Xanthogalenol and 4′-O-methylxanthohumol Content in Some American *Humulus*

Kim E. Hummer and James M. Oliphant
USDA ARS, National Clonal Germplasm Repository 33447 Peoria Road, Corvallis Oregon 97333-2521, USA

Alan W. Taylor, Linus Pauling Institute, Oregon State University, Corvallis, OR 97331 USA

Max L. Deinzer, Department of Chemistry, Oregon State University, 153 Gilbert Hall Corvallis, OR 97331, USA

Jodi M. Smith, Department of Horticulture, Oregon State University, Agricultural Life Sciences, Corvallis, OR 97331, USA

Keywords: *Humulus lupulus* var. *lupuloides*, *Humulus lupulus* var. *pubescens*, *Humulus lupulus* var. *neomexicanus*, prenylated flavonoids, xanthogalenol, xanthohumol, chemotaxonomy, hops, biogeography.

Abstract

The resins produced by either lupulin or leaf glands of more than 41 genotypes of botanical varieties of *Humulus lupulus* L. collected from the wild in 9 mid-western and western American states were analyzed for the presence of prenylated flavonoids. Xanthogalenol and other 4′-O-methylchalcones are fixed in *H. lupulus* var. *lupuloides* E. Small and in *H. l.* var. *pubescens* E. Small. Most of the *H. l.* var. *neomexicanus* Nelson and Cockerell collected east of the Rocky Mountains contained 4′-O-methylchalcones, while most specimens of *H. l.* var. *neomexicanus* from west of the Rocky Mountains did not. In 2 localities, the Kaibab Plateau, Arizona, and Phantom Canyon, Colorado, individuals of *H. l.* var. *neomexicanus* containing 4′-O-methylchalcones and those without, were sympatric. We have expanded the results of Stevens et al. (2000) who reported that European hops (*Humulus lupulus* var. *lupulus* L.), like most *H. l.* var. *neomexicanus* from west of the Rocky Mountains, lack 4′-O-methylchalcones, whereas Japanese hops (*H. l.* var. *cordifolius* (Miquel) Maximowicz) contain 4′-O-methylchalcones, like the majority of North American hop samples east of the Rocky Mountains. This geographic disjunction and polymorphism in the occurrence of 4′-O-methylchalcones suggests a complex evolutionary history in *Humulus lupulus*. Further morphological and molecular evaluations of this wild collected germplasm are in progress.

INTRODUCTION

The species *Humulus lupulus* L. in North America currently has 3 recognized varieties (Small 1978, 1997). Stevens et al. (2000) recognized a geographic dichotomy of phenotypes in Native American hops regarding their prenylflavonoid composition. They observed that, while all *Humulus lupulus* varieties contain xanthohumol, the presence of xanthogalenol (3′-prenyl-4′-O-methylchalconaringenin), 4′-O-methylxanthohumol, and 4′, 6′-di-O-methylchalconaringenin was limited to North American hops from the Missouri and Mississippi River drainages (*H. l.* var. *lupuloides* E. Small, *H. l.* var. *neomexicanus* Nelson and Cockerell east of the Rocky Mountains, and *H. l.* var. *pubescens* E. Small) and to the Japanese hops (*H. l.* var. *cordifolius* (Miquel) Maximowicz). Neither Southwestern American hops (*H. l.* var. *neomexicanus* west of the Rocky Mountains) nor European hops (*Humulus lupulus* var. *lupulus* L.) produced 4′-O-methylchalcones (Stevens et al., 2000). Small (1978) considered the Native American hop *H. l.* var. *lupuloides* to be more closely related to European hop (*H. l.* var. *lupulus*) than to the other two North American botanical varieties. Hampton et al. (2002), however, found that

unlike European hops, all 109 *H. l.* var. *lupuloides* cone samples they analyzed produced 4′-O-methylchalcones, in agreement with findings of Stevens et al. (2000). The objectives of our research were to analyze additional samples of Native American hops, including *H. l.* var. *lupuloides*, *H. l.* var. *pubescens*, and *H. l.* var. *neomexicanus*, particularly those collected in watersheds on either side of the Rocky Mountains, and from isolated mountains in New Mexico and Arizona; and to examine the pattern of geographic distribution of 4′-O-methylchalcones in American sub-specific taxa.

MATERIALS AND METHODS

Hop cones were obtained from hop plants collected from the wild in 9 mid-western and western American states. One hop cone, or about 0.1 g hops, was immersed in 100 ml of MeOH-HCOOH (99:1 by vol.). The cone was extracted for 2 hours, with occasional agitation during the first hour. For the leaf gland extracts, about 30 glands were removed from the abaxial side of young leaves with a solid needle at 7x (stereomicroscope), transferred to a 0.25 ml sample vial, and extracted with MeOH-HCOOH (99:1, 0.1 ml) at room temp for 2 h with occasional agitation during the first hour.

Hop extracts were analyzed for prenyl flavonoids using liquid chromatography-tandem mass spectrometry (LC-MS) as described by Stevens et al. (1999) and Stevens et al. (2000). In brief, an aliquot of each hop extract was separated on a 250 x 4.6 mm Synergi Hydro-RP column (Phenomenex, Torrance, CA) using a gradient of 40 – 100% acetonitrile in 0.5% formic acid (aq) over 15 minutes, followed by 7 min at 100% MeCN, at a flow of 0.8 ml/min. The effluent from the HPLC was introduced to a PE-Sciex API III+ triple quadrupole mass spectrometer via an atmospheric pressure chemical ionization interface. Prenylflavonoids were detected in the multiple reaction monitoring mode: (1) xanthohumol and xanthogalenol, *m/z* 355.2 to 179.0; (2) desmethylxanthohumol, *m/z* 341.2 to 165.0; (3) 4'-O-methylxanthohumol, *m/z* 369.2 to 193.0; and (4) 4', 6'-di-O-methylchalconaringenin, *m/z* 301.2 to 181.0.

Taxonomy and species identification followed Small (1997).

RESULTS AND DISCUSSION

The presence and absence of 4′-O-methylchalcones of 41 genotypes of North American *H. lupulus* (Tables 1 and 2) and the geographical distribution of these samples are reported. Xanthogalenol and other 4′-O-methylchalcones were fixed in *H. l.* var. *lupuloides* and in *H. l.* var. *pubescens*. *H. l.* var. *neomexicanus* was polymorphic for the presence of the 4′-O-methylchalcones. The majority of specimens east of the Rocky Mountains, including *H. l.* var. *neomexicanus,* had xanthogalenol and 4′-O-methylxanthohumol while most of the specimens west of the Rocky Mountains did not. In 2 localities, the Kaibab Plateau, Arizona, and Phantom Canyon, Colorado, individuals of *H. l.* var. *neomexicanus* containing 4′-O-methylchalcones and those without, were sympatric. The degree of polymorphism and heritability for 4′-O-methyl substitutions in populations of *H. l.* var. *neomexicanus* is unknown. Further sampling within populations and controlled crosses are planned. Most individuals of *H. l.* var. *neomexicanus* from the western United States and all native European hops (Stevens et al., 2000) lack any 4′-O-methyl substitutions. *H. l.* var. *lupuloides and H. l.* var. *pubescens,* share the presence of 4′-O-methyl chalcones with *H. l.* var. *cordifolius* from Japan (Stevens et al., 2000). This geographic disjunction and polymorphism in the occurrence of 4′-O-methylchalcones suggests a complex evolutionary history in *Humulus lupulus* and possible introgression among North American taxa. Molecular markers will be applied to further examine the relationships of this species complex.

Based on material of *H. l.* var. *neomexicanus* that we collected throughout the western United States, much variability was observed for other traits besides the chemical polymorphism mentioned above. While Small (1978, 1997) describes this variety as usually having well defined, 5-lobed leaves, our observations suggest this variety exhibits a wide range of leaf and cone morphology (data not shown). This suggests that *H. l.* var.

neomexicanus may be the most genetically diverse North American hop variety. If introgression and past hybridization has occurred then morphological characters may also show variation intermediate between taxa. A critical assessment of the variation within populations is necessary to elucidate useful and stable taxonomic characters. We feel that further molecular and morphological examination is warranted for this variety utilizing the recently expanded USDA germplasm collection.

ACKNOWLEGEMENTS

We thank USDA ARS CRIS 5358-21000-033-00D for financial support for this project and the Department of Chemistry, Oregon State University for laboratory analyses.

Literature Cited

Hampton, R., Nickerson, G.B., Whitney, P. and Haunold, A. 2002. Comparative chemical attributes of native North American hop, *Humulus lupulus* var. *lupuloides* E. Small. Phytochemistry 61:855-862.

Small, E. 1978. A numerical and nomenclatural analysis of morpho-geographic taxa of Humulus. Syst. Bot. 3:37-76.

Small, E. 1997. Cannabaceae. p.381-387. In: Flora of North America Editorial Committee, eds. Flora of North America North of Mexico. New York and Oxford.

Stevens, J., Taylor, A.W. and Deinzer, M.L. 1999. Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 832:97-107.

Stevens, J.F., Taylor, A.W., Nickerson, G.B., Ivancic, M., Henning, J., Haunold, A. and Deinzer, M.L. 2000. Prenylflavonoid variation in *Humulus lupulus*: distribution and taxonomic significance of xanthogalenol and 4′-O-methylzanthohumol. Phytochemistry 53:759-775.

Tables

Table 1. North American *Humulus lupulus* samples lacking 4′-O-methyl chalcones.

Local no.	Taxon	Origin
1428	*H. l.* var. *neomexicanus*	Alpine, Arizona
60	*H. l.* var. *neomexicanus*	Big Springs, Kaibab National Forest, Arizona
1437	*H. l.* var. *neomexicanus*	Frye Canyon, Arizona
1429	*H. l.* var. *neomexicanus*	Gilita Creek, Arizona
1426	*H. l.* var. *neomexicanus*	Macks Crossing, Arizona
1440	*H. l.* var. *neomexicanus*	Pitchfork Canyon, Arizona
1424	*H. l.* var. *neomexicanus*	Oak Creek Canyon, Arizona
1363	*H. l.* var. *neomexicanus*	Aspen, Colorado
1347	*H. l.* var. *neomexicanus*	Near Axial, Colorado
1352	*H. l.* var. *neomexicanus*	Buford, Colorado
1377	*H. l.* var. *neomexicanus*	Chimney Rock, Colorado
1382	*H. l.* var. *neomexicanus*	Las Huertes Creek, Colorado
1378	*H. l.* var. *neomexicanus*	Leopard Creek, Colorado
1395	*H. l.* var. *neomexicanus*	Phantom Canyon, Colorado
1371	*H. l.* var. *neomexicanus*	Poncha Creek, Colorado
46	*H. l.* var. *neomexicanus*	Five miles NE of San Luis, Colorado
92	*H. l.* var. *neomexicanus*	Blue Water, New Mexico
70	*H. l.* var. *neomexicanus*	Three miles East of Eagles Nest, New Mexico
84	*H. l.* var. *neomexicanus*	Near Taos, New Mexico
1433	*H. l.* var. *neomexicanus*	Windy Point, New Mexico

Table 2. North American *Humulus lupulus* samples containing 4′-O-methyl chalcones.

Local no.	Taxon	Origin
758	*H. l.* var *lupuloides*	Guttenberg, Iowa
21605	*H. l.* var *lupuloides*	White Earth, Minnesota
793	*H. l.* var. *lupuloides*	Blackleaf, Montana
1003	*H. l.* var *lupuloides*	Burlington, North Dakota
1004	*H. l.* var *lupuloides*	Minot, North Dakota
792	*H. l.* var *lupuloides*	Souris, North Dakota
1441	*H. l.* var. *neomexicanus*	Lookout Canyon, Arizona
1348	*H. l.* var. *neomexicanus*	Axial, Colorado
23	*H. l.* var. *neomexicanus*	Buckhorn Canyon near Ft. Collins, Colorado
1368	*H. l.* var. *neomexicanus*	Cochetopa Creek, Colorado
8	*H. l.* var. *neomexicanus*	35 miles south of Denver, Colorado
9	*H. l.* var. *neomexicanus*	35 miles south of Denver, Colorado
1427	*H. l.* var. *neomexicanus*	McNary, Arizona
1393	*H. l.* var. *neomexicanus*	Phantom, Colorado
1338	*H. l.* var. *neomexicanus*	Redstone, Colorado
1339	*H. l.* var. *neomexicanus*	Redstone, Colorado
1373	*H. l.* var. *neomexicanus*	Sangre de Cristo, Colorado
1391	*H. l.* var. *neomexicanus*	Wootten, Colorado
91	*H. l.* var. *neomexicanus*	Sherman Mountains, Wyoming
780	*H. l.* var. *pubescens*	Big Lake, Missouri
21550M	*H. l.* var. *pubescens*	Rulo, Nebraska