hydroxy-xanthohumol M (**25**), xanthohumol I (**26**), xanthohumol O (**27**), xanthohumol L (**28**), xanthohumol M (**29**), isoxanthohumol M (**30**), 2″,3″-dehydrocyclohumulohydrochinon (**31**), 1-methoxy-4-prenylphloroglucinol (**32**), *cis*-/*trans*-*p*-coumaric acid (**33a**/**b**), *cis*-/*trans*-*p*-coumaric acid methyl ester (**34a**/**b**), *cis*-/*trans*-*p*-coumaric acid ethyl ester (**35a**/**b**), quercetin (**36**), quercetin-3-*O*-*β*-D-glucopyranoside (**37**), kaempferol-3-*O*-*β*-D-glucopyranoside (**38**), kaempferol-3-*O*-*β*-D-(6″-malonyl)glucopyranoside (**39**), 1-*O*-*β*-D-(2-methylpropanoyl)phloroglucinol glucopyranoside (co-multifidol glucopyranoside) (**40a**), 1-*O*-*β*-D-(2-methylbutyryl)phloroglucinol glucopyranoside (ad-multifidolglucopyranoside) (**40c**), phloroisovalerophenon-3,5-di-*C*-*β*-D-glucopyranoside (*n*-multifidol-di-*C*-glucopyranoside) (**41b**), and *trans*-*N*-feruloyltyramine (**42**).

Germany). Water for high-performance liquid chromatography (HPLC) and medium-pressure liquid chromatography (MPLC) separation was purified by means of a Milli-Q water advantage A 10 water system (Millipore, Molsheim, France), and bottled water was used for sensory studies (Evian, Danone, Wiesbaden, Germany). Solvents for HPLC were of HPLC grade and for LC-MS were of MS grade (J.T.Baker, purchased through Sigma-Aldrich).

Hop pellets type 90 and spent hop pellets, which were obtained after supercritical carbon dioxide extraction of hops, were delivered from Joh. Barth & Sohn GmbH & Co. KG (Nuremberg, Germany); XanthoFlav 15, XanthoFlav 75, hop tannin extracts (from both aroma and bitter hops), and resin extracts (from both aroma and bitter hops) were obtained from Hopsteiner (Simon H. Steiner GmbH, Mainburg, Germany). A novel *ε*-extract was produced in cooperation with Joh. Barth & Sohn GmbH & Co. KG and NateCO_2 GmbH & Co. KG

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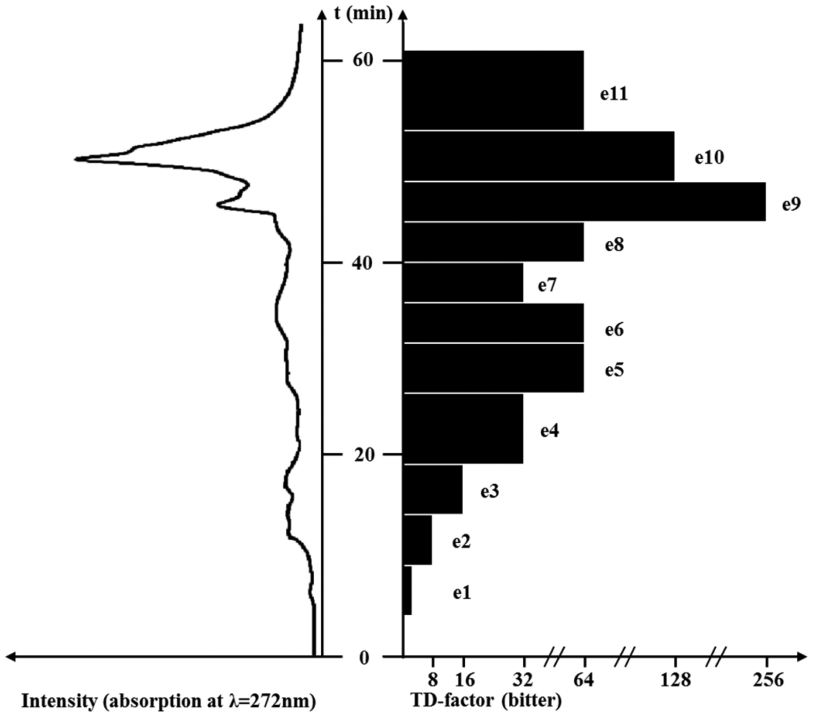

Figure 2. RP-MPLC chromatogram (left) and taste dilution chromatogram (right) of the ε-resin fraction isolated from spent hops.

(Wolnzach, Germany) by means of a supercritical CO_2 extraction using ethanol as a modifier.[27] Reference substances for individual iso-α-acids were purified from an iso-α-acid extract (30%; Hopsteiner), and purified α- and β-acids were isolated from an ethanolic hop extract (Hopsteiner, Simon H. Steiner GmbH, Mainburg, Germany) following the protocol reported recently.[28] Xanthohumol was purified from a commercial xanthohumol extract, and isoxanthohumol was prepared from xanthohumol by alkaline cyclization as reported recently.[28] 8-Prenylnaringenin was prepared from isoxanthohumol by demethylation using scandium trifluoromethanesulfonate.[29] Experimental brewing trails were conducted in the Research Brewery St. Johann St. Johann GmbH & CO. KG (Train-St. Johann, Germany). Unhopped "zero" beer was obtained from the Chair of Brewing and Beverage Technology, Technische Universität München (Freising, Germany).

Preparative Isolation of Soft Resin and δ- and ε-Hard Resin. According to the literature with some modifications,[30] spent hops (100 g) were exhaustively extracted with methanol/diethyl ether (1/5, v/v; 900 mL) for 1 h while stirring. After the addition of hydrochloric acid (300 mL; 0.1 mol/L), the mixture was stirred for additional 10 min and then filtered (320 mm, Macherey-Nagel, Düren, Germany), and the organic layer was extracted with hydrochloric acid (3 × 100 mL; 0.1 mol/L) before the solvent was separated in vacuum. To remove waxes, the residue was solubilized in methanol (400 mL), and, after incubation at −20 °C overnight and filtration (round filter paper, 320 mm, Roth, Karlsruhe, Germany), the solution was applied onto 55 μm, 70A, Strata C18-E Giga Tube cartridges (10 g, 60 mL; Phenomenex, Aschaffenburg, Germany), conditioned with methanol. Chlorophylls were separated by flushing the cartridges with methanol (150 mL), and the effluent was collected and separated from solvent in vacuum. The resin obtained was suspended in hexane and filtered (185 mm, Macherey-Nagel, Düren, Germany). The filtrate was collected and separated from solvent in vacuum to give the soft resin (91.9% yield), whereas the residue was extracted with hexane (50 mL) to give the nonsoluble hard resin (8.1% yield). Following a literature protocol for ε-resin isolation,[31,32] the hard resin was suspended in water at 40 °C during ultrasonification. After filtration (185 mm, Macherey-Nagel, Düren, Germany), the aqueous phase was collected, whereas the residue was again suspended in water (40 °C). After this process was repeated three times, the combined aqueous layer was freeze-dried to obtain the δ-resin (20.2% of total hard resin), whereas lyophilization of the residual material afforded the ε-resin (79.8% of total hard resin).

Fractionation of the ε-Resin by Medium-Pressure Liquid Chromatography (MPLC). Aliquots of the ε-resin (0.5 g) were dissolved in methanol/water (40/60, v/v; 20 mL) and separated on a 460 × 16 mm glass column (Büchi, Flawil, Switzerland) filled with 25−40 μm LiChroprep RP18 material (Merck KGaA, Darmstadt, Germany) using an MPLC system (Büchi Sepacore, Flawil, Switzerland) consisting of two C-605 pumps, a C-620 control unit, a C-660 fraction collector, a 6-way-injection valve, and a C-635 UV detector monitoring the effluent at 272 nm. Data acquisition was performed by means of Sepacore Control Chromatography Software, Version 1.0. Operating with a flow rate of 30.0 mL/min and using 0.1% aqueous formic acid as solvent A and methanol containing 0.1% formic acid as solvent B, chromatography was performed starting with 40% solvent B for 1 min and, then, increasing solvent B to 60% within 6 min, to 70% within 30 min and, finally, to 100% within an additional 20 min, followed by isocratic elution for 20 min. After 86.0 min, solvent B decreased again to 40% and was kept for 10 min prior to the next injection. A total of 11 fractions (e1−e11) were collected, separated from solvent in vacuum, and then used for a taste dilution analysis[33−35] to locate fractions e2 (yield: 3.4%), e3 (yield: 3.0%), e4 (yield: 3.2%), e5 (yield: 3.6), e6 (yield: 3.2%), e7 (yield: 2.6%), e8 (yield: 2.7%), e9 (yield: 18.4%), e10 (yield: 31.3%), and e11 (yield: 22.9%) with the highest bitter impact (Figure 2).

Isolation of Key Bitter Molecules from MPLC Fractions of ε-Resin. MPLC fractions were separated by means of preparative HPLC on a 250 × 21.2 mm, 5 μm, Luna Phenyl-Hexyl column (Phenomenex, Aschaffenburg, Germany) operated with a flow rate of 15 mL/min and using 0.1% aqueous formic acid and acetonitrile containing 0.1% formic acid as solvents. Separation of MPLC fractions e2 to e11 gave subfractions e2-1 to e2-14, e3-1 to e3-13, e4-1 to e4-5, e5-1 to e5-5, e6-1 to e6-11, e7-1 to e7-16, e8-1 to e8-8, e9-1 to e9-9, e10-13 to e10-13, and e11-1 to e11-15, all of which were collected, separated from solvent in vacuum, and subjected to a taste dilution analysis (Figure 3).

Structure Determination of Bitter Compounds in ε-Resin Subfractions. The individual ε-resin subfractions isolated above were directly analyzed by means of LC-TOF-MS and 1D/2D-NMR spectroscopy and, if needed, further purified by means of rechromatography using a semipreparative 250 × 10.0 mm Hypersil RP-18 column (Thermo Hypersil GmbH, Kleinostheim, Germany) using a solvent

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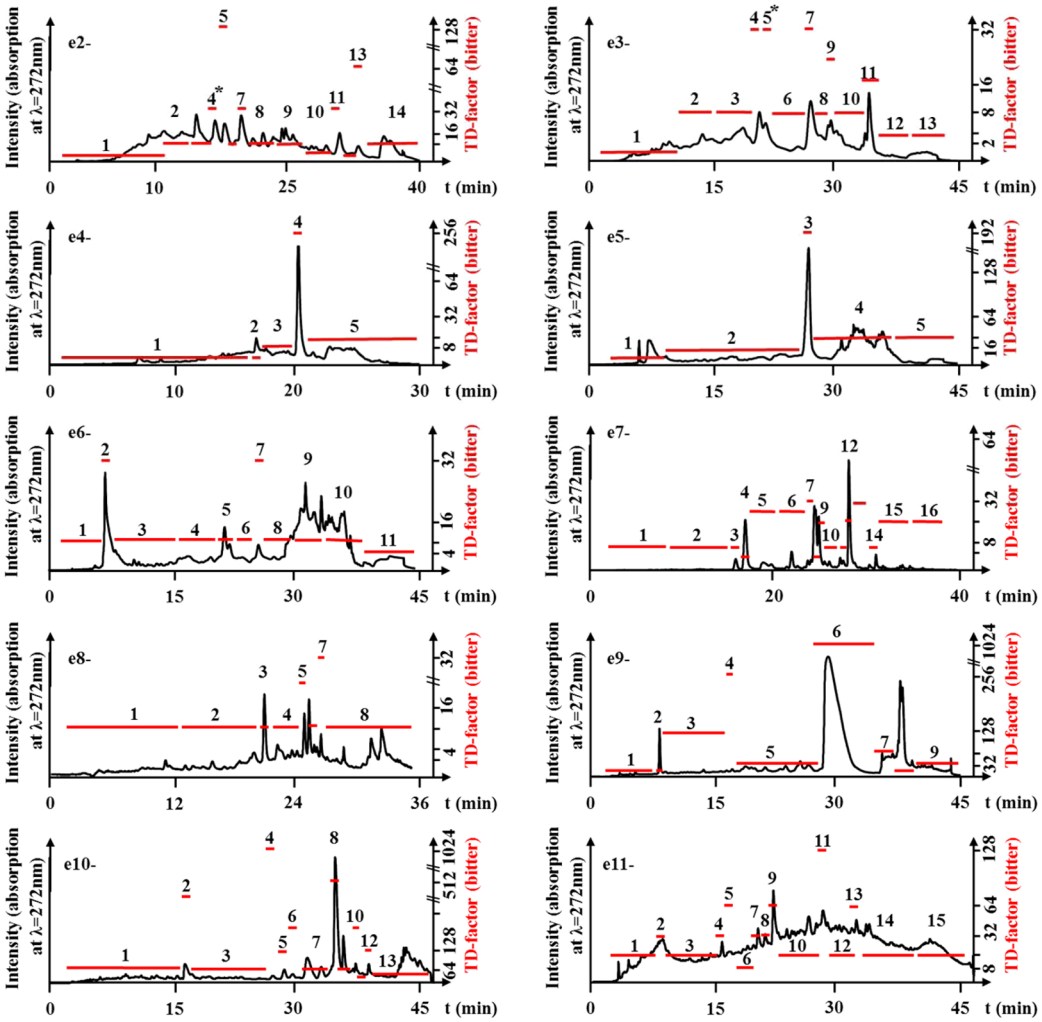

Figure 3. RP-HPLC-TDA chromatogram of the ε-resin subfractions e2−e11 isolated from spent hops. * Fraction with an astringent taste.

gradient of aqueous 0.1% formic acid and methanol containing 0.1% formic acid (flow rate: 4.7 mL/min). Compounds **5**−**7**, **9**−**12**, **14**, **17**−**22**, **25**−**29**, **31**−**34**, **36**, **37**, **40a**, and **42**−**44** were isolated from the subfractions as summarized in Table 1 in a purity of more than 98% and, after the solvent was separated in vacuum and freeze-dried twice, were used for structure determination by means of UV/vis, LC-MS/MS, UPLC-TOF-MS, and 1D/2D NMR, as well as for sensory experiments. Based on the comparison of spectroscopic (UV/vis, LC-TOF-MS, $^{1}H/^{13}C$ NMR) and chromatographic data with those recorded for references, previously reported compounds were confirmed as xanthohumol (**5**),[24] isoxanthohumol (**6**),[24] desmethylxanthohumol (**7**),[25] 6-prenylnaringenin (**9**),[24] 1″,2″-dihydroxanthohumol C (**12**),[23,36,37] 1″,2″-dihydroxanthohumol K (**14**),[36,37] 5′-prenylxanthohumol (**17**),[24,25] xanthohumol D (**19**),[25] xanthohumol B (**20**),[24,25] xanthohumol C (**21**),[24] xanthohumol H (**22**),[23,36,37] 2″-hydroxyxanthohumol M (**25**),[38] xanthohumol I (**26**),[23,36,37] xanthohumol O (**27**),[39] xanthohumol L (**28**),[39] *cis*-/*trans*-*p*-coumaric acid (**33a**/**b**),[29] *cis*-/*trans*-*p*-coumaric acid methyl ester (**34a**/**b**),[29] quercetin (**36**),[40] quercetin-3-*O*-β-D-glucopyranoside (**37**),[41] 1-*O*-β-D-(2-methylpropanoyl)-phloroglucinol glucopyranoside (co-multifidol glucopyranoside) (**40a**),[43] *trans*-*N*-feruloyltyramine (**42**),[23,36,37] cohumulon (**41a**),[13] *n*-humulon (**41b**),[13] adhumulon (**41c**),[13] colupulon (**44a**),[13] *n*-lupulon (**44b**),[13] and adlupulon (**44c**).[13] The chemical structures of compounds **10**, **11**, **18**, **29**, **31**, and **32** have been determined by means of UV/vis, LC-MS, and 1D/2D NMR experiments and, to the best of our knowledge, have not been earlier reported (Figure 4).

4′-Hydroxytunicatachalcone, 10 (Figure 4). UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 406 nm. LC-MS (ESI$^-$): *m*/*z* (%) 421 (12, [M − H]$^-$), 119 (100), 93 (3), 217 (2), 245 (1). LC-TOF-MS (ESI$^-$): *m*/*z* 421.2014 ([M − H]$^-$, measured), *m*/*z* 421.2015 ([M − H]$^-$, calcd for $C_{26}H_{29}O_5^-$). ^{1}H NMR (500 MHz, MeOD; COSY): δ (ppm) 1.56 [s, 4 × 3H, H−C(4″, 4″a, 5″, 5″a)], 2.58 [m, 3H, H−C(1″, 1″a)], 3.99 [s, 3H, H−C(1‴)], 4.84 [m, 2H, H−C(2″, 2″a)], 5.56 [s, 1H, H−C(6)], 6.85 [m, 2H, *J* = 8.9 Hz, H−C(3′, 5′)], 7.54 [m, 2H, *J* = 8.9 Hz, H−C(2′, 6′)], 7.61 [d, 1H, *J* = 15.7 Hz, H−C(3)], 7.88 [d, 1H, *J* = 15.7 Hz, H−C(2)]. ^{13}C NMR (125 MHz, MeOH; HSQC, HMBC): δ (ppm) 17.9 [C(5″, 5″a)], 25.8 [C(4″, 4″a)], 39.5 [C(1″, 1″a)], 57.1 [C(1‴)], 62.6 [C(8)], 98.9 [C(6)], 106.1 [C(10)], 116.6 [C(3′, 5′)], 118.8 [C(2″, 2″a)], 119.1 [C(3)], 128.1 [C(1′)], 131.8 [C(2′, 6′)], 136.2 [C(3″, 3″a)], 145.9 [C(2)], 161.9 [C(4′)], 174.3 [C(5)], 180.3 [C(4)], 199.8 [C(7)], 205.9 [C(9)].

Isoxantholupon, 11 (Figure 4). UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 308 nm. LC-MS (ESI$^-$): *m*/*z* (%) 475.2 (100, [M − H]$^-$), 243 (95), 119 (55), 363 (29), 286 (26), 257 (15). LC-TOF-MS (ESI$^-$): *m*/*z* 475.2492 ([M − H]$^-$, measured), *m*/*z* 475.2484 ([M − H]$^-$, calcd for $C_{30}H_{35}O_5^-$). ^{1}H NMR (500 MHz, MeOD; COSY): δ (ppm) 1.51/1.53/1.56/1.59 [s, 4 × 3H, H−C(4‴, 4‴a, 5‴, 5‴a)], 1.55 [s, 3H, H−C(4″)], 1.61 [s, 3H, H−C(5″)], 2.60/2.62 [d, 2 × 2H, *J* = 6.75 Hz, *J* = 7.35 Hz, H−C(1‴, 1‴a)], 2.96 [dd, 1H, *J* = 7.0 Hz, *J* = 18.2 Hz, H−C(3eq)], 3.00 [d, 2H, *J* = 7.0 Hz, H−C(1″)], 3.16 [dd, 1H, *J* = 9.8 Hz, *J* = 18.2 Hz, H−C(3ax)], 4.71/4.82 [t, 2 × 2H, *J* = 6.75 Hz, *J* = 7.35 Hz, H−C(2‴, 2‴a)]; 4.89 [t, 2H, *J* = 7.0 Hz, H−C(2″)]; 5.3 [dd, 1H, *J* = 4.0 Hz, *J* = 9.8 Hz, H−C(2)], 6.82 [m, 2H, *J* = 8.9, H−C(3′, 5′)], 7.28 [m, 2H, *J* = 8.9, H−C(2′, 6′)]. ^{13}C NMR (125 MHz, MeOD; HSQC, HMBC): δ (ppm) 17.9/18.1 [C(4″, 4‴, 4‴a)], 22.2 [C(1″)], 25.9/26.0 [C(5″, 5‴, 5‴a)], 38.5 [C(3)], 39.1/39.7 [C(1‴, 1‴a)], 61.9 [C(6)], 78.3 [C(2)],

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Table 1. Orosensory Quality and Human Recognition Threshold Concentration of Taste Compounds Identified in Hops' Hard Resin and Hop Extracts

compound (no.)[a]	sensory quality[b]	fraction no.[c]	TC[d] [μmol/L]
cis-*p*-coumaric acid (**33a**)	as	e2-4	22
trans-*p*-coumaric acid (**33b**)	as	e2-4	22
quercetin-3-*O*-*β*-D-glc*p*[e] (**37**)	bi/as	e2-5; –[f]	28/2
kaempferol-3-*O*-*β*-D-glc*p* (**38**)	bi/as	–[f]	29/1
kaempferol-3-*O*-*β*-D-(6″-malonyl)-glc*p* (**39**)	bi/as	–[f]	35/5
co-multifidol glc*p* (**40a**)	bi	e2-7; –[f]	5
ad-multifidol glc*p* (**40c**)	bi	–[f]	10
n-multifidol di-*C*-glc*p* (**41b**)	bi	–[f]	37
trans-*N*-feruloyltyramine (**42**)	bi	e2-11	10
xanthohumol L (**28**)	bi	e2-13	8
xanthohumol I (**26**)	bi	e3-4	29
quercetin (**36**)	as	e3-5	15
1-methoxy-4-prenylphloroglucinol (**22**)	bi	e3-7	44
cis-*p*-coumaric acid methyl ester (**34a**)	bi	e3-7	184
trans-*p*-coumaric acid methyl ester (**34b**)	bi		189
cis-*p*-coumaric acid ethyl ester (**35a**)	bi	–[g]	195
trans-*p*-coumaric acid ethyl ester (**35b**)	bi	–[g]	198
xanthohumol O (**27**)	bi	e3-9	9
1″,2″-dihydroxanthohumol K (**14**)	bi	e3-11; e4-4; e7-3	16
isoxanthohumol (**6**)	bi	e4-2; e5-3; e6-5; e9-2	16
xanthohumol H (**22**)	bi	e6-7; e7-4	25
isoxanthohumol H (**23**)	bi	–[g]	25
xanthohumol B (**20**)	bi	e7-7	15
2″-hydroxyxanthohumol M (**25**)	bi	e7-8	9
xanthohumol D (**19**)	bi	e7-9	24
2″,3″-dehydrocylcohumulohydrochinon (**31**)	bi	e7-11	22
desmethylxanthohumol (**7**)	bi	e7-12	16
1″,2″-dihydroxanthohumol F (**18**)	bi	e7-14	12
xanthohumol M (**29**)	bi	e8-3; e9-4	14
isoxanthohumol M (**30**)	bi	–[g]	19
6-prenylnaringenin (**9**)	bi	e8-5	10
8-prenylnaringenin (**8**)	bi	–[g]	8
xanthohumol (**5**)	bi	e8-7; e9-6; e10-2	10
1″,2″-dihydroxanthohumol C (**12**)	bi	e10-4; e11-4	8
xanthohumol C (**21**)	bi	e10-5; e11-5	6
1″,2″-dihydroisoxanthohumol C (**13**)	bi	–[g]	6
4′-hydroxytunicatachalcone (**10**)	bi	e10-6	7
n-humulon (**1b**)	bi	e10-8; e11-8	21
adhumulon (**1c**)	bi	e10-9; e11-9	21
5′-prenylxanthohumol (**17**)	bi	e10-10; e11-8	7
isoxantholupon (**11**)	bi	e10-12	6
colupulon (**2a**)	bi	e11-7	17
n-lupulon (**2b**)	bi	e11-11	35
adlupulon (**2c**)	bi	e11-13	37
xanthohumol N (**24**)	bi	–[g]	13
xanthohumol P (**15**)	bi	–[g]	20
isoxanthohumol P (**16**)	bi	–[g]	24

[a]Chemical structures are given in Figure 1. [b]Sensory quality: astringent (as), bitter (bi). [c]Number of HPLC fraction referring to Figure 3. [d]Taste recognition threshold concentrations (TC) were determined in aqueous ethanolic solution (5%, v/v; pH 4.4). [e]glc*p*: glucopyranoside. [f]Compound isolated from hop tannin extract. [g]Compound synthesized.

105.3 [C(10)], 114.8 [C(8)], 116.5 [C(3′, 5′)], 118.9/119.0 [C(2‴, 2‴a)], 123.5 [C(2″)], 129.1 [C(2′, 6′)], 130.3 [C(1′)], 131.9 [C(3″)], 136.1/136.2 [C(3‴, 3‴a)], 159.2 [C(4′)], 165.7 [C(9)], 185.7 [C(4)], 198.6 [C(7)].

*1″,2″-Dihydroxanthohumol F, **18** (Figure 4).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 326 nm. LC-MS (ESI$^-$): *m*/*z* (%) 421 (100, [M − H]$^-$), 119 (98), 365 (10). LC-TOF-MS (ESI$^-$): *m*/*z* 421.2008 ([M − H]$^-$, measured), *m*/*z* 421.2015 ([M − H]$^-$, calcd for $C_{26}H_{29}O_5^-$). ^{1}H NMR (500 MHz, MeOD; COSY): *δ* (ppm) 1.22 [s, 2 × 3H, H−C(4″, 5″)], 1.68 [s, 3H, H−C(4‴)], 1.76 [s, 3H, H−C(5‴)], 1.79 [t, 2H, *J* = 6.8 Hz, H−C(2″)], 2.65 [t, 2H, *J* = 6.8 Hz, H−C(1″)], 3.30 [2H, H−C(1‴)], 3.61 [s, 3H, H−C(1‴)], 5.20 [m, 1H, H−C(2‴)], 6.80 [m, 2H, H−C(3′, 5′)], 6.82 [d, 1H, *J* = 15.9 Hz, H−C(2)], 7.25 [d, 1H, *J* = 15.9 Hz, H−C(3)], 7.43 [m, 2H, H−C(2′, 6′)]. ^{13}C NMR (125 MHz, MeOH; HSQC, HMBC): *δ* (ppm) 17.98 [C(5‴)], 18.20 [C(1″)], 23.53 [C(1‴)], 25.91 [C(4‴)], 26.79 [C(4″, 5″)], 33.12 [C(2″)], 63.09 [C(1‴)], 75.38 [C(3″)], 106.57 [C(8)], 115.06 [C(6)], 116.43 [C(10)], 116.97 [C(3′, 5′)], 124.76 [C(2‴)], 127.03 [C(1′)], 127.48 [C(3)], 131.48 [C(2′, 6′)], 131.92 [C(3‴)], 147.57 [C(2)], 151.71 [C(9)], 156.33 [C(5)], 156.44 [C(7)], 161.50 [C(4′)], 198.33 [C(4)].

*Xanthohumol M, **29** (Figure 4).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 368 nm. LC-MS (ESI$^-$): *m*/*z* (%) 385 (100, [M − H]$^-$), 119 (87), 265 (2), 163 (1). LC-TOF-MS (ESI$^-$): *m*/*z* 385.1664 ([M − H]$^-$, measured), *m*/*z* 353.1657 ([M − H]$^-$, calcd for $C_{22}H_{25}O_6^-$). ^{1}H NMR (500 MHz, MeOD; COSY): *δ* (ppm) 1.23 [s, 6H, H−C(4″, 5″)], 1.65 [m, 2H, H−C(2″)], 2.57 [m, 2H, H−C(1″)], 3.28 [s, 3H, H−C(6″)], 3.91 [s, 3H, H−C(1‴)], 6.04 [s, 1H, H−C(6)], 6.83 [m, 2H, *J* = 8.5 Hz, H−C(3′, 5′)], 7.50 [m, 2H, *J* = 8.5 Hz, H−C(2′, 6′)], 7.67 [d, 1H, *J* = 15.6 Hz, H−C(3)], 7.80 [d, 1H, *J* = 15.6 Hz, H−C(2)]. ^{13}C NMR (125 MHz, MeOD; HSQC, HMBC): *δ* (ppm) 18.6 [C(1″)], 26.4 [C(4″, 5″)], 39.4 [C(2″)], 50.6 [C(6″)], 57.1 [C(1‴)], 77.5 [C(3″)], 92.5 [C(6)], 110.8 [C(8)], 117.8 [C(3′, 5′)], 126.7 [C(3)], 129.3 [C(1′)], 132.1 [C(2′, 6′)], 144.3 [C(2)], 161.9 [C(4′)], 163.3 [C(5)], 164.7 [C(9)], 167.1 [C(7)], 195.0 [C(4)].

*2″,3″-Dehydrocyclohumulonhydrochinon, **31** (Figure 4).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 280 nm. LC-MS (ESI$^-$): *m*/*z* (%) 291 (100, [M − H]$^-$), 289 (95, [M − 3H]$^-$), 207 (25, [M − H]$^-$), 235 (21), 136 (20). LC-TOF-MS (ESI$^-$): *m*/*z* 291.1222 ([M − H]$^-$, measured), *m*/*z* 291.1238 ([M − H]$^-$, calcd for $C_{16}H_{19}O_5^-$). ^{1}H NMR (500 MHz, MeOD; COSY): *δ* (ppm) 1.41 [s, 6H, H−C(1′, 2′)], 1.64 [s, 3H, H−C(4″)], 1.76 [s, 3H, H−C(5″)], 2.67 [s, 2H, H−C(3)], 3.20 [d, 2H, *J* = 7.3 Hz, H−C(1″)], 5.15 [m, 1H, *J* = 7.3 Hz, H−C(2″)]. ^{13}C NMR (125 MHz, MeOD; HSQC, HMBC): *δ* (ppm) 16.6 [C(5″)], 21.2 [C(1″)], 24.5 [C(4″)], 25.5 [C(1′, 2′)], 49.5 [C(3)], 78.0 [C(2)], 101.0 [C(10)], 107.8 [C(8)], 122.8 [C(2″)], 129.9 [C(3″)], 147.6 [C(5)], 151.7 [C(9)], 154.1 [C(7)], 197.3 [C(4)].

*1-Methoxy-4-prenylphloroglucinol, **32** (Figure 4).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 292 nm. LC-MS (ESI$^-$): *m*/*z* (%) 207 (100, [M − H]$^-$), 137 (25), 121 (14), 109 (10), 149 (9). LC-TOF-MS (ESI$^-$): *m*/*z* 207.1035 ([M − H]$^-$, measured), *m*/*z* 207.1027 ([M − H]$^-$, calcd for $C_{12}H_{15}O_3^-$). ^{1}H NMR (500 MHz, MeOD; COSY): *δ* (ppm) 1.65 [s, 3H, H−C(4′)], 1.75 [s, 3H, H−C(5′)], 3.2 [d, 2H, *J* = 7.3 Hz, H−C(1′)], 3.83 [s, 3H, H−C(1″)], 5.21 [m, 1H, *J* = 7.3 Hz, H−C(2′)], 5.42 [s, 1H, H−C(2)], 6.04 [s, 1H, H−C(6)]. ^{13}C NMR (125 MHz, MeOD; HSQC, HMBC): *δ* (ppm) 17.8 [C(5′)], 22.0 [C(1′)], 25.9 [C(4′)], 56.2 [C(1″)], 93.4 [C(6)], 99.8 [C(2)], 105.9 [C(4)], 123.2 [C(2″)], 132.4 [C(3′)], 159.6 [C(1)], 167.9 [C(5)], 173.3 [C(3)].

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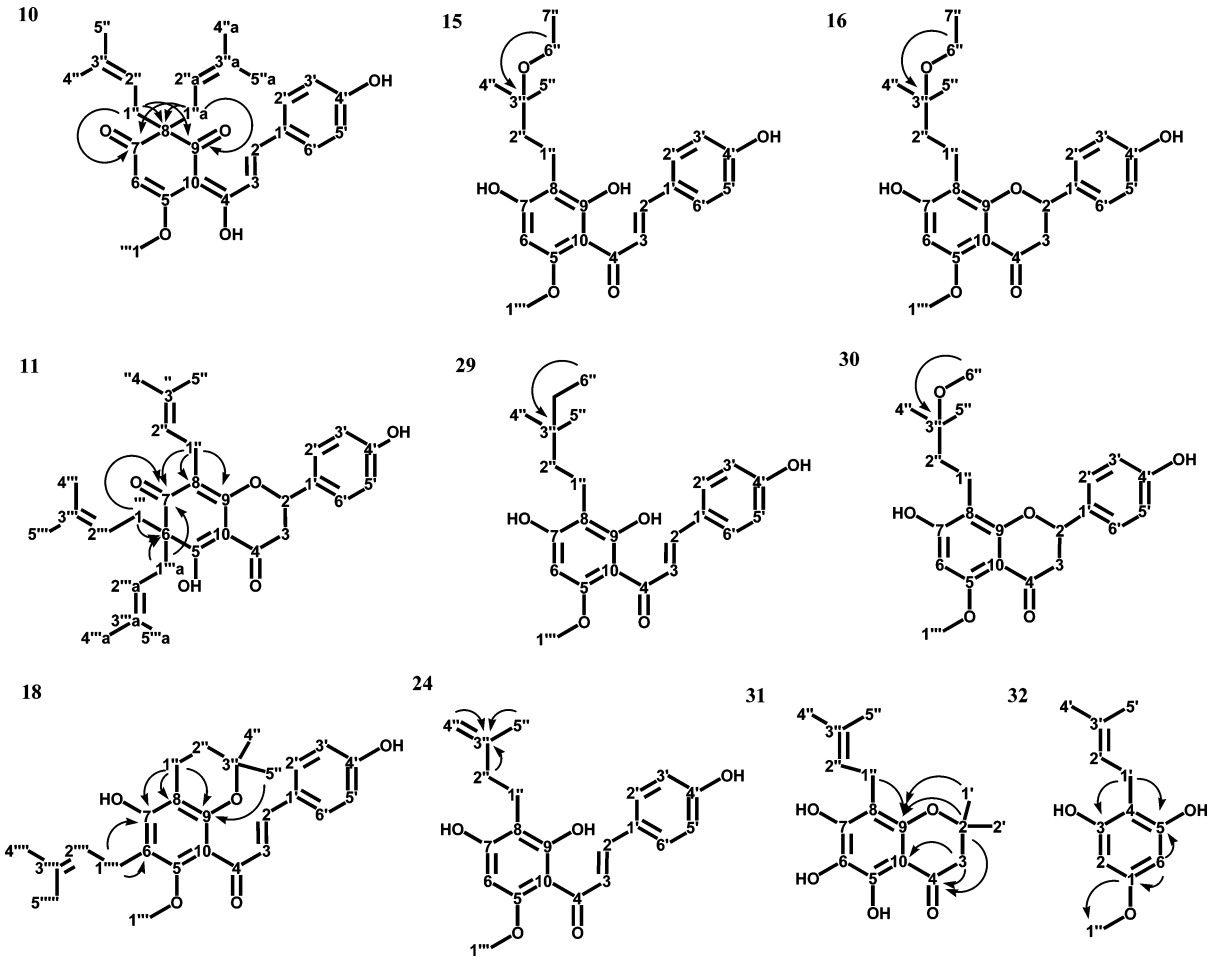

Figure 4. Chemical structures of compounds **10**, **11**, **15**, **17**, **18**, **24**, and **29**–**32** with heteronuclear key correlations (HMBC experiment) highlighted.

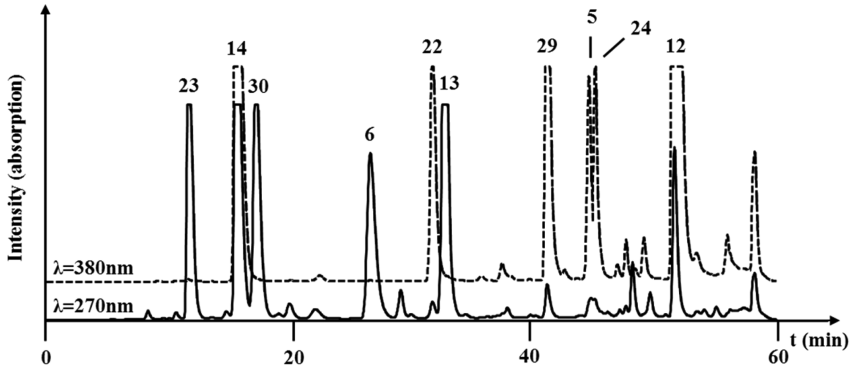

Figure 5. RP-HPLC/UV chromatogram of a methanolic solution of xanthohumol (**5**) after treatment (45 min, rt) with hydrobromic acid solution (33% in acetic acid). Numbering of compounds refer to structures given in Figure 1

Isolation of Compounds from Hop Tannin Extract. Aliquots of the hop tannin extract (200 μL), diluted with water (500 μL), were separated by means of HPLC on a 250 × 21.2 mm, 5 μm, Luna Phenyl-Hexyl column (Phenomenex, Aschaffenburg, Germany) operated with a flow rate of 15 mL/min and using 0.1% aqueous formic acid and acetonitrile containing 0.1% formic acid as solvents. Chromatography was performed by starting with 5% solvent B for 0.5 min, then increasing solvent B to 15% within 0.5 min, and, after keeping at 15% for 34.0 min, solvent B was increased to 25% within additional 7 min, then increased to 100% within 1 min, followed by isocratic elution for 3.0 min. After 46 min, solvent B decreased again to 5% within 1.0 min and was kept for 3.0 min prior to the next injection. The compounds eluting at 24.5 (**37**), 27.0 (**40a**), 30.0 (**38**), and 32.0 min (**39**) were collected and separated from solvent in vacuum and freeze-dried twice. For preparative isolation of additional compounds, the following gradient was used: 5% solvent B for 0.5 min and then increasing solvent B to 15% within 0.5 min. Solvent B was kept for 41.0 min at 15% and, finally, increased to 100% within an additional minute, followed by isocratic elution for 3.0 min. After 46 min, solvent B decreased again to 5% within 1.0 min and was kept for 3.0 min prior to the next injection. Compounds eluting after 31.5 (**40a**), 32.3 (**38**), 33.0 (**40c**), and 33.3 min (**41b**) were collected, separated from solvent under vacuum, and freeze-dried twice. Compounds **37**–**41b** (>98% purity) were analyzed by means of UV/vis, LC-MS/MS, UPLC-TOF-MS, and 1D/2D NMR. Based on the comparison of spectroscopic and chromatographic data with those recorded for references, previously reported compounds were confirmed as

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quercetin-3-*O*-*β*-D-glucopyranoside (**37**),[41] kaempferol-3-O-*β*-D-glucopyranoside (**38**),[41] kaempferol-3-*O*-*β*-D-(6″-malonyl)glucopyranoside (**39**),[42] 1-*O*-*β*-D-(2-methylpropanoyl)phloroglucinol glucopyranoside (co-multifidol glucopyranoside, **40a**),[43] 1-*O*-*β*-D-(2-methylbutyryl)-phloroglucinol glucopyranoside (ad-multifidolglucopyranoside, **40c**),[43] and phloroisovalerophenon-3,5-di-*C*-*β*-D-glucopyranoside (*n*-multifidol-di-*C*-glucopyranoside) (**41b**).[43]

Synthetic Preparation of Xanthohumol Derivatives 12−14, 22−24, 29, and 30. An aliquot (50 mg) of xanthohumol (**5**) was solved in a mixture of hydrobromic acid solution (33% in acetic acid; 5 mL) and methanol (5 mL) and stirred for 45 min at room temperature. The reaction mixture was separated by semipreparative HPLC on a 250 × 10.0 mm Hypersil RP-18 column (Thermo Hypersil GmbH, Kleinostheim, Germany) using water (0.1% formic acid) as solvent A and methanol (0.1% formic acid) as solvent B at a flow rate of 4.7 mL/min. Chromatography was performed using the following gradient: 62% solvent B for 4.5 min, then increasing solvent B to 64% within 5.5 min. After 3.0 min solvent B was increased to 70% within 10 s, then to 72% within 5.3 min, to 80% within 0.5 min, kept for 6.0 min and, finally, to 100% within an additional 1.5 min, followed by isocratic elution for 2.5 min. After 29.0 min, solvent B decreased again to 62% within 1 min and was kept for 3.0 min prior to the next injection. Besides xanthohumol (**5**) and isoxanthohumol (**6**), the effluent of the peaks of compounds **12−14**, **22−24**, **29**, and **30** (Figure 5) was collected and, after the solvent was removed in vacuum and freeze-dried twice, the individual compounds (>98% purity) were used for sensory and identification experiments (UV/vis, LC-MS/MS, UPLC-TOF-MS, 1D/2D NMR). Based on the comparison of spectroscopic (UV/vis, LC-TOF-MS, $^{1}H/^{13}C$ NMR) and chromatographic data (retention time) with those recorded for reference substances, compounds reported earlier in the literature were identified as 1″,2″-dihydroxanthohumol C (**12**),[23,36,37] 1″,2″-dihydroisoxanthohumol C (**13**),[45] 1″,2″-dihydroxanthohumol K (**14**),[36,37] xanthohumol H (**22**),[23,36,37] and isoxanthohumol H (**23**).[39] Furthermore, compound **29** was identified as xanthohumol M based on the comparison with the compound isolated above form the *ε*-resin, whereas compounds **24** and **30** have not been previously reported (Figure 4).

*Xanthohumol N, **24** (Figure 4).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 368 nm. LC-MS (ESI^-): *m/z* (%) 353 (100, $[M-H]^-$), 119 (35), 163 (15), 233 (12), 283 (8). LC-TOF-MS (ESI^-): *m/z* 353.1394 ($[M-H]^-$, measured), *m/z* 353.1394 ($[M-H]^-$, calcd for $C_{21}H_{21}O_5^-$). ^1H NMR (500 MHz, MeOD; COSY): *δ* (ppm) 1.79 [s, 3H, H−C(5″)], 2.18 [m, 2H, *J* = 8.1, H−C(2″)], 2.69 [m, 2H, *J* = 8.1, H−C(1″)], 3.28 [s, 3H, H−C(6″)], 3.90 [s, 3H, H−C(1‴)], 4.66 [s, 2H H−C(4″)], 6.03 [s, 1H, H−C(6)], 6.83 [m, 2H, *J* = 8.7 Hz, H−C(3′, 5′)], 7.50 [m, 2H, *J* = 8.7 Hz, H−C(2′, 6′)], 7.67 [d, 1H, *J* = 15.5 Hz, H−C(3)], 7.80 [d, 1H, *J* = 15.5 Hz, H−C(2)]. ^{13}C NMR (125 MHz, MeOD; HSQC, HMBC): *δ* (ppm) 22.1 [C(1″)], 22.6 [C(5″)], 37.9 [C(2″)], 56.2 [C(1‴)], 91.6 [C(6)], 106.5 [C(10)], 109.7 [C(8)], 110.1 [C(4″)], 116.9 [C(3′, 5′)], 125.9 [C(3)], 128.5 [C(1′)], 131.2 [C(2′, 6′)], 143.3 [C(2)], 161.0 [C(4′)], 162.5 [C(5)], 163.9 [C(7)], 166.2 [C(9)], 194.1 [C(4)].

*Isoxanthohumol M, **30** (Figure 4).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 289 nm. LC-MS (ESI^-): *m/z* (%) 385 (8, $[M-H]^-$), 119 (100), 265 (19), 165 (6), 163 (5), 197(4). LC-TOF-MS (ESI^-): *m/z* 385.1664 ($[M-H]^-$, measured), *m/z* 353.1657 ($[M-H]^-$, calcd for $C_{22}H_{25}O_6^-$). ^1H NMR (400 MHz, MeOD; COSY): *δ* (ppm) 1.13 [s, 6H, H−C(4″, 5″)], 1.60 [m, 2H, H−C(2″)], 2.53 [m, 2H, H−C(1″)], 2.66 [dd, 1H, *J* = 3.0, *J* = 16.9, H−C(3eq)], 3.00 [dd, 1H, *J* = 12.9, *J* = 16.9, H−C(3ax)], 3.06 [s, 3H, H−C(6″)], 3.80 [s, 3H, H−C(1‴)], 5.30 [dd, 1H, *J* = 3.0, *J* = 12.9, H−C(2)], 6.13 [s, 1H, H−C(6)], 6.81 [m, 2H, *J* = 8.7 Hz, H−C(3′, 5′)], 7.33 [m, 2H, *J* = 8.7 Hz, H−C(2′, 6′)]. ^{13}C NMR (100 MHz, MeOD; HSQC, HMBC): *δ* (ppm) 18.1 [C(1″)], 25.6/27.2 [C(4″, 5″)], 38.7 [C(2″)], 46.2 [C(3)], 49.3 [C(6″)], 56.0 [C(1‴)], 76.4 [C(3″)], 80.1 [C(2)], 93.5 [C(6)], 105.9 [C(10)], 110.5 [C(8)], 116.2 [C(3′, 5′)], 129.0 [C(2′, 6′)], 131.4 [C(1′)], 158.9 [C(4′)], 161.9 [C(5)], 163.9 [C(9)], 164.4 [C(7)], 192.9 [C(4)].

Synthetic Preparation of *cis*/*trans*-*p*-Coumaric Acid Ethyl Ester (35a/b). An aliquot (10 mL) of an ethanolic solution of *trans*-*p*-coumaric acid (**33b**; 100 mmol/L) was acidified with 0.1 mL of sulfuric acid. The solution was stirred under reflux for 24 h. Afterward the reaction mixture was subjected to a solid phase extraction using ethanol as an eluent. A HPLC separation with two injections of the crude extract after 0.0 and 3.0 min, using a gradient as follows, was applied to achieve fast separation of the *cis*- and *trans*-isomers: 41% solvent B for 20 min and, then, increasing solvent B to 100% within 0.5 min, followed by an isocratic elution for 1 min. After 21.5 min, solvent B decreased again to 41% within 0.1 min and was kept for 3.0 min prior to the next injection. After removing the solvent in vacuum and freeze-drying twice, compounds **35a** and **35b** were isolated (>98% purity) and were used for sensory experiments as well as UV/vis, TOF-MS, and 1D/2D NMR. Based on the comparison of spectroscopic and chromatographic data with those recorded for references, compounds reported earlier in the literature were identified as *cis*-/*trans*-*p*-coumaric acid ethyl ester (**35a**/**b**).[29]

Synthetic Preparation of Xanthohumol P (15). An aliquot (25 mg) of xanthohumol (**5**) and Amberlyst 15 (1 g) was stirred in ethanol (10 mL) for 4 h at 80 °C. HPLC purification of **15** was achieved using a gradient as follows: 30% solvent B for 2.0 min and, then, increasing solvent B to 80% within 12.5 min and, finally, increasing solvent B to 100% within 1.0 min, followed by isocratic elution for 2.5 min. After 16.0 min, solvent B decreased again to 30% within 1.0 min and was kept for 3.0 min prior to the next injection. The compound eluting after 15.3 min was isolated (>98% purity) and, after the solvent was removed in vacuum and freeze-dried twice, was used for sensory experiments. Structure verification by means of UV−vis, LC-MS/MS, UPLC-TOF-MS, and 1D/2D NMR undoubtedly identified the compound as xanthohumol P, **15** (Figure 4), which has to the best of our knowledge not been described yet in the literature. In addition, compounds **6** and **12−14** were isolated from the reaction mixture.

*Xanthohumol P, **15** (Figure 1).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 367 nm. LC-MS (ESI^-): *m/z* (%) 399 (57, $[M-H]^-$), 119 (100), 353 (64), 232 (20), 174 (13). LC-TOF-MS (ESI^-): *m/z* 399.1802 ($[M-H]^-$, measured), *m/z* 399.1808 ($[M-H]^-$, calcd for $C_{23}H_{27}O_6^-$). ^1H NMR (400 MHz, MeOD; COSY): *δ* (ppm) 1.18 [t, 3H, H−C(7″)], 1.23 [s, 6H, H−C(4″, 5″)], 1.65 [m, 2H, *J* = 7.1, *J* = 8.5, H−C(2″)], 2.58 [m, 2H, *J* = 7.1, *J* = 8.5), H−C(1″)], 3.53 [dd, 2H, H−C(6″)], 3.90 [s, 3H, H−C(1‴)], 6.03 [s, 1H, H−C(6)], 6.87 [m, 2H, *J* = 8.7 Hz, H−C(3′, 5′)], 7.50 [m, 2H, *J* = 8.7 Hz, H−C(2′, 6′)], 7.67 [d, 1H, *J* = 15.5 Hz, H−C(3)], 7.80 [d, 1H, *J* = 15.5 Hz, H−C(2)]. ^{13}C NMR (100 MHz, MeOD; HSQC, HMBC): *δ* (ppm) 16.3 [C(7″)], 17.8 [C(1″)], 26.3 [C(4″, 5″)], 39.0 [C(2″)], 56.2 [C(1‴)], 57.5 [C(6″)], 76.4 [C(3″)], 91.7 [C(6)], 106.5 [C(10)], 110.0 [C(8)], 116.9 [C(3′, 5′)], 125.9 [C(3)], 128.5 [C(1′)], 131.3 [C(2′, 6′)], 143.4 [C(2)], 161.1 [C(4′)], 162.4 [C(5)], 163.8[C(9)], 166.2 [C(7)], 194.1 [C(4)].

Alkaline Isomerization of Compounds 9, 12, 15, and 29. An aliquot (10 mg) of the compound **9**, **12**, **15**, and **29**, respectively, was dissolved in 5 mL of methanol and, after adjusting the pH to a value of 14 with potassium hydroxide (0.1 mol/L), stirred for 30 min in a closed glass vial at 60 °C. The reaction mixture was diluted with water and subjected to a solid phase extraction using 55 *μ*m, 70A, Strata C18-E Giga Tube cartridges (200 mg, 3 mL; Phenomenex, Aschaffenburg, Germany) and methanol as an eluent, prior to HPLC separation on a 250 × 21.2 mm, 5 *μ*m, Luna Phenyl-Hexyl column (Phenomenex, Aschaffenburg, Germany). The HPLC system was operated with a flow rate of 15 mL/min, using 0.1% aqueous formic acid and acetonitrile containing 0.1% formic acid as solvents, and using a gradient as follows: 20% solvent B for 2.5 min and then increasing solvent B to 100% within 20.0 min followed by isocratic elution for 2.5 min. After 25.0 min, solvent B decreased again to 20% within 2.0 min and was kept for 3.0 min prior to the next injection. After the solvent was removed in vacuum and freeze-dried twice, compounds **7**, **8**, **13**, **16**, and **30** (>98% purity) were used for sensory experiments and structure determination. Based on the comparison of spectroscopic (UV/vis, LC-TOF-MS, $^{1}H/^{13}C$ NMR) and chromatographic data (retention time) with those recorded for reference substances, the following compounds were identified: desmethylxanthohumol (**7**),[25] 8-prenylnaringenin (**8**),[25] 1″,2″-dihydroisoxanthohumol C (**13**),[45] and isoxanthohumol M (**30**). To the best of our knowledge, isoxanthohumol P, **16** (Figure 4), has not been earlier reported in the literature.

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Table 2. Concentrations of Taste-Active Soft and Hard Resin Components in Commercial Hop Products

	concn (μmol/100 g)											
	tannin extract		hard resin		pellets	Xantho Flav extract		resin extract		soft resin		
tastant[a]	aroma hops	bitter hops	Perle	Taurus	Herkules	15%	75%	aroma hops	bitter hops	Perle	Taurus	ε-extract
1a	799.5	559.0	681.4	694.9	15987	590.1	578.3	8727	8216	5820	5786	251.7
1b	798.2	818.3	857.3	833.2	15964	731.6	639.2	12139	11665	9533	12327	423.8
1c	378.8	363.9	362.0	450.9	7573	342.6	307.3	5403	4898	3904	4083	173.9
2a	730.4	650.7	679.7	785.6	14605	815.6	644.4	14808	14720	12603	19220	614.2
2b	644.6	441.1	453.7	724.8	12892	825.9	606.5	15763	9396	7971	11818	456.5
2c	279.1	233.9	212.6	428.8	5584	419.4	268.7	5101	3783	4259	6364	188.9
3a	8.91	4.54	2.89	2.64	178.1	1.47	3.04	14.09	7.13	14.56	5.28	0.70
3b	6.91	5.88	16.05	17.40	137.9	2.76	4.93	57.21	41.93	15.85	21.09	1.07
3c	10.83	13.54	19.14	14.03	216.6	6.33	5.00	129.45	96.96	28.90	16.85	1.60
4a	0.98	0.34	1.47	0.66	19.7	0.21	0.69	6.18	6.57	2.86	3.86	0.46
4b	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
4c	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5	101.3	100.5	62811	33031	4993	35953	207646	1082	736.1	35.18	629.6	8601
6	30.83	45.44	7202	4817	265.7	13916	9754	112.8	98.08	1.57	126.5	7671
7	<0.01	17.14	93.68	15.61	57.25	<0.01	670.1	<0.01	5.50	<0.01	<0.01	21.06
8	11.41	18.05	3449	653.6	57.84	1319	3478	32.23	25.26	1.18	34.80	658.9
9	55.26	49.97	6532	1067	139.1	2524	13873	73.35	57.15	3.01	52.50	1154
10	<0.01	<0.01	16383	241.4	34.56	156.1	140.2	4.84	36.83	2.18	1193	<0.01
11	<0.01	<0.01	<0.01	<0.01	1575	8142	<0.01	<0.01	<0.01	<0.01	<0.01	6879
12	<0.01	<0.01	20347	4571	3.56	140.3	70.73	0.98	1.08	2.26	41.16	66.13
13	<0.01	<0.01	733.3	218.5	<0.01	317.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
14	<0.01	<0.01	7540	21723	6.55	112.9	717.5	2.01	<0.01	2.38	<0.01	37.74
15	<0.01	<0.01	919.8	363.4	180.3	601.9	397.4	11.26	11.43	0.55	<0.01	453.6
16	5.00	<0.01	341.2	271.4	32.09	896.0	752.1	5.95	2.82	<0.01	13.67	762.1
17	20.94	<0.01	4107	20445	464.0	1149	504.7	58.27	90.45	20.48	1320	7155
18	<0.01	<0.01	511.6	1967	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	87.09
19	<0.01	33.88	1651	1139	758.4	2306	1797	14.41	12.02	<0.01	114.2	438.5
20	<0.01	<0.01	1017	745.7	133.2	1004	570.1	20.24	8.45	<0.01	<0.01	259.7
21	14.43	7.33	370.5	5165	303.7	983.8	815.1	42.05	31.31	11.90	74.18	1206
22	<0.01	<0.01	7331	1032	8.9	<0.01	1171	<0.01	<0.01	<0.01	<0.01	50.85
23	7.58	<0.01	1715	466.8	<0.01	<0.01	98.55	<0.01	<0.01	<0.01	<0.01	41.41
24	<0.01	3.02	1316	1238	166.2	1562	330.5	16.89	12.26	0.21	6.56	245.5
25	<0.01	<0.01	2797	2491	116.0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	<0.01	<0.01	289.2	601.9	34.27	2854	4548	17.57	7.08	<0.01	<0.01	681.3
27	<0.01	<0.01	1459	626.3	97.36	757.9	850.4	<0.01	21.19	<0. 01	<0.01	<0.01
28	27.97	41.93	983.5	2138	67.85	1676	11656	27.61	17.65	<0.01	<0.01	569.3
29	<0.01	<0.01	20354	1344	<0.01	23.96	22.40	1.02	<0.01	0.47	15.94	15.52
30	<0.01	<0.01	827.3	110.0	261.1	1429	95.80	0.56	3.73	<0.01	<0.01	38.88
31	<0.01	<0.01	<0.01	1924	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
32	<0.01	<0.01	<0.01	1022	68.38	1568	1418	<0.01	<0.01	<0.01	<0.01	2086
33a/b	<0.01	<0.01	1065	2357	227.2	<0.01	2997	156.4	<0.01	<0.01	<0.01	<0.01
34a/b	<0.01	<0.01	1777	3801	<0.01	276.8	<0.01	<0.01	<0.01	<0.01	<0.01	691.0
35a/b	<0.01	42.55	<0.01	<0.01	<0.01	<0.01	229.7	<0.01	<0.01	2.59	<0.01	183.5
36	<0.01	127.5	1041	507.8	26.12	<0.01	1239	27.16	<0.01	<0.01	<0.01	<0.01
37	9775	5927	831.4	4189	5622	2063	661.8	28.98	55.21	3.32	293.7	<0.01
38	14541	4623	3436	5447	31869	82522	45105	197.5	15.06	<0.01	401.5	<0.01
39	4280	813.2	254.88	1318	250.0	351.8	124.4	12.02	<0.01	<0.01	<0.01	<0.01
40a	1363	4802	297.88	2906	4639	1864	2968	20.42	79.87	3.85	129.9	3408
40c	585.1	1235	219.35	1820	1415	1178	800.6	19.94	21.80	1.31	146.6	124.08
41b	428.7	1038	<0.01	252.0	263.2	348.1	<0.01	8.95	<0.01	<0.01	<0.01	<0.01
42	<0.001	<0.01	2999	1637	100.6	380.7	1264	17.19	7.27	<0.01	<0.01	<0.01

[a]Numbering of molecules is referring to structures given in Figure 1.

*Isoxanthohumol P, **16** (Figure 1).* UV/vis (ACN/water; 0.1% formic acid): λ_{max} = 287 nm. LC-MS (ESI$^-$): *m/z* (%) 399 (10, [M − H]$^-$), 119 (100), 265 (21). LC-TOF-MS (ESI$^-$): *m/z* 399.1804 ([M − H]$^-$, measured), *m/z* 399.1808 ([M − H]$^-$, calcd for $C_{23}H_{27}O_6^-$). ^{1}H NMR (400 MHz, MeOD; COSY): δ (ppm) 1.12 [s, 6H, H−C(4″, 5″)], 1.12 [t, 3H, H−C(7″)], 1.60 [m, 2H, H−C(2″)], 2.54 [m, 2H, H−C(1″)], 2.67 [dd, 1H, *J* = 3.2, *J* = 16.9, H−C(3eq)], 3.02 [dd, 1H, *J* = 12.8, *J* = 16.9, H−C(3ax)], 3.34 [dd, 2H, H−C(6″)], 3.79 [s, 3H, H−C(1‴)], 5.32 [dd, 1H, *J* = 3.2, *J* = 12.8, H−C(2)], 6.12 [s, 1H, H−C(6)], 6.81 [m, 2H, *J* = 8.8 Hz, H−C(3′, 5′)], 7.33 [m, 2H, *J* = 8.8 Hz, H−C(2′, 6′)]. ^{13}C NMR (100 MHz, MeOD; HSQC, HMBC): δ (ppm) 15.8 [C(7″)], 17.5 [C(1″)], 25.3/27.1 [C(4″)/C(5″)], 38.0 [C(2″)], 45.9 [C(3)], 55.3

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Table 3. Concentration of Hop-Derived Taste Components in a Commercial Pilsner-Type Beer and Beer Samples I–IV from a Brewing Trial Using ε-Extract and Spent Hops, Respectively

	concn (μmol/L)[c]				
tastant[a]	Pilsner beer[b]	beer I	beer II	beer III	beer IV
1a	1.30	3.34	23.56	11.63	7.50
1b	0.99	0.68	28.14	6.85	4.55
1c	0.17	0.16	4.53	1.10	0.83
2a	0.08	1.45	0.95	0.44	0.34
2b	0.05	0.04	0.37	0.08	0.10
2c	0.01	0.02	0.10	<0.01	0.02
3a	16.49	17.63	0.96	6.34	2.07
3b	24.32	26.17	1.65	9.09	3.31
3c	9.71	7.16	0.55	1.93	0.83
4a	5.10	5.23	0.83	3.86	1.10
4b	6.24	7.44	0.69	3.86	1.24
4c	<0.01	<0.01	<0.01	<0.01	<0.01
5	0.04	0.31	56.12	4.16	2.86
6	5.54	<0.01	4.02	0.46	0.92
7	0.01	0.08	0.10	0.21	0.07
8	0.05	<0.01	<0.01	0.02	0.01
9	0.06	0.09	3.25	0.50	0.80
10	<0.01	0.59	0.08	0.11	0.17
11	<0.01	0.39	<0.01	<0.01	<0.01
12	<0.01	0.05	0.06	0.04	0.02
13	0.03	0.18	10.44	3.35	2.55
14	0.01	<0.01	1.16	0.91	1.25
15	<0.01	0.01	1.61	0.30	0.12
16	0.04	0.03	0.87	9.84	9.68
17	<0.01	0.22	4.12	0.47	0.11
18	<0.01	<0.01	<0.01	0.07	<0.01
19	<0.01	<0.01	3.69	0.76	0.67
20	0.01	0.04	4.00	0.88	1.02
21	0.01	0.28	3.84	0.98	0.74
22	0.01	0.05	0.94	0.65	1.20
23	0.02	2.27	78.97	96.74	110.8
24	<0.01	0.02	0.12	0.03	0.08
25	<0.01	<0.01	<0.01	<0.01	11.43
26	0.03	0.02	<0.01	<0.01	<0.01
27	<0.01	<0.01	0.18	0.87	0.29
28	0.05	<0.01	8.62	32.37	9.33
29	<0.01	0.03	0.01	0.01	0.01
30	0.01	0.22	8.87	12.71	13.15
31	<0.01	0.20	4.02	<0.01	0.96
32	0.10	0.22	8.29	7.07	6.17
33a/b	0.01	34.83	48.32	69.36	46.24
34a/b	<0.01	0.03	0.104	0.12	0.09
35a/b	0.01	<0.01	1.83	0.51	2.06
36	0.01	0.49	0.947	15.14	0.79
37	3.54	1.42	4.64	566.4	46.82
38	12.03	4.50	9.42	663.3	43.74
39	0.38	0.12	0.364	24.08	2.03
40a	3.93	0.69	20.85	261.7	45.36
40c	0.90	0.12	8.52	142.0	17.93
41b	0.13	0.16	0.717	111.1	6.67
42	0.48	1.02	7.59	23.71	7.57

[a]Chemical structures are given in Figure 1. [b]Commercial Pilsner-type beer. [c]Beers I–IV were produced by the Research Brewery St. Johann (Germany) and their International Bitterness Units (IBU) adjusted as follows: beer I (reference) was produced using an Isohop extract (30%) added to the beer to adjust an IBU value of 23. Beers II–IV were produced using the same wort as for beer I just differing in the hop products. For beer II (27 IBU) and beer IV (27 IBU), an ε-extract was added to the wort postboiling and at the beginning of the kettle boiling, respectively. Beer III (51 IBU) was made by adding spent hops, obtained by exhaustive carbon dioxide extraction, to the wort prior to boiling.

[C(6″)], 56.4 [C(1‴)], 75.6 [C(3″)], 79.8 [C(2)], 93.8 [C(6)], 105.3 [C(10)], 109.9 [C(8)], 115.8 [C(3′, 5′)], 128.4 [C(2′, 6′)], 130.6 [C(1′)], 158.2 [C(4′)], 161.2[C(5)], 163.3 [C(9)], 163.2 [C(7)], 192.4 [C(4)].

Quantitation of Bitter Compounds in Hop Extracts and Beer by LC-MS/MS. For quantitation of bitter compounds, aliquots of hop pellets (1.5 g), hop extracts (100 mg), and samples (40 mg) of the advanced hop extracts XanthoFlav 15, XanthoFlav 75, and polyphenol extracts, respectively, were exhaustively extracted for 1 h with methanol (4 × 25 mL) during ultrasonification. After filtration, the extracts were diluted (1:100) with methanol and, after membrane filtration (0.45 μm, Sartorius, Göttingen, Germany), samples (5 μL) were directly injected into a Dionex UltiMate 3000 series UHPLC system consisting of two pumps, a degasser, a column compartment, and an autosampler (Dionex, Idstein, Germany) connected to an API 4000 Q-TRAP mass spectrometer (SCIEX, Darmstadt, Germany) which was equipped with an electrospray ionization (ESI) source and operated in negative ionization mode.

For the analysis of beer samples, the beverage was degassed by ultrasonification and membrane filtered (0.45 μm, Sartorius, Göttingen, Germany), and aliquots (5 μL) were directly injected into the SCIEX LC-MS system. The temperature of the autosampler was set to 5 °C and of the column compartment to 40 °C. The declustering potential (DP), the cell exit potential (CXP), and the collision energy (CE) of the mass spectrometer were optimized for each substance to induce fragmentation of the pseudo molecular ion $[M - H]^-$ to the corresponding target product ions after collision-induced dissociation. Quantitative analysis was performed by means of multiple reaction monitoring (MRM) mode using optimized fragmentation parameters and retention times of the corresponding reference compounds. The ion spray voltage was set to −4500 V, and dwell time for each mass transition was 2.5×10^{-3} s. Nitrogen was used as the collision gas (4×10^{-5} Torr). Using unhopped beer as a blank matrix, 8-point external matrix calibration curves were recorded for the individual analytes **1**–**42** in low (2.5 nmol/L to 200 μmol/L) and high concentrations (0.2 mmol/L to 20 mmol/L) by means of UPLC-MS/MS. Quantitation of the co- and ad-congeners of α-acids (**1**), β-acids (**2**), and *cis*-/*trans*-iso-α-acids (**3**, **4**) was performed by using the calibration curve of the *n*-congeners. Calibration functions were forced through zero, thus leading to correlation coefficients of >0.99 for all the reference compounds in unhopped beer. Concentrations of *cis*-/*trans*-*p*-coumaric acid (**33a**/**b**) were corrected for amounts detected in the nonhopped beer (control). The limit of detection was determined to be 0.3 nmol/L (S/N ratio: 3) for all compounds (**1**–**42**), and the limit of quantification was accordingly estimated to be 0.001 μmol/L (S/N ratio: 10).[46] Data processing and integration was performed by means of Analyst software version 1.5 (AB Sciex Instruments, Darmstadt, Germany). As stationary phase, a 150 × 2.0 mm, 4 μm, Synergi Hydro-RP column (Phenomenex, Aschaffenburg, Germany) was used. The mobile phase consisted of acetonitrile (0.1% formic acid) as solvent A and water (0.1% formic acid) as solvent B. Using a flow rate of 0.6 mL/min, chromatographic separation was achieved (Figure 5) by gradient elution increasing solvent A from 10 to 40% within 15 min and further increased to 60% in 13 min, to 70% in 20 min, and finally to 100% within 2 min. It was maintained at 100% for 1 min, following by readjustment to 10% within 5 min and re-equilibrated for 4 min prior to the next injection.

Sensory Analysis. *General Conditions and Panel Training.* Twelve subjects (7 women and 5 men, ages 23–29 years), who gave

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consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, participated for at least two years in weekly training sessions and were recruited from the Chair of Food Chemistry and Molecular Sensory Science (Freising, Germany). To familiarize the subjects with the bitter taste of hop-derived compounds and to get them trained in recognizing bitterness by using the sip-and-spit method,[33] xanthohumol (**5**) and *trans*-isocohumulone (**4a**) were used as references. The sensory experiments were performed at 20−22 °C. To prevent cross-modal interactions with olfactory or visual inputs, the panelists used nose clips and yellow light or whenever necessary red light, respectively, was used.

Pretreatment of Fractions and Pure Compounds. Prior to sensory analysis, the fractions or isolated compounds were suspended in water, and, after removal of the volatiles under high vacuum (<5 hPa), were freeze-dried twice. ^{1}H NMR spectroscopy, GC/MS, and high-performance ion chromatographic analysis of an aliquot revealed that fractions treated by that procedure are essentially free of solvents and buffer compounds used. Formic acid, which is GRAS listed as a flavoring agent for food and feed applications, was used to adjust the pH value of solutions to be sensorially evaluated, because trace amounts of this acid do not influence the sensory profile of the test solution.

Sensory Analysis of Isolated Hop Resins. The individual resin fractions isolated from hops as described above were evaluated in their bitter intensity. To achieve this, each resin sample was taken up in 5% aqueous ethanol adjusted to pH 4.4 with trace amounts of 10% aqueous formic acid. The bitter intensity of these solutions was judged on a scale from 0 (not detectable) to 5 (strongly detectable).

Taste Dilution Analysis (TDA)..[33−35] An aliquot of each fraction isolated from hops was freeze-dried twice and dissolved in water containing 5% ethanol (pH 4.4) in "natural" concentration ratios matching the amounts present in 2.0 g of hops hard resin. After each fraction had been sequentially diluted 1 + 1 with water (pH 4.4), the serial dilutions of each of these fractions were presented to the sensory panel in order of ascending concentration. Using a duo test, panelists were asked to determine the dilution step at which a difference between sample and blank could be detected. This so-called taste dilution (TD) factor[33] determined by the 12 sensory assessors was averaged and did not differ by more than plus/minus one dilution step between the trained individuals.

Bitter Recognition Threshold Concentrations. Bitter taste threshold concentrations of the purified compounds were determined by 12 panelists using a duo test. The geometric mean of the last and the second to last concentrations was calculated and taken as the individual recognition threshold. The threshold value of the sensory group was approximated by averaging the threshold values of the individuals in three independent sessions and did not differ by more than plus/minus one dilution step. Based on the three independent repetitions, the confidence interval (α = 0.05) was calculated for each compound.

Taste Re-Engineering Experiments. To investigate the impact of the hop constituents on the bitter perception of beer, four taste recombinants were produced by spiking unhopped "zero" beer with bitter taste molecules in concentrations as determined in a standard Pilsner-type beer (Table 3) by means of LC-MS/MS. Recombinant A: "zero" beer was spiked with iso-α-acids (**3a−c**, **4a−c**). Recombinant B: recombinant A was spiked with xanthohumol (**5**) and isoxanthohumol (**6**). Recombinant C: recombinant A was spiked with prenylated flavonoids **5−32**. Recombinant D: recombinant A was spiked with compounds **5−42**. Using a linear 5-point scale, the bitterness of these recombinants A−D was then compared with that of a standard Pilsner-type beer (reference).

Sensomics Heatmapping. Data analysis was performed within the programming and visualization environment R (version 2.13.2).[47] The sensomics heatmap was calculated using the heatmap.2 function of R based on the concentration data after scaling the sum of all sensometabolites to an equal value for each beer sample. The dendrograms were constructed by means of an agglomerative linkage algorithm specifying the distance between two clusters as the increase in the error sum of squares after fusing two clusters into a single cluster and seeking for a minimum distance at each clustering step.[48]

Liquid Chromatography/Mass Spectrometry (LC-MS/MS). For compound identification, mass and product ion spectra were acquired on an API 4000 Q-Trap triple quadrupole/linear ion trap mass spectrometer (SCIEX, Darmstadt, Germany). Isolated fractions were dissolved in methanol/water (50/50, v/v) and directly introduced into the mass spectrometer by flow infusion using a syringe pump. Data were acquired in full-scan mode with negative electrospray ionization (−4500 V). Both quadrupoles operated at unit mass resolution. Nitrogen served as curtain gas (30 psi) and as turbo gas (450 °C). Fragmentation of the pseudo molecular ions $[M-H]^-$ into specific product ions was induced by collision with nitrogen (4.5×10^{-5} Torr). Data acquisition and instrumental control were performed with Analyst 1.5 software (SCIEX).

UPLC/Time-of-Flight Mass Spectrometry (UPLC/TOF-MS). Mass spectra of the compounds were measured on a Synapt G2 HDMS mass spectrometer (Waters, Manchester, U.K.) coupled to an Acquity UPLC core system consisting of a binary solvent manager, sample manager, and column oven. The compounds were dissolved in acetonitrile (1 mL), and aliquots (1−5 μL) were injected into the UPLC-TOF-MS system equipped with a 2 × 150 mm, 1.7 μm, BEH C18 column (Waters). Operated with a flow rate of 0.3 mL/min at 40 °C, the following gradient was used for chromatography: starting with a mixture (5/95, v/v) of acetonitrile and aqueous formic acid (0.1%, pH 2.5), the acetonitrile content was increased to 100% within 5 min and held at 100% for 1 min. Measurements were performed using negative ESI and the resolution modus consisting of the following parameters: capillary voltage −3.0 kV, sampling cone 30, extraction cone 4.0, source temperature 150 °C, desolvation temperature 450 °C, cone gas 30 L/h, and desolvation gas 850 L/h. The instrument was calibrated over an m/z range of 100−1200 using a solution of sodium formate (0.5 mM) in a 2-propanol/water mixture (9/1, v/v). All data were lock mass corrected using leucine enkephalin as the reference (m/z 554.2615, $[M-H]^-$). Data processing was performed by using MassLynx 4.1 (Waters) and the tool "elemental composition".

Nuclear Magnetic Resonance Spectroscopy (NMR). 1D/2D-^{1}H and ^{13}C NMR spectra were acquired on a 400 MHz DRX and a 500 MHz Avance III spectrometer (Bruker, Rheinstetten, Germany), respectively. CD_3OD, D_2O, or DMSO-d_6 was used as solvent, and chemical shifts are reported in parts per million relative to the solvent signal. For signal assignment, 2D-NMR experiments (COSY, HMQC, HMBC) were carried out using the pulse sequences taken from the Bruker software library. Data processing was performed by using XWin-NMR software (version 3.5; Bruker, Rheinstetten, Germany) as well as MestReNova 5.1.0-2940 (Mestrelab Research S.L., Santiago de Compostela, Spain).

RESULTS AND DISCUSSION

As the use of the hop's hard resin fraction for beer manufacturing was reported to reveal beers with an intense and pleasant bitter profile,[26] the sensory impact of the hard resin fractions should be evaluated. To achieve this, the hard resin fraction was isolated from hops,[30] chlorophylls and hop waxes were separated by means of solid phase extraction, and the resin was separated to give the water-soluble δ-resin and the nonsoluble ε-resin fraction by extraction with water at 40 °C.[31,32] Aliquots of the δ-resin, ε-resin, hard resin, soft resin, and total hop resin, respectively, were solubilized in 5% ethanolic solution in their "natural" concentration ratio (0.2/0.8/1/9/10; w/w), and the pH was adjusted to 4.4 to match the pH value of a standard beer. The trained sensory panelists, who were asked to rate the bitter intensity on a linear scale from 0 (not detectable) to 5.0 (very intense), judged the bitterness of the soft resin solution with the highest score of 4.25 (±0.25), followed by the ε-resin and the hard resin with 0.51 (±0.08) and 0.5 (±0.25), whereas bitterness was almost not detectable (0.08 ± 0.04) in the solution containing the δ-resin (data not shown). These data confirm the previously reported major bitter contribution of the soft resin

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components and indicated the ε-resin to account for the bitterness of the hop's hard resin fraction. The ε-resin was, therefore, further separated to locate and identify the key bitter molecules in that hydrophobic fraction.

Identification of Key Bitter Compounds in the ε-Resin. Fractionation of the ε-resin by means of MPLC on RP18 material revealed 11 fractions (e1−e11), which were dissolved in 5% ethanolic solution in their natural concentration ratio and, then, evaluated in their bitter impact by means of a taste dilution analysis (TDA).[33−35] Fractions e5, e6, and e8−e11 were judged with the highest TD factors of 64 and 256, respectively, followed by fractions e2−e4 judged with TD factors between 6 and 32 (Figure 2). Aimed at locating the key contributors to the bitter taste of fractions e2−e11, each MPLC fraction was separated by means of preparative RP-HPLC and the subfractions were again analyzed by means of TDA to locate most intense bitter tastants (Figure 3).

Comparison of spectroscopic data (UV/vis, LC-MS/MS, $^{1}H/^{13}C$ NMR) of xanthohumol (**5**) in subfractions e8-7, e9-6, and e10-2, isoxanthohumol (**6**) in subfractions e4-2, e5-3, e6-5, and e9-2, desmethylxanthohumol (**7**) in subfraction e7-12, 8-prenyl- (**8**) and 6-prenylnaringenin (**9**) in subfraction e8-5, 5′-prenylxanthohumol (**17**) in subfractions e10-10 and e11-8, xanthohumol D (**19**) in e7-9, xanthohumol B (**20**) in e7-7, and xanthohumol C (**21**) in e10-5 and e11-5 with the literature data[24,25] confirmed the identity of these compounds in the ε-resin (Table 1). In addition, 1″,2″-dihydroxanthohumol C (**12**) was identified in subfractions e10-4 and e11-4, 1″,2″-dihydroxanthohumol K (**14**) in e4-2, e5-3, and e7-3, xanthohumol H (**22**) in e6-7 and e7-4, xanthohumol I (**26**) in e3-4, and 2″-hydroxyxanthohumol M (**25**) in e7-8 (Table 1), the spectroscopic data of which confirmed previously published data.[23,36−38,45] Another polar bitter compound in fraction e2 was identified as co-multifidol glucopyranoside **40a**[43] in subfraction e2-7 (Table 1). Some trace amounts of α-acids (**1a**/**b**/**c**) were identified in e10-8/9 and e11-8/9, and β-acids (**2a**/**b**/**c**) in e11-7, e11-11, and e11-13, thus indicating some residual amounts of soft resin components in the hard resin fraction. Next to these bitter compounds, some astringent molecules were identified as *cis*/*trans*-*p*-coumaric acid (**33a**/**b**)[49] in subfraction e2-4, quercetin (**36**)[40] in e3-5, and quercetin-3-*O*-β-D-glucopyranoside (**37**)[41] in e2-5 (Table 1). The bitter molecules ad-multifidol glucopyranoside (**40c**)[43] and *n*-multifidol-di-*C*-glucopyranoside (**40b**)[44] as well as the astringent molecules *trans*-*N*-feruloyltyramine (**42**),[23,36,37] kaempferol-3-*O*-β-D-glucopyranoside (**38**),[41] and kaempferol-3-*O*-β-D-(6″-malonyl)glucopyranoside (**39**)[42] were isolated from a hop polyphenol extract and were also identified as hard resin constituents (Table 2). For unequivocal structure elucidation by means of NMR spectroscopy, glycosides **37**−**41** were isolated in larger quantities from the hop tannin extract.

In contrast to the above-mentioned taste molecules, compounds **10**, **11**, **18**, **23**, **27**−**29**, **31**, and **32** isolated from the ε-resin have not yet been identified as natural phytochemicals in hops and beer, respectively. Although not yet reported as hop components, xanthohumol O (**27**), xanthohumol L (**28**), and isoxanthohumol H (**23**) were found earlier as metabolites in feces of xanthohumol-fed rats.[39] The chemical structures of the previously not reported hop bitter constituents **10**, **11**, **18**, **29**, **31**, and **32** were determined by means of LC-MS and 1D/2D-NMR experiments.

NMR analysis of compound **29**, isolated from subfractions e8-3 and e9-4, showed similar ^{1}H and ^{13}C shifts as found for xanthohumol H (**22**), thus suggesting a similar chemical structure. However, an additional proton signal for H−C(6″) resonating at 3.28 ppm with an integral of three protons and an additional ^{13}C resonance signal for C(6″) at 50.6 ppm indicated compound **29** as the 3″-methoxy adduct of xanthohumol H (**22**). This was confirmed by the heteronuclear coupling observed between carbon atom C(3″) and the protons of the methyl group H−C(6″) in the HMBC spectrum (Figure 4). To confirm the natural occurrence of **29** and to exclude any artifact formation during workup, hop was extracted with ethanol instead of methanol. LC-MS/MS analysis of this ethanolic extract unequivocally identified xanthohumol M (**29**) in hops.

Compound **18** showed similar ^{1}H and ^{13}C NMR shifts for the naringenin moiety and the cyclic prenylic side chain as found for 1″,2″-dihydroxanthohumol K (**14**) and 1″,2″-dihydroxanthohumol C (**12**), respectively. But in comparison to **12** and **14**, any ^{1}H NMR signal for H−C(6) was lacking, whereas additional signals were observed for a second prenyl side chain as known for 5′-prenylxanthohumol (**17**). The HMBC spectrum indicated a heteronuclear coupling between carbon atom C(6) and the protons at C(1‴) and confirmed the attachment of a prenyl side chain to carbon C(6) (Figure 4). The hypsochromic shift of the UV maximum of xanthohumol (**5**) and 1″,2″-dihydroxanthohumol C (**12**), respectively, from ∼370 to 326 nm was well in line with the intramolecular cyclization of the prenyl side chain attached to carbon C(8) with the hydroxyl group bound to carbon C(9).[50] In consequence, the structure of this previously unknown compound was assigned as 1″,2″-dihydroxanthohumol F, **18** (Figure 4).

The spectroscopic data of compound **10**, showing major differences compared to those reported for other hop constituents in the past, indicated the presence of two prenyl side chains, a *p*-coumaroyl moiety, and a methoxy group in the HSQC spectrum. The HMBC spectrum revealed both prenyl side chains to be attached to carbon C(8) and to show connectivity to two keto functions, thus indicating a cyclohexenedione structure (Figure 4). In contrast to the keto function at C(4) as found in xanthohumol derivatives, carbon C(4) showed a ^{13}C chemical shift as expected for a hydroxylated carbon. However, the proton signal of H−C(2) showed an extraordinarily strong low-field shift as found for xanthohumol due to the *peri*-effect[51] caused by the keto function at C(9). Taking all spectroscopic data into consideration, the target bitter compound was identified as the previously not reported 4′-hydroxytunicatachalcone, **10** (Figure 4), which is the 4′-hydroxy derivative of tunicatachalcone identified earlier.[52]

The ^{1}H and ^{13}C NMR spectrum of compound **11** showed very similar chemical shifts as found for 5′-prenylxanthohumol (**17**) but additional resonance signals indicating the presence of another prenylic side chain and a keto function. Furthermore, an HSQC experiment revealed compound **11** to be a flavonoid showing the characteristic proton resonances at 2.96 and 3.16 ppm for the diastereotopic protons H−C(3). The HMBC spectrum showed a heteronuclear coupling between carbon C(9) and one prenylic side chain, and between carbonyl carbon C(7), resonating at 198.6 ppm, to three prenylic side chains (Figure 4). Taking all spectroscopic data into consideration, the structure of compound **11** was determined to be 5-hydroxy-2-(4-hydroxyphenyl)-6,6,8-triprenyl-6*H*-chroman-4,7-dione, coined isoxantholupon, **11** (Figure 4), which to the best of our knowledge has not previously been reported.

Compared to xanthohumol (**5**), compound **31** was missing the typical ^{1}H shifts of the *p*-coumaric acid moiety. Furthermore, the

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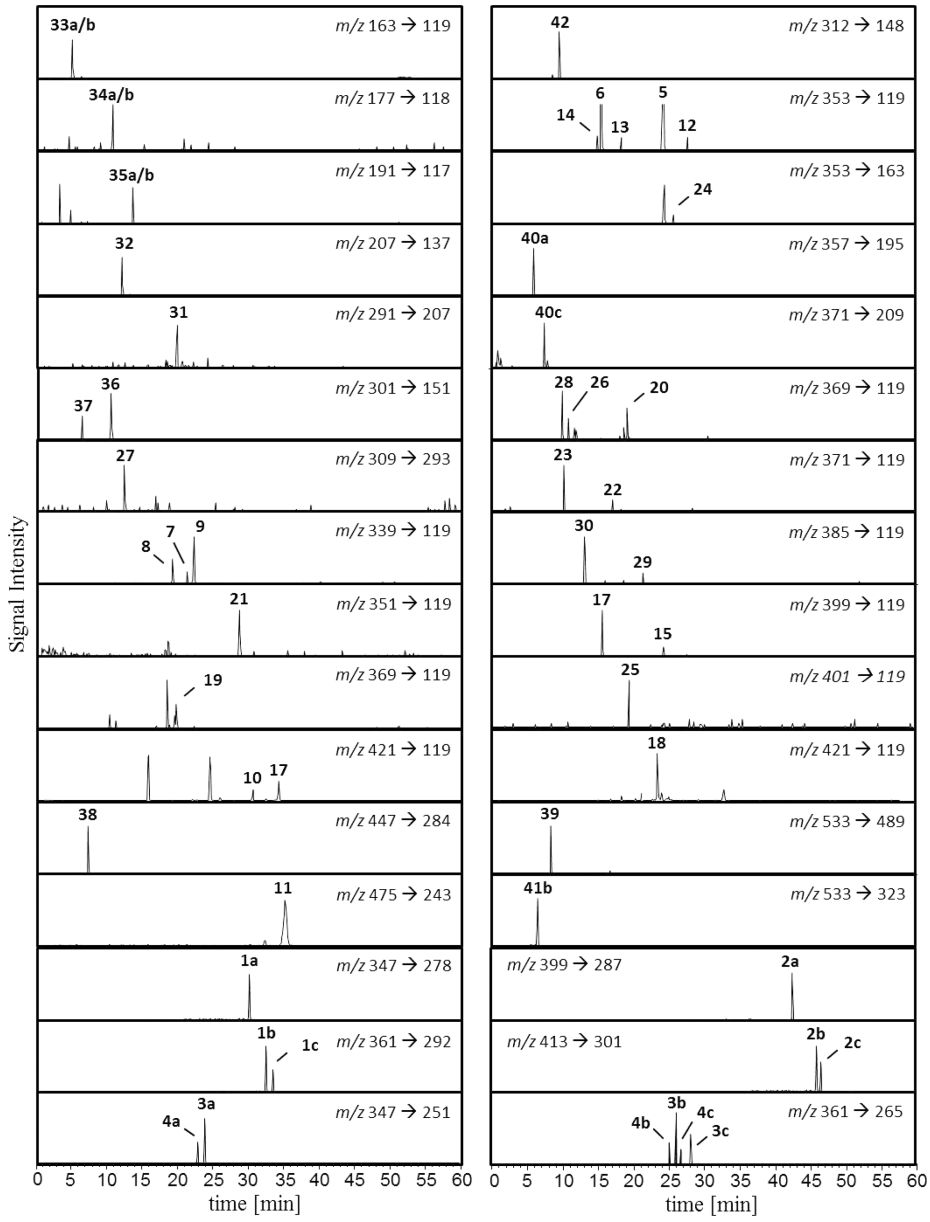

Figure 6. RP-HPLC-MS/MS mass chromatogram of taste compounds in hop extract. Numbering of compounds refers to chemical structures given in Figure 1.

spectra also showed that a substitution had occurred at carbon atom C(6) due to the lacking proton signal at this carbon atom. But interestingly, compound **31** exhibited typical ^{1}H shifts of an intact prenylic side chain as well as signals of two chemically identical methyl groups which showed no ^{1}J-coupling with any other proton. A long-range coupling of carbon C(9) and the methyl protons H−C (1′, 2′) in the HMBC spectrum revealed the structure of compound **31** to be 2,2-dimethyl-5,6,7-trihydroxy-8-(3-methylbut-2-enyl)chroman-4-one, which is coined 2″,3″-dehydrocyclohumulohydrochinone, **31** (Figure 4), due to its structural similarity with humulohydrochinon.[53] As LC-MS-MS analysis undoubtedly confirmed the presence of this compound in hops and excluded its formation as workup artifact (data not shown), compound **31** is considered a previously not reported hop constituent.

The ^{1}H NMR data of compound **32**, showing a molecular composition of $C_{12}H_{16}O_3$ (TOF-MS), revealed large similarities with those recorded for xanthohumol (**5**), but showed an additional singlet at 5.4 ppm and lacked the typical aromatic proton shifts of the *p*-coumaric acid moiety. Heteronuclear couplings in the HMBC spectrum indicated that a prenylic side chain is attached at carbon C(4) and that a methoxy group is connected to carbon C(1). Taking all spectroscopic data into account, the target compound was identified as the previously not reported 1-methoxy-4-prenylphloroglucinol, **32** (Figure 4). It is proposed in the literature that xanthohumol is biosynthesized from coumaroyl-CoA and three malonyl-CoA to form chalconaringenine, followed by a prenylation reaction to form first desmethylxanthohumol (**7**) and, thereafter, xanthohumol (**5**) by additional methylation.[54] Interestingly, the occurrence of compound **32** in hops provides evidence that the prenylation and methoxylation of the phloroglucin moiety during the biosynthesis of **5** can occur prior to its reaction with *p*-coumaroyl-CoA.

Synthetic Preparation of Xanthohumol Derivatives 12−16, 22−24, 29, and 30. In order to screen the hop fractions for additional xanthohumol derivatives, xanthohumol P

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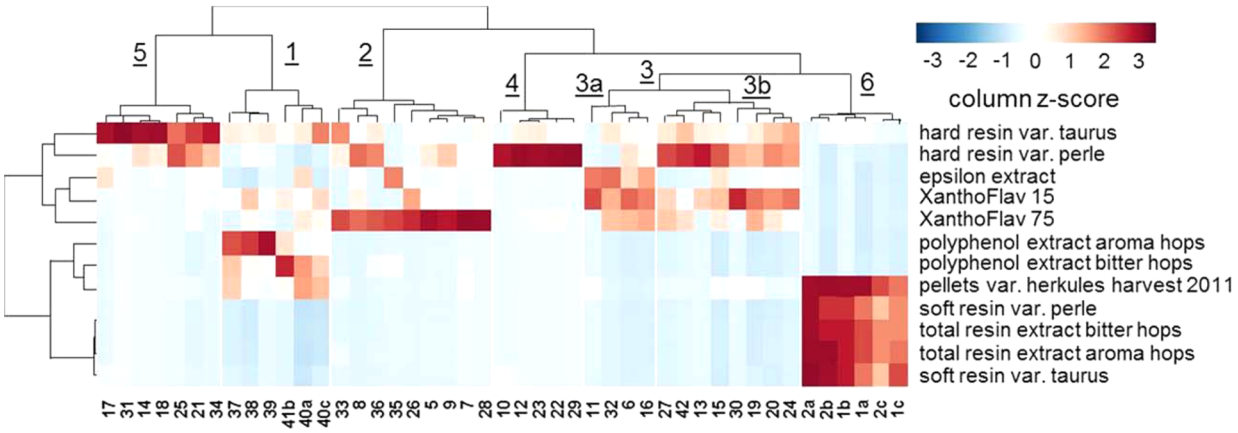

Figure 7. Sensomics heatmapping of concentrations of taste compounds in hop products/extracts taken from Table 3. The dendrogram is based on an agglomerative linkage algorithm.[48] Numbering of compounds refers to structures given in Figure 1

(**15**) was synthesized by heating an ethanolic solution of xanthohumol (**5**) for 4 h at 80 °C in the presence of the acidic cation exchanger Amberlyst 15, while isoxanthohumol P (**16**) and isoxanthohumol M (**30**) were obtained after alkaline isomerization of xanthohumol P (**15**) and xanthohumol M (**29**), respectively. Reaction of xanthohumol (**5**) with hydrobromic acid in methanol/acetic acid revealed, besides compounds **6**, **15**, **16**, **29**, and **30**, additional major transformation products, namely, **12**, **14**, and **22**−**24** (Figure 5). These compounds were identified as 1″,2″-dihydroxanthohumol C (**12**) and K (**14**), and xanthohumol H (**22**) by LC-MS and 1D/2D-NMR experiments as well as by cochromatography with the corresponding reference substance (Figure 4). Isoxanthohumol H (**23**) and 1″,2″-dihydroisoxanthohumol C (**13**) were isolated and identified by comparing the LC-MS and NMR data to those reported in literature.[39,45] Reaction product **24** exhibited similar ^{1}H and ^{13}C shifts as found for the naringenin substructure in xanthohumol (**5**) but with a different proton signal pattern recorded for the prenylic side chain. Instead of two nonaromatic signals integrating for three protons, only one signal at 1.79 ppm with an integral of three protons was observed. In addition, resonance signals integrating for two protons were observed at 2.18 and 4.66 ppm, respectively, suggesting that the double bond in **24** is located between C(3″) and C(4″). The heteronuclear coupling between C(3″) and H−C(4″) observed in the HMBC spectrum (Figure 4) confirmed the structure of **24** as xanthohumol N (Figure 1), which to the best of our knowledge has not yet been reported earlier.

Sensory Evaluation of Purified Hop Components. Prior to sensory analysis, the purity of each compound was confirmed by HPLC-MS as well as ^{1}H NMR spectroscopy to be more than 98%. To determine the human threshold concentrations for the bitter sensation induced by the hop derivatives identified, aqueous solutions of target compounds were evaluated in serial dilutions by means of a duo test (Table 1). The bitter threshold concentrations determined for the α-acids (**1**), β-acids (**2**), xanthohumol (**5**), and isoxanthohumol (**6**) by means of the duo test were comparable to those recorded recently by means of a triangular test,[15−17] thus confirming the suitability of the duo test setup.

Except for the *p*-coumaric acid esters (**34a**/**b**, **35a**/**b**), the human bitter threshold concentrations were relatively low and ranged from 5 to 44 μmol/L (Table 1). Comparably high threshold concentrations (>20 μmol/L) were found for compounds **15**, **16**, **19**, **22**, **23**, **26**, and **30** exhibiting a modified prenylic side chain, while an intramolecular cyclization (**12**, **14**, **20**, **28**) seemed not to influence the bitter threshold levels. More lipophilic molecules like compound **12** that elute later on RP-18 material seem to have a lower human threshold concentration than structurally related earlier eluting substances like compound **14**. Interestingly, the polar *O*-β-D-glycosides of the multifidol congeners **40a** and **40c** feature surprisingly low human threshold concentrations of 5 and 10 μmol/L, respectively (Table 1). As the α/β-acids, iso-α-acids, xanthohumol, and isoxanthohumol activate three bitter taste receptors, namely, hTAS2R1, hTAS2R14, and hTAS2R40 receptor,[21] these data suggest the involvement of another bitter taste receptor responding to the bitter β-glycosides **40a** and **40c**. One candidate receptor would be the hTAS2R16, which has been reported to be activated by β-glycosides like salicin.[55]

Quantitation and Sensomics Heatmapping of Hop-Derived Bitter Compounds in Hop Products and Beer. To gain insight into the occurrence and concentrations of the individual hop-derived bitter compounds in beer, compounds **1**−**42** (Figure 1) were quantitated in hop extracts as well as a Pilsner-type beer by means of HPLC-MS/MS in a single chromatographic run. Operating the mass spectrometer in the multiple reaction monitoring (MRM) mode, a highly selective and sensitive quantitation of the analytes could be accomplished (Figure 6).

As expected, xanthohumol (**5**) was the most predominant compound (>30 mmol/100 g) found in the hard resin (Table 2). With the exception of the very lipophilic isoxantholupon (**11**) and *cis*-/*trans*-*p*-coumaric acid ethyl ester (**35a**/**b**), all other compounds identified above were found in the hard resin as well. 1-Methoxy-4-prenyl-phloroglucinol (**32**) and 2″,3″-dehydrocyclohumulohydrochinon (**31**) were only detectable in the hard resin isolated from the bitter hop variety Taurus (Table 2). As expected, trace amounts of most of the prenylated flavonoid were found in the soft resins too. Only the nonpolar 4′-hydroxytunicatachalcone (**10**) and 5′-prenylxanthohumol (**17**), respectively, were observed in considerable amounts of 1193 and 1320 μmol/100 g in the soft resin of the bitter hop variety Taurus. Analysis of the commercially available pure resin extracts contained high amounts of >700 μmol/100 g of xanthohumol (**5**), whereas all other compounds (**6**−**42**) were present in comparatively low amounts (Table 2).

Quantitative analysis of the XanthoFlav 75% extract revealed, next to xanthohumol (**5**), high concentrations of isoxanthohumol (**6**, 9754 μmol/100 g), desmethylxanthohumol (**7**, 13873

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μmol/100 g), xanthohumol L (**28**, 11656 μmol/100 g), and kaempferol-3-*O*-β-D-glycopyranoside (**38**, 45105 μmol/100 g). With the exception of xanthohumol (**1**), the XanthoFlav 15% extract did not differ much in its composition from the xanthohumol enriched extract XanthoFlav 75%. The only significant difference was the presence of much higher amounts (8142 vs 3.87 μmol/100 g) of isoxantholupon (**11**) in the extract XanthoFlav 15%. The ε-extract[27] contained quite the same compounds as the XanthoFlav extracts, however, xanthohumol (**5**) and isoxanthohumol (**6**) were present in comparably low amounts of 8601 and 7671 μmol/100 g, respectively, and the polar compounds **10**, **34**−**39**, **41b**, and **42** were not detectable at all (Table 2).

Commercially available tannin extracts revealed very high amounts of the flavonol glycosides **37**−**39** (813.2−14541 μmol/100 g) and the intensely bitter tasting multifidol glycosides **40a**, **40b**, and **41b** (428.7−4802 μmol/100 g). Interestingly, the extract prepared from an aroma hop variety has higher amounts of the flavononol glycosides **37**−**39** (18.60 vs 11.36 mmol/100 g), whereas the extract made from a bitter hop variety showed higher levels of the multifidol glycosides **40a**, **40b**, and **41b** (2.38 vs 7.08 mmol/100 g). Both extracts contained only minor amounts (<105 μmol/100 g) of xanthohumol (**5**) and its derivatives.

To examine the multivariate distances between the individual sensometabolites throughout the set of different hop products, the concentrations determined for hard resin-derived compounds (**5**−**42**) were scaled, followed by a hierarchical cluster analysis to arrange the taste compounds into six clusters labeled 1−6 as visualized in a sensomics heatmap (Figure 7). Cluster 1 consisted of the glycosides **37**−**41b** present in high concentrations in the tannin extracts and in hop pellets, thus demonstrating the quantitative dominance of glycosides over the xanthohumol derivatives. Cluster 2 comprised xanthohumol (**5**), desmethylxanthohumol (**7**), its isomerization products 8-(**8**) and 6-prenylnaringenin (**9**), xanthohumol I (**26**), *cis*-/*trans*-*p*-coumaric acid ethyl ester (**35a**/**b**), and quercetin (**36**) being the characteristic attribute of the XanthoFlav extract 75%. In comparison, the large cluster 3 consisted of isoxanthohumol (**6**), isoxantholupon (**11**), isoxanthohumol P (**16**), and 1-methoxy-4-prenylphloroglucinol (**32**) separating in subcluster 3a and 1″,2″-dihydroisoxanthohumol C (**13**), xanthohumol P (**15**), xanthohumol D (**19**), xanthohumol B (**20**), xanthohumol N (**24**), xanthohumol O (**27**), isoxanthohumol M (**30**), and *N*-*trans*-feruloyltyramine (**42**) in subcluster 3b. Whereas higher concentrations of compounds in subcluster 3a seem to be characteristic for the ε-extract and the XanthoFlav 75% extract, the extract XanthoFlav 15% and the hard resins showed high concentrations of compounds of subcluster 3b. Interestingly, the hard resin fraction prepared from the hop varieties Taurus and Perle showed significant differences in clusters 4 and 5. Compounds 4′-hydroxytunicatachalcone (**10**), 1″,2″-dihydroxanthohumol C (**14**), xanthohumol H (**22**), isoxanthohumol H (**23**), and xanthohumol M (**29**) are grouped in cluster 4 and are representative for the Perle hard resin, whereas 1″,2″-dihydroxanthohumol K (**14**), 5′-prenylxanthohumol (**17**), xanthohumol C (**21**), 2″-hydroxyxanthohumol M (**25**), 2″,3″-dehydrocyclohumulohydrochinon (**31**), and *cis*-/*trans*-*p*-coumaric acid methyl ester (**34a**/**b**), classified in cluster 5, seem to be typical for the Taurus hard resin. As expected, the α-acids (**1a**/**b**/**c**) and β-acids (**2a**/**b**/**c**) are characteristic for the total resin extract and the soft resin and are therefore grouping together in cluster 6. These data clearly show that the various hop products are distinctive for their content of specific hard resin derived compounds and are a useful resource to adjust levels of desired compounds in final hop-containing products like beer.

The quantitative analysis of a traditional Pilsner beer (Table 3) confirmed that the iso-α-acids (**3a**/**b**/**c**, **4a**/**b**/**c**) are the quantitatively predominant bitter tasting compounds[12−14] with concentrations between 5.0 and 24.5 μmol/L.[56] Due to the isomerization reaction during wort boiling, significant amounts (5.5 μmol/L) of isoxanthohumol (**6**) are present in the beer sample. Next to the iso-α-acids (**3a**/**b**/**c**, **4a**/**b**/**c**) and isoxanthohumol (**6**), the glycosides (**37**−**41b**) are the third largest group with concentrations between 0.9 and 4.0 μmol/L. All other xanthohumol and isoxanthohumol derivates (**5**, **8**−**30**) are present in concentrations below 0.6 μmol/L. With the exception of the iso-α-acids (**3a**/**b**/**c**, **4a**/**b**/**c**), none of the other compounds were detected in beer above their taste threshold concentration (Table 1). In order to investigate whether the subthreshold prenylated flavonoids and glycosides contribute additively to the beer's bitterness, taste re-engineering experiments were performed in the following.

Taste Re-Engineering Experiments. Samples of unhopped "zero" beer were spiked with natural concentrations (Table 3) of the individual purified iso-α-acids **3** and **4** to give recombinant A, the iso-α-acids (**3a**−**c**, **4a**−**c**), xanthohumol (**5**), and isoxanthohumol (**6**) to give recombinant B, the iso-α-acids (**3a**−**c**, **4a**−**c**) and the prenylated flavonoids **5**−**32** to give recombinant C, and the iso-α-acids (**3a**−**c**, **4a**−**c**) plus all the identified compounds **5**−**42** to give recombinant D. Using a linear 5-point scale, the bitterness of these recombinants A−D was then compared with that of a standard Pilsner-type beer as reference (Figure 8). Without any further addition, the bitterness

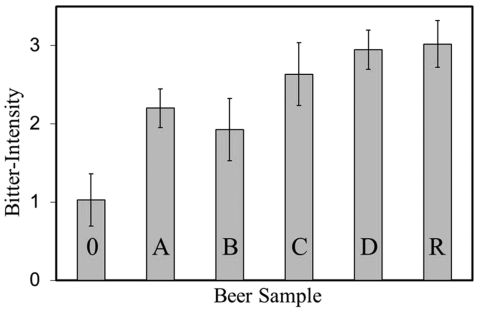

Figure 8. Bitter taste intensity of (R) standard Pilsner-type (reference), (0) unhopped "zero" beer and "zero" beer spiked with (A) iso-α-acids (**3a**/**b**/**c**, **4a**/**b**/**c**), (B) iso-α-acids (**3a**/**b**/**c**, **4a**/**b**/**c**), xanthohumol (**5**), and isoxanthohumol (**6**), (C) iso-α-acids (**3a**/**b**/**c**, **4a**/**b**/**c**) and compounds **5**−**35** and **42**, and (D) iso-α-acids (**3a**/**b**/**c**, **4a**/**b**/**c**) and all hard resin-derived compounds **5**−**42** (Table 1). Confidence interval (α = 5%).

of the "zero" beer was rated with a low score of 1.03 ± 0.33 compared to the reference Pilsner-type beer (3.02 ± 0.29), thus demonstrating that a majority of the beer's bitterness is due to hop-derived components while a marginal portion of remaining bitterness comes from other sources such as, e.g., yeast and malt. Recombinant A containing the iso-α-acids showed an increased intensity of 2.20 ± 0.25 for a scratchy bitterness which was perceived with an instantaneous onset but a short offset. Addition of xanthohumol (**5**) and isoxanthohumol (**6**) to recombinant A induced a marginally decreased bitter intensity of recombinant B (1.93 ± 0.40) which was reported by the panelists to exhibit a more long lasting and less sharp bitter profile when compared to

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recombinant A. Recombinant C containing iso-α-acids plus compounds **5**−**32** showed a typical beer-like, well-rounded bitterness with a medicinal-herbal-like off-taste, and the bitter intensity increased and was determined to be 2.64 ± 0.25. Sensory evaluation of the total taste recombinant D revealed a bitterness of 2.95 ± 0.26, which did not differ significantly from the bitter intensity of the reference beer (3.02 ± 0.29) and was described as beer-like, long lasting, and well-rounded without any specific off-taste. Taking all these sensory data into account, iso-α-acids (**3a**−**c**, **4a**−**c**) and the identified compounds **5**−**42** were demonstrated for the first time to be truly sufficient for constructing the authentic bitter percept of beer. The knowledge on the structure and concentration of these bitter compounds paves the way for the targeted manufacturing of tailored taste profiles of beer and hop-containing foods and beverages.

Sensory and Quantitative Analysis of Hop-Derived Taste Compounds in Beer Manufactured with ε-Extract and Spent Hops, Respectively. It has been recently reported that the use of hop's hard resin instead of the soft resin fraction for beer production revealed a beer exhibiting an intense and pleasant bitter character.[26] As the above-mentioned ε-extract was found to be enriched in bitter tasting prenylated flavonoids, it was interesting to quantitate the bitter molecules and to sensorially evaluate the bitter taste of a beer manufactured by using this ε-extract as the only hop source. To achieve this, four different beers (beers I−IV) were produced and their International Bitterness Units (IBU) were determined by a trained sensory panel.[57] Beer I was produced as a bitter reference using an Isohop extract (30%) added to the final, fermented beer to adjust an IBU value of 23. Beers II−IV were produced using the same wort as for beer 1 just differing hop products. For manufacturing of beer II (27 IBU) and beer IV (27 IBU), the ε-extract was added to the wort postboiling and at the beginning of the kettle boiling, respectively. Beer III (51 IBU) was made by adding spent hops, which were obtained by exhaustive carbon dioxide extraction, to the wort prior to boiling. Descriptive sensory analysis revealed beer I (23 IBU) and beer IV (27 IBU) as well-rounded with a pleasant and typical Pilsner-type bitter profile. In comparison, beer III produced from spent hops (51 IBU) was described by the trained panelists as strongly bitter with a harsh bitter and irritating character perceived at the back of the tongue, while beer II (27 IBU) was reported to exhibit a medicinal or herbal bitter taste profile.

To gain some more insight into the concentrations of the individual hop-derived bitter compounds in beer, compounds **1**−**42** were quantitated in beers I−IV and a standard Pilsner-type beer by means of HPLC-MS/MS in a single chromatographic run (Table 3). Analysis of beer I revealed about 60 μmol/L iso-α-acids (**3**, **4**) as well as small amounts of 4′-hydroxytunicatachalcone (**10**) and isoxantholupon (**11**). Van Opstaele[58] already described that iso-α-acids extracts also contain some other hop-derived compounds which are accordingly introduced into a beer spiked with an iso-α-acids extract. All other compounds of the hard resin (**5**−**9**, **12**−**42**) were present just in trace amounts or were not detectable. In comparison, beer II contained significant amounts of the nonisomerized xanthohumol derivatives **5**, **12**, **14**, **15**, **17**, **19**−**22**, **24**, and **28**, besides α-acids (**1**) and β-acids (**2**), being well in line with manufacturing the beer by using a xanthohumol derivative enriched extract after wort boiling. Adding the ε-extract at the beginning of the wort boiling procedure (beer IV) resulted in rather low amounts of xanthohumols but significantly increased amounts of isoxanthohumol derivatives, namely, isoxanthohumol (**6**), 6-prenylnaringenin (**9**), 8-prenylnaringenin (**8**), isoxanthohumol H (**23**), and isoxanthohumol M (**30**). Interestingly, high concentrations of isoxanthohumol P (**16**) and *cis*-/*trans*-*p*-coumaric ethyl ester (**35a**/**b**) were found in beer IV, which might be explained by postfermentation reactions with ethanol produced by yeast.

The concentrations of most compounds (**5**−**35**) found in beer III, which was produced with spent hops only, did not significantly differ from those found in beer IV, thus confirming that all target compounds are already present in the spent material and are extracted and/or isomerized during kettle boiling. Interestingly, *trans*-*N*-feruloyltyramine (**42**), quercetin (**36**), and the bitter glycosides **37**−**41b** were also found in beer III in up to 10-fold higher concentrations than in beer IV. With the exception of the multifidol glycosides **40a** and **40c**, these very polar compounds were not present in the ε-extract and are extracted during wort boiling from spent hops. As beer III and beer IV showed quite similar concentrations of prenylated flavonoids (Table 3), but do differ drastically in the content of glycosides **37**−**41**, it is assumed that the overall harsh and unpleasant bitter taste sensation perceived for beer IV is due to the rather high levels of these glycosides.

With the exception of isoxanthohumol (**6**) and 8-prenylnaringenin (**8**), the concentrations of compounds **5**−**42** were much higher in beer IV than in the standard Pilsner-type beer, e.g., beer IV contained 68, 240, and 5500 times higher levels of xanthohumol, isoxanthohumol P, and isoxanthohumol H when compared to the standard Pilsner beer (Table 3). Since prenylated flavonoids have been shown to possess health beneficial effects in vitro[23,45,59,60] but a 60-fold enrichment of xanthumol derivatives is necessary to benefit from the proposed activities,[61] these results impressively demonstrate the possibility to produce beverages strongly enriched with prenylated hop flavonoids.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Chromatographic parameters for HPLC separation of MPLC subfractions. Spectroscopic data of all hard resin derived compounds. Comprehensive spectroscopic data of isolated hop compounds. Table S1: Optimized mass spectrometric parameters for the quantitative analysis of taste-active hop-derived compounds by means of LC-MS/MS using negative electrospray ionization (ESI^-). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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