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The Constituents of Hops (*Humulus lupulus* L.). VII.[1]
The Rapid Analysis of Volatile Components

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The volatile constituents from several varieties of Hops have been investigated by the direct loading of fresh plant tissue through GC-MS. The Saaz variety was distinguished among the examined varieties by its high content of β-farnesene. Hallertau was characterized by a low content of myrcene and a high content of humulenes. Shinshu-wase differed in its high content of myrcene and its low content of humulenes. Northern Brewer was rather close to Hallertau, whereas Yakima appeared to be closely related to Shinshu-wase. The myrcene content of hop oil was observed to correlate competitively with the total amount of humulenes, caryophyllene, and farnesene. 2-Methylbutyl isobutyrate accumulates in company with myrcene. The biosynthetic implication of these relationships have been briefly discussed.

Hop oil is conventionally obtained by the steam distillation of hops involving solvent extraction. The analytical results of the volatiles have, therefore, usually been affected by various treatments after the picking of the cones. Recently, Hautke *et al.*[2] reported a headspace analysis useful for the discrimination of hop varieties, but the identification of the volatile compounds has generally been limited to the major constituents. The technique[3] to release the volatile constituents from fresh plant tissue seemed to demand a simple and rapid analysis of the hop aroma by combined gas chromatography-mass spectrometry (GC–MS), as has been previously described.[1] The present work will report the analytical results by GC–MS from several varieties of hops, using a solid-sampler for direct volatilization from fresh plant tissue "lupulin". The correlation between the characteristic volatile constituents and the varieties of hops has also been attempted in order to connect this experiment with phylogeny.

Experimental

Commercial hop samples harvested in 1971 were used for the experiment (Table 1). Lupulin, glands of hop oil, were sifted from hop cones after manual grinding under freezing with liquid nitrogen or dry ice. For the quantitative glc analysis, a Hitachi Model K-53 apparatus equipped with an inlet-arrangement "solid-sampler" (an optional part of the JEOL SS-801) and a flame ionization detector was used in connection with a digital integrator (Takedariken TR-2213). The capillary column used for the analysis was 45 m×0.25 mm; it was made of stainless steel and was coated with HB 2000. A precolumn trap system was provided for the condensation of the volatiles. The trapped volatiles were then evaporated at once; the temperature was programmed so

TABLE 1. SAMPLES FROM SEVERAL VARIETIES OF HOPS HARVESTED IN 1971

Variety	Origin	Oil yield (%)
Saaz	Czechoslovakia	2.2
Hallertau	Germany	1.8
Northern Brewer	Germany	2.2
Yakima	U.S.A.	2.2
Shinshu-wase	Japan	1.7

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[1] Previous paper of this series: Y. Naya and M. Kotake, This Bulletin, **44**, 3116 (1971).

[2] P. Hautke and D. Petříček, *Wallerstein Commun.*, **33**, 89(1970); *Monatsschrift für Brauerei*, **23**, 241 (1970).

[3] E. von Rudloff, *J. Gas. Chromatog.*, **3**, 390 (1965).

TABLE 2. PERCENTAGE ABUNDANCE OF THE CONSTITUENTS DRIVED FROM THE FIVE VARIETIES OF HOPS BY DIRECT LOADING GC–MS AND SUBSEQUENT PEAK AREA MEASURMENT

Peak No	Constituent	Saaz	Hallertau	Northern brewer	Yakima	Shinshu-wase
1	Acetone		○		○	○
2	Methyl isopropyl ketone		○			○
3	Methyl isobutyl ketone	0.33	0.80	0.05	○	0.08
4	Dimethylvinylcarbinol	0.40	0.97	0.64	0.71	0.27
5	α-Pinene	0.03	0.04	0.13	0.38	0.06
6	67 (B) 124$^{a)}$		0.08		0.19	
7	Isobutyl isobutyrate		0.06	0.29	0.13	0.39
9	β-Pinene	0.36	0.46	0.71	1.09	0.86
10	2-Methyl-1-butanol					0.67
10	Methyl hexanoate			0.15		
11	Myrcene	29.44	24.09	42.67	52.03	73.41
12	2-Methylbutyl isobutyrate		0.26	1.85	2.13	3.15
13	Limonene	0.05	0.16	0.29	0.49	0.38
14	β-Phellandrene$^{a)}$	0.05	0.12	0.30	0.45	0.41
15	Methyl 5-methylhexanoate				0.61	
15	3-Methyl-2-buten-1-ol	0.06	○	0.29		0.09
16	105 (B) 120$^{a)}$	○	○	0.06	0.13	
17	2,2,7,7-Tetramethyl-1,6-dioxaspiro-[4.4]-nona-3,8-diene	0.11	0.08	0.92		
17	β-Ocimene$^{a)}$				0.61	0.17
18	Methyl heptanoate	0.13	0.14	0.30	0.18	0.07
19	Furfural				○	
20	Methyl 4-methyl-2-hexenoate	0.19	0.26	0.39	0.44	
20	2-Methylbutyl 2-methylbutyrate					0.22
21	2-Methylbutyl isovalerate			0.12	0.15	0.05
22	Methyl 6-methylheptanoate	0.16	0.05	0.18	0.36	0.18
23	2-Nonanone	0.18	0.18	0.08	○	○
24	Methyl octanoate	0.11	0.18	0.14	0.35	0.02
24	Perillene					
26	4,4-Dimethylcrotonolactone	0.09	0.37	0.05	0.05	0.07
27	Methyl 7-methyloctanoate				0.12	
28	Hexyl isobutyrate			0.02		
29	2-Decanone	0.15	○		○	○
30	Linalool	0.40	0.51	0.30	0.23	0.55
31	Methyl nonanoate	0.17		0.09	0.10	
32	α-Cubebene$^{a)}$	○	0.05	0.03		0.03
33	9-Methyldecanone-2	0.29	0.04	0.07	○	0.06
34	α-Ylangene	0.27	0.20	0.17	0.24	0.09
35	Methyl 8-methylnonanoate	0.39		0.43	0.32	0.20
35	α-Copaene		0.59			
36	2-Undecanone	0.67	0.60	0.52	0.20	0.07
37	Methyl decanoate				0.23	
38	Methyl 4-decenoate	0.81	0.25	0.33	0.86	0.09
39	Myrtenol$^{a)}$	0.24				
39-1	Isocaryophyllene$^{a)}$	0.22				
40	Caryophyllene	8.73	13.08	10.44	9.68	3.68
41	85 (B) 182$^{a)}$		○			0.20
42	Humulene	28.84	41.72	29.38	20.47	5.29
43	β-Farnesene	16.19				
43	Nerol					0.10
44	γ-Muurolene	1.10	1.89	1.04	0.61	0.49
46	Germacrene-D$^{a)}$	0.53	1.01	0.74	0.79	
46	β-Selinene					1.73
47	α-Muurolene$^{a)}$	0.68	0.93			
47	α-Selinene	○		0.62	0.87	1.98
48	Geraniol					○
48	β-Bisabolene$^{a)}$	0.29				
49	δ-Cadinene	2.03	3.68	2.35	1.53	1.29

Peak No	Constituent	Saaz	Hallertau	Northern brewer	Yakima	Shinshu-wase
51	δ_2-Cadinene[a]	0.16	0.22	0.16	0.08	0.06
52	α-Cadinene	0.32		0.16		0.46
53	2-Tridecanone	0.24	0.36	0.23	0.34	
53	Geranyl isobutyrate					○
54	Calamenene	0.05	0.12	0.07	○	0.02
55	79 (B) 210[a]	0.24	○	0.02	○	0.02
56	γ-Calacorene		○			
57	α-Calacorene	0.03	0.11	0.06	0.06	0.02
60	Caryophyllene oxide	0.16	0.29	0.07	0.04	○
61	Humulene epoxide-I	0.11	0.42	0.05		
62	Humulene epoxide-II	0.62	1.72	0.29	0.11	0.05
64	epi-Cubenol	○	0.07	0.03	○	○
65	43 (B) 224[a]	0.22	0.04	0.08	○	
66	79 (B) 222[a]	0.51	0.03	0.16	○	0.02
67	Humulol	0.09	0.06	0.02		
68	γ-Eudesmol	0.03				
69	T-cadinol	0.15	0.17	0.11		0.08
70	T-muurolol	0.04		0.02		
71	δ-Cadinol	○	0.04	0.05		
71	Cadalene	○				
72	α-Eudesmol	0.22		0.07		
73	α-Cadinol	0.10	0.17	0.09		0.19}
74	Junipercamphor					
75	Humulenol-II	0.18	1.46	0.11		
76	Humulene dioxide	○	0.21			
	Total	97.16	98.34	97.99	97.37	97.30

a) Tentatively identified.

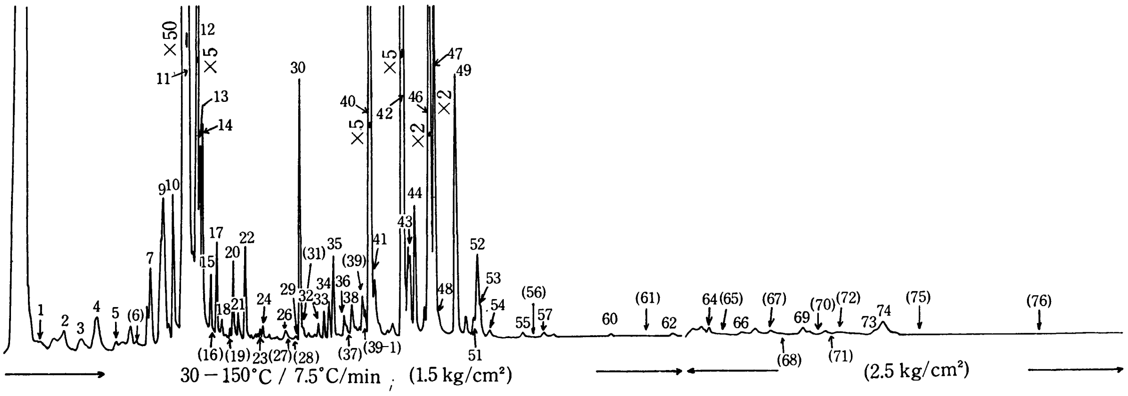

Fig. 1. Gas chromatographic record obtained from 5 mg of lupulin from Shinshu-wase, through capillary column (HB 2000, 45 m × 0.25 mm) with the vapor splitting assembly (1 : 70). The peak numbers correspond to those of Table 2. Those in parentheses were found in the different varieties.

that the volatiles could be detected as very sharp bands. 5 mg of lupulin was placed in the solid-sampler, and then the solid-sampler placed inside the injection block heated at 180°C for 1.5 min under a nitrogen-carrier gas-inlet pressure of 1.5 kg/cm^2, which swept the volatilized constituents onto the trapping column cooled by dry ice–isopropanol. After the cooling jacket had been taken off, the whole column system was heated by temperature programming from 30° to 150°C at the rate of 7.5°C/min. It was necessary to heat the injection block at around 180°C and to withdraw the plunger within 2 min in order to obtain a sufficient volatility of the volatiles without any thermal decomposition.

For the GC-MS analysis, a capillary column (45 m × 0.5 mm) coated with HB 2000 was used. The sample amount was about 10 mg for each analysis, which was done by the technique described above (hellium-carrier gas-inlet pressure, 0.7 kg/cm^2; temperature programming, 30—150°C at 5°C/min. The mass spectrometer was a Hitachi Model RMU-6 apparatus, and the operating conditions were as follows: ionization energy, 80 eV; acceleration voltage, 2000 V. The compounds were identified by the agreement of the mass spectra, as well as by that of the retention time on glc, with those of authentic samples or of published standard references.

Results and Discussion

In our study, 10 mg of lupulin were sufficient for the identification of about fifty constituents, amounting to more than 97% of the total oil (Table 2). 5 mg of lupulin were required for a satisfactory gas chromatogram, obtained through a capillary column equipped with a vapor-splitting assembly (1: 70). The gas chromatogram (Fig. 1) obtained for 5 mg of lupulin from Shinshu-wase is shown as an example. The chromatogram obtained using 1 mg of the sample without the splitting assembly was still sufficient for the purpose of comparison. A chromatogram using an essential oil from the same variety, obtained by the conventional method, was compared with the above chromatogram; it was found that low-boiling constituents were inevitably lost to a certain extent in the course of steam distillation and solvent extraction.

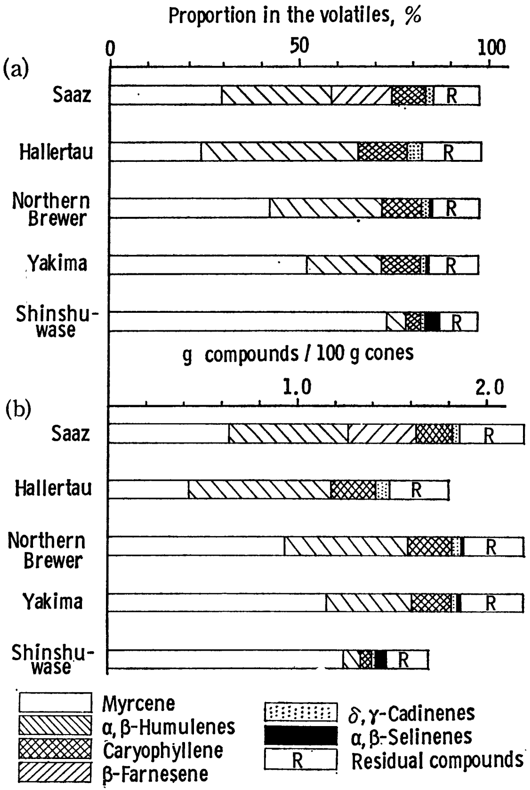

Fig. 2. Variation in the varieties and correlation among the contents of some hydrocarbons: (a) Proportion in the volatiles. (b) amounts calculated for the yield of essential oil.

The results (Fig. 2) indicate the differences among the varieties. A substantial amount of β-farnesene is contained only in the lupulin from Saaz. This fact is in agreement with the report of Šorm *et al.*[4] though not with that of Haward *et al.*[5] In this point, Saaz can be clearly distinguished from Hallertau, Northern Brewer, Yakima, and Shinshu-wase. The major hydrocarbons of Shinshu-wase, caryophyllene, and a mixture of α- and β-humulenes have been shown[1] to be characteristic constituents throughout the whole plant and to be no concern in ripening, while the amount of myrcene increases greatly in the course of ripening. Thus, Shinshu-wase is characterized by a high content of myrcene and a low content of humulenes. On the contrary, Hallertau is characterized by a low content of myrcene and a high content of humulenes. Furthermore, the composition of the hydrocarbons from Northern Brewer was similar to that of Hallertau, whereas Yakima appeared to be closely related to Shinshu-wase. The myrcene content of hop oil is observed to correlate competitively with the total amount of humulenes, caryophyllene, and farnesene. The biosyntheses of myrcene, humulenes, caryophyllene, and farnesene possibly proceed through enzymic transformations in the limited supply of the common intermediate. Accordingly, an increase in the amount of the one must cause a corresponding decrease in the amount of the rest. It is also observed that the selinene group competes with cadinenes from the common intermediate, the germacrenium ion.[6] The myrcene-rich varieties show a high content of selinenes, whereas the humulene-rich varieties are characterized by a high content of cadinenes. We can not explain readily the correlation in the contents between myrcene and selinenes as well as between humulene and cadinenes.

The oxygenated compounds can be divided into the two groups based on the difference in development. The one formed by the deterioration of the hop consists of oxidized volatiles (caryophyllene-oxide, humulene-epoxide-I and -II, humulenol-II, *etc.*) and the decomposed bitter principles[7] (dimethylvinylcarbinol, dimethylcrotonolactone *etc.*) (Fig. 3). The other group, in the course of the maturing of the cones, probably contains some monoterpenic alcohols, methyl ketones, and methyl esters and higher esters of various fatty acids. 2-Methylbutyl isobutyrate, which was the most rich component among the esters, can be correlated with myrcene. The myrcene-rich varieties show high contents of this esters (Fig. 4). Therefore, 2-methylbutyl isobutyrate must correspond to the Peak II-substance,[5] which accumulates in company with myrcene according to Haward *et al.* In fact, the amount of

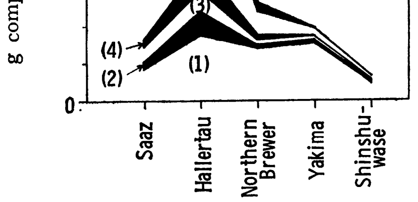

Fig. 3. Proportion of some oxygenated compounds caused from deterioration of hop. (1): dimethylvinylcarbinol, (2): 4,4-dimethylcrotonolactone, (3): caryophyllene-oxide, (4): humulene-epoxide-I, (5): humulene-epoxide-II, (6): humulenol-II.

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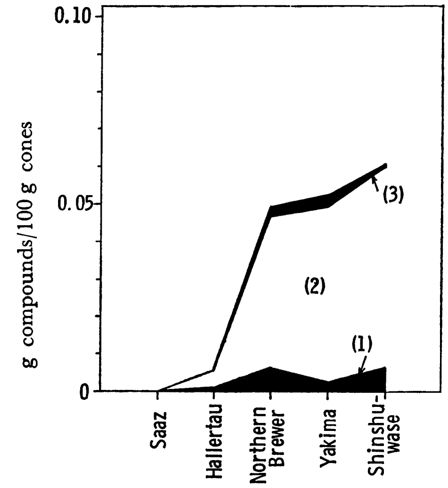
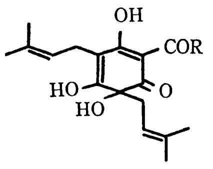


Fig. 4. Proprotion of some higher esters in the volatiles. (1): isobutyl isobutyrate, (2): 2-methylbutyl isobutyrate, (3): 2-methylbutyl isovalerate.

2-methylbutyl isobutyrate increases in the course of ripening in proportion to the formation of myrcene.[1] It has been shown that[8] a representative group of bitter principles, alpha-acid (I), and essential oil do not all reach a maximum at the same time. That is, the volatiles are accumulated later than alpha-acid. The various pieces of evidence above suggest that the formation of 2-methylbutyl isobutyrate is possibly connected with the biosynthesis of the acyl side-chains[9] of bitter principles, while myrcene is connected with isoprenyl units of them. Further study is now under way.



(I)	Humulone	R: *i*-Bu
	Cohumulone	*i*-Pr
alpha-Acid	Adhumulone	*sec*-Bu
	Posthumulone	*i*-Val
	Prehumulone	Et

In spite of the short history of hop cultivation in Japan (since 1915),[10] the phylogeny of Japanese hops is uncertain. In order to elucidate the phylogeny of these hops, investigation[11] has been done on the basis of cytology. It is said that the sex chromosome types of wild and garden male hops are the same and are characteristic of their geographical groups namely, those of European origin are of the XY^w type, those of American origin are of the XY^h and XY^{nw} types, and those of Japanese origin are of the XA^vAY^a type. Only a few simple XY^h and XY^{nw} types were found in Japanese garden hops. This shows that intercrossings between the cultivated and wild hops have actually occurred. Thus, when the hops were introduced to a new region, acclimated new varieties have grown in the new habitat. From the viewpoint of phylogeny, our study lends support to the assumption that the local varieties in Europe named Saaz and Hallertau went over to America and then came to Japan, where they developed into a new local variety.

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