On the Essential Oil Components from *Humulus lupulus* L. var. *neomexicanus* Nels. & Cockerell. I. Contribution

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Essential Oil, *Humulus lupulus* var. *neomexicanus* Nels. & Cockerell, Terpenes, GC and GC-MS Data

The essential oil of strobiles from *Humulus lupulus* L. var. *neomexicanus* Nels. & Cockerell, grown in the semi-arid climate of Central Colorado, was isolated by steam distillation. A characteristic seasonal dependence of oil production was observed over two vegetation periods. – The main components of the essential oil were identified structurally by means of GC and GC-MS analysis. About 40 different compounds, including monoterpenoic hydrocarbons and alcohols, ketones, methylesters of saturated and unsaturated carboxylic acids were detected. Other substances were esters of short chain acids with monoterpene alcohols, plus sesquiterpene hydrocarbons.

Introduction

Humulus lupulus L. var. *neomexicanus* Nels. & Cockerell, known commonly as "Wild Hop-Vine" or "Wild Hops", is the only native genus and species of the Cannabaceae (Moraceae) family within the central Rocky Mountains, where it grows at an altitude from 1 300 to 3 000 m.

It is found in canyons and foothills and is sometimes seen climbing over bushes [1, 2]. The annual aerial climbing stems arize from the perennial rhizome and become 5 to 10 m long. The opposite leaves are lobed palmately. Two of the female flowers sit together under a large persistent bract. At maturity the bracts from the conelike "hops" change colors and appear as papery and conspicuous clusters [1, 2] known as strobiles.

The essential oil is produced by the female inflorescences, the strobiles ("strobuli lupuli") [3] which contain the oil bearing glands ("glandulae lupuli") [4].

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For hundreds of years strobiles from *H. lupulus* have been used to flavor beer and to calm nervous disorders. The essential oils are known to possess a narcotic as well as diuretic, antiseptic and bittertonic actions.

The strobiles from *H. lupulus* L. var. *neomexicanus* reveal a different scent if compared to *H. lupulus* L. So far nothing is known on the composition of its essential oil. This first contribution was undertaken to determine the different volatile substances and to analyze quantitatively the occurance of its several components, and to possibly observe variations over the seasonal life of the hops.

Materials and Methods

Biological material

Humulus lupulus L. var. *neomexicanus* Nels. & Cockerell was collected in western El Paso County of central Colorado from January 1980 through October 1981. The collection site was located at 2423 m elevation on a north facing slope. The soil consisted mainly of decomposed granite with sand as the major texture component. On each collection date, 11 to

Fig. 1. *Humulus lupulus* var. *neomexicanus*, courtesy of Colorado Associated University Press; about 1/3 natural size.

40 g fresh weight of strobiles were picked and were air to freeze dried. Freeze dried strobiles were first frozen in liquid nitrogen and then lyophilized for 6 to 8 h. Dried strobiles were sealed in air tight plastic bags until extraction for the essential oils.

Climatic data

The semi-arid climate of central Colorado is characterized as follows. Sunshine: Mean sunshine/day averaged over the month of August = 14.0 h, and of September = 12.1 h. Temperature see Fig. 2. Precipitation: 4 cm/year with 50–80% coming during the summer (Fig. 2).

Quantitative extraction of the essential oils

The essential oils were extracted quantitatively using the method given by the German Pharmacopeia No. 7 (DAB 7) [5]. The essential oils were extracted from 10 to 15 g of dried strobiles by steam distillation for 2 h. The essential oils were collected by dissolving in 0.20 ml xylene within a graduated capillary tube. The total volume of essential oil was thus collected and its volume determined quantitatively.

Gas chromatographic analysis

I) Packard 427, 2 m glass column, 3 mm ID, 5% OV 17 on chromosorb, 20 ml N_2/min; II) Perkin Elmer Sigma 1, 17 m glass WCOT capillary SE 30, 0.25 mm ID, 1.0 bar N_2; III) Carlo Erba 2150, 50 m WCOT glass capillary WG 11, 1.0 bar N_2.

All gaschromatographic separations were run by use of a temperature program, the capillary chromatograms by split injection.

Gas chromatography – mass spectrometry

GC-MS combination Finnigan 3200 E and Finnigan data system 6000, 27 m WCOT glass capillary column SE 30, 16 psiq He, all glass interface, 70 eV spectra, 3–4 seconds per scan.

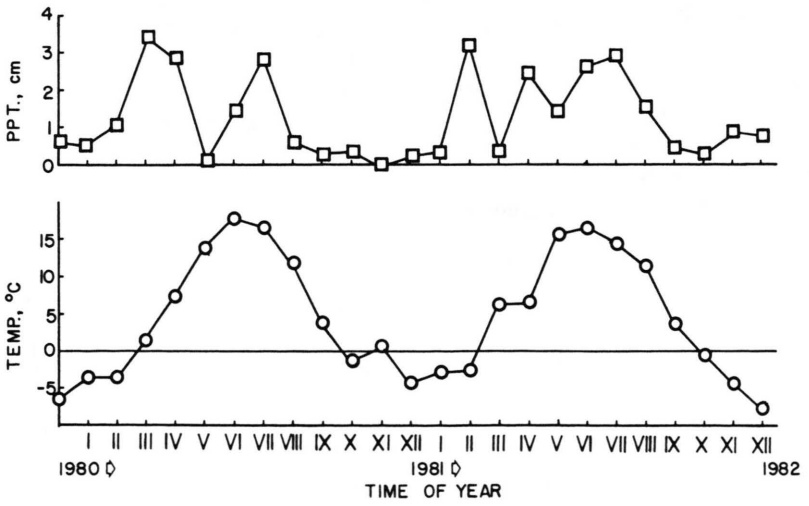

Fig. 2. Average monthly temperature and precipitation from Jan. 1980 to Jan. 1982 at the growing area of *H. lupulus* var. *neomexicanus*, Green Mtn. Falls, El Paso-County, Central Colorado.

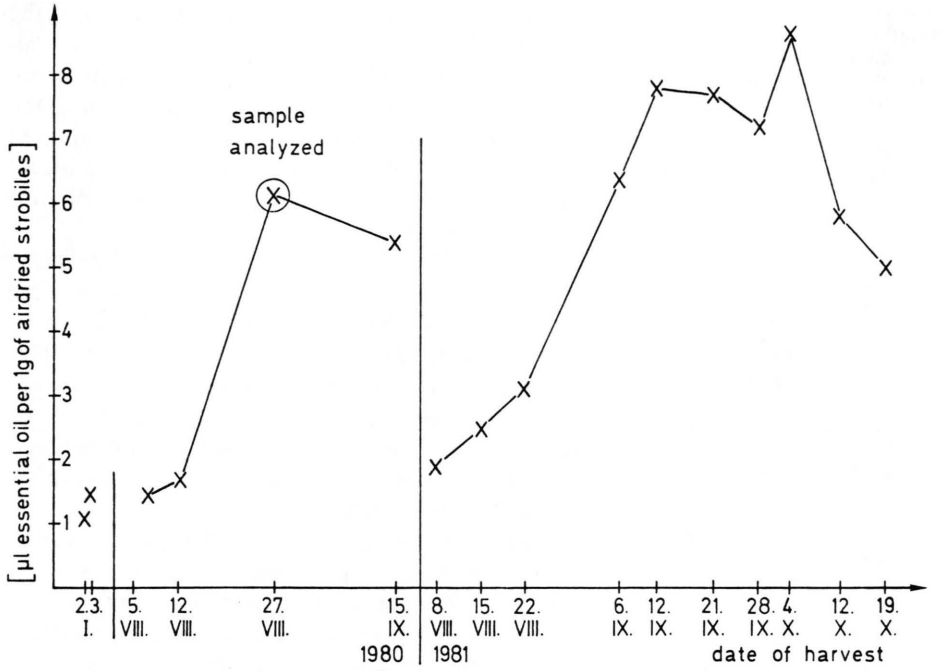

Fig. 3. Seasonal dependence of essential oil content from strobiles of *H. lupulus* var. *neomexicanus* grown in Central Colorado at 2423 m elevation.

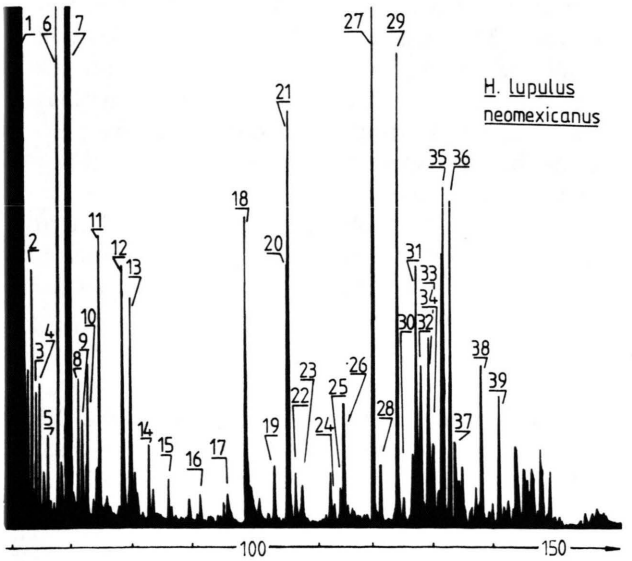

Fig. 4. Gas chromatogram of the essential oil of *H. lupulus* var. *neomexicanus*, harvested August 27, 1980. WCOT glass capillary column SE 30, temperatue programmed 50–220 °C, rate 1.5 °C/min (the signal numbers refer to Table I).

Results and Discussion

Quantitative determinations on the essential oil of the hops revealed a seasonal dependence in oil biosynthesis. It was observed that samples taken in October, as seen for the last time from the sample collected at October 19, 1981, were already airdried due to the arid climate of Central Colorado. The hops content in essential oil was about 1 to 2 µl per 1 g of airdried samples collected during the winter season, and obviously stayed at this level until end of July (Fig. 3). Biosynthesis of the oil in female buds during the next vegetation period started again in early August and reached a maximum of 6 to 8 µl per 1 g of dried material in late summer (Fig. 3) depending very likely on the year's climatic data.

All samples collected (see Fig. 3) were chromatographed comparatively on a WG 11 capillary column (comparable to FFAP phase) and on a packed column OV 17. Thereby the main components of the oil were determined quantitatively (fid values). The oil from the sample taken on August 27, 1980 (see Fig. 3) was chosen for the complete analysis of its different components by use of GC-MS spectra (Fig. 4).

Table I. Composition of the essential oil of *H. lupulus* var. *neomexicanus*. The compound identified are presented quantitatively in percentage values. Methods applied and references for structure elucidation are included. Xylene has been used as the solvent. The numbers refer to signals given in Fig. 4.

No.	Compound	%	ident. by	retention index RI
1	xylene			
2	C_9H_{12}	0.53	GCMS	
3	$C_7H_{16}O_2$	0.33	GCMS	
4	α-pinene	0.26	GCMS[a, b]	942[c]
5	C_9H_{12}	0.21	GCMS	
6	sabinene	1.39	GCMS[a, b]	976[c]
7	myrcene	62.18	GCMS[a, b]	
8	isobutyrate	0.38	GCMS	
9	methyl heptanoate	0.37	GCMS[b]	1006[c]
10	limonene	0.54	GCMS[a, b]	1030[c]
11	(*E*)-ocimene	0.95	GCMS[a, b]	1038[c]
12	branched methyl octanoate	0.95	GCMS	
13	linalool	1.09	GCMS[a, b]	1092[c]
14	methyl octanoate	0.24	GCMS[b]	1107[c]
15	branched methyl nonanoate	0.14	GCMS	
16	methyl nonanoate	0.12	GCMS[b]	1207[c]
17	ketone $C_{11}H_{23}O$	0.05	GCMS	
18	geraniol	1.52	GCMS[a, b]	1243[c]
19	undecan-2-one	0.24	GCMS[b]	1276[c]
20	methyl decenoate	1.10	GCMS[b]	
21	methyl decadienoate	1.65	GCMS	
22	methyl decadienoate	0.22	GCMS	
23	methyl decanoate	0.31	GCMS[b]	1307[c]
24	acetate of nerol or geraniol	0.24	GCMS[a, b]	
25	α-ylangene	0.13	GCMS[a, b]	
26	α-copaene	0.52	GCMS[a, b]	1398[c]
27	β-caryophyllene	2.90	GCMS[a, b]	1428[c]
28	probably β-cubebene	0.26	GCMS[b]	
29	humulene	2.12	GCMS[a, b]	1465[c]
30	propionate of geraniol or nerol	0.36	GCMS	
31	probaly γ-muurolene	1.21	GCMS[b]	1475[c]
32	$C_{15}H_{24}$	0.87	GCMS	
33	$C_{15}H_{24}$	0.98	GCMS	
34	isobutyrate of geraniol or nerol	0.51	GCMS	
35	γ-cadinene and calamenene	2.36	GCMS[a, b]	1518[c]
36	δ-cadinene	1.43	GCMS[a, b]	1524[c]
37	$C_{15}H_{24}$	0.45	GCMS	
38	unknown	0.92		
39	unknown	0.70		

[a] Spectrum of authentic sample;
[b] spectrum in ref. [6];
[c] retention indices on OV 101, ref. [8].

For identification of the main components of the oil, the mass spectra were compared to spectra already reported in the literature [6] and to those obtained with authentic samples [7]. In addition, the elution sequence of each compound was examined by comparison with data obtainable from the literature concerning the retention sequence from columns of similar polarity [8].

Myrcene (**7**, 62.18%) was identified as the main substance, other monoterpene hydrocarbons were α-pinene (**4**), sabinene (**6**), limonene (**10**) and (*E*)-ocimene (**11**), (total amount ~ 3%). Linalool (**13**, 1.09%) and geraniol (**18**, 1.52%) were the only monoterpene alcohols we could detect. *I.e.*, the oil of the variety *neomexicanus* seems to be similar compared to other species of hops [9] as far as its monoterpene composition is concerned.

We also identified the so-called typical ingredients of the oil from the hops, as there are methylesters of heptanoic (**9**), octanoic (**14**), nonanoic (**16**) and decanoic acid (**23**), both the methylbranched alkanoic methylesters (**12**) and (**15**), the methylesters of the unsaturated carboxylic acids (**20**), (**21**) and (**22**), as well as the acetate (**24**), propanoate (**30**) and isobutyrate (**34**) of geraniol or nerol.

The sesquiterpene fraction of the oil investigated turned out to be relative complex. We are able to identify α-ylangene (**25**), α-copaene (**26**), β-caryophyllene (**27**), humulene (**29**), γ-cadinene (**35**) and δ-cadinene (**36**) by means of comparison with authentic samples. β-cubebene (**28**), γ-muurolene (**31**) and calamene (**35**) could be assigned only by comparing the mass spectra with published data [6] or known retention indices [8].

In a paper to be published later we would like to report in detail on the complete spectrum of the oil composition of *H. lupulus* L. var. *neomexicanus* Nels. & Cockerell.

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[1] H. D. Harrington, Manual of the Plants of Colorado, The Swallow Press Inc., Chicago 1964.
[2] W. A. Weber, Rocky Mountain Flora, Colorado Associated University Press, Boulder, Colorado 1976.
[3] H. Wagner, Pharmazeutische Biologie, **Vol. 2** (Drogen und ihre Inhaltstoffe); Gustav Fischer Verlag, Stuttgart, New York 1980.
[4] E. Teuscher, Pharmazeutische Biologie (H. Borris, ed.), Friedr. Vieweg & Sohn, Braunschweig, Wiesbaden 1979.
[5] H. Böhme and K. Hartke, Deutsches Arzneibuch, 7th Ed., 1968, Commentary; Wissenschaftliche Verlagsanstalt mbH, Stuttgart, Govi-Verlag GmbH, Frankfurt/Main 1971.
[6] A. F. Thomas and B. Willhalm, Helv. Chim. Acta **47,** 475 (1964); E. Stenhagen, S. Abrahamsson, and W. F. McLafferty, Registry of Mass Spectral Data, J. Wiley & Sons, New York 1974; A. Cornu and R. Massot, Compilation of mass spectral data, Heyden Sons Ltd, 1966.
[7] O. Vostrowsky, K. Michaelis, H. Ihm, R. Zintl, and K. Knobloch, Z. Lebensm. Unters. Forsch. **173,** 365 (1981).
[8] W. Jennings and T. Shibamoto, Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography, Academic Press, New York 1980.
[9] R. Stevens, Chem. Rev. **67,** 19 (1967); R. G. Buttery, W. H. McFadden, D. R. Teranishi, and M. P. Kealy, Nature **200,** 435 (1963); Y. Masada, Analysis of essential oils by gaschromatography and mass spectrometry, p. 226, John Wiley & Sons, New York 1967; G. A. Howard and A. R. Tatchell, J. Inst. Brewing **62,** 158 (1956); F. Sharpe and D. R. J. Laws, J. Inst. Brewing **87,** 96 (1981); W. Goedkoop, Intern. Tijdschr. Brouw. en Mout. **20,** 39 (1960).