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The Constituents of Hops. IV.[1] The Volatile Composition of *Humulus Lupulus* L. *var. cordifolius* Maxim.

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The steam-volatile constituents of *Humulus Lupulus* L. *var. cordifolius* Maxim. have been studied, and 66 compounds of the oil have been identified. Besides the previously-reported principal hop components (myrcene, β-humulene, caryophyllene, β-selinene, δ- and γ-cadinenes), evidence was found for the presence in an abnormally high concentration of others: zingiberene, α-curcumene together with β-bisabolene, and β-sesquiphellandrene, selina-3,7(11)-diene, and selina-4(14),7(11)-diene. A number of oxygenated sesquiterpenoids were detected. The major one was found to be caryophyllene-oxide. The isolated components were identified by spectroscopic or chemical means.

The steam volatile constituents of the horticultural Japanese hop, "Shinshu-wase" (*Humulus Lupulus* L.), were examined; the characterization of most compounds in the oil has already been reported.[1–5] In the present paper, the isolation and identification of volatile compounds contained in the cone oil of a wild variety, "Karahanaso" (*Humulus Lupulus* L. *var. cordifolius* Maxim.), will be reported for the first time.

Experimental

Separation. The air-dried material (9.2 kg) was distilled with steam, and then the distillate was treated by usual methods to remove any acidic compounds.

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[1] Previous paper of this series: Y. Naya and M. Kotake, *Nippon Kagaku Zasshi*, **91**, 275 (1970).

[2] Y. Naya and M. Kotake, *ibid.*, **89**, 1113 (1968).

[3] Y. Naya and M. Kotake, *ibid.*, **88**, 1302 (1967).

[4] N. Shigematsu and Y. Kitazawa, *Bull. of Brew. Sci.*, **8**, 23 (1962).

[5] R. G. Buttery, R. E. Lundin and L. Ling, *Agri. Food Chem.*, **15**, 58 (1967).

The residual oil (17.8 g)(0.19%) was readily separated into the hydrocarbon and the oxygenated constituents by selective adsorption on neutral alumina. The amount of the hydrocarbon fraction was four times that of the oxygenated fraction. The hydrocarbon fraction was divided into three parts by fractional distillation under a pressure of 3 mmHg. Each part was separated into its constituents by a combination of adsorption chromatography (silica-gel or silica-gel impregnated with silver nitrate) and preparative gas-liquid chromatography (SF 96 and a Carbowax 20 M columns). Similar methods were applied to oxygenated compounds, using appropriate adsorbents and columns.

Apparatus. The analytical GLC was carried out using a Hitachi Model K-53 gas chromatograph provided with a flame-ionization detector. The stainless-steel capillary column (0.25 mm in diameter and 45 m in length) used for the analysis was coated with HB 2000; the column temperature was held isothermally at 100°C, and then it was flushed to 150°C. The nitrogen-carrier gas-inlet pressure was set at 1.5 kg/cm^2 for the initiation and 2.5 kg/cm^2 at the end.

For preparative gas chromatography, a Model 90-P Varian Aerograph equipped with a thermal conductivity detector was employed. Usually a 10-ft. × 3/8 in. aluminum column packed with 20% Carbowax 20 M on Chromosorb W (60—80 mesh) was used. Helium was the carrier gas, and the column temperature was 100—220°C.

The NMR spectra were measured in CCl_4, using TMS as an internal reference at 60 MHz on a JEOL Model C-60 spectrometer.

The mass spectra were measured with a Hitachi Model RMU-6 Mass Spectrometer; the operating conditions were as follows: ionization energy, 80 eV; ion-accelerating voltage of 2000 V, at temperatures of 250°C and 150°C in the ionization chamber and the sample injection block respectively.

Table 1. The constituents of hydrocarbons

Peak No. in Fig. 1	Identified compound
1	α-Pinene
2	β-Pinene
3	Myrcene
4	Undecane
6	Limonene
7	β-Phellandrene
8	γ-Terpinene
9	Dodecane
15	Tridecane
19	Tetradecane
23	α-Copaene
27	Pentadecane
27	*trans*-α-Bergamotene
28	Caryophyllene
32	β-Humulene
32	β-Farnesene
34	γ-Muurolene
35-1	Hexadecane
37	α-Selinene
37	β-Selinene
38	Zingiberene
39	β-Bisabolene
40	α-Curcumene
40-1	δ-Cadinene
41	γ-Cadinene
43	β-Sesquiphellandrene
44	Selina-4(14),7(11)-diene
45	Selina-3,7(11)-diene
47	Calamenene
49	Heptadecane
53	Octadecane
58-1	Nonadecane

Results and Discussion

The constituents are listed in Tables 1—3, together with the peak number on the programmed

Table 2. The constituents of oxygenated compounds

Peak No.	Identified compound
1-1	2-Methyl-1-butanol
5	3-Methyl-2-buten-1-ol
7-1	2,2,7,7-Tetramethyl-1,6-dioxaspiro[4.4]-non-3-ene
8	2,2,7,7-Tetramethyl-1,6-dioxaspiro[4.4]-nona-3,8-diene
10	Methyl 4-methyl-2-hexenoate
12	Methyl octanoate
14	*trans*-2-Methyl-2-vinyl-5-hydroxyisopropyl-tetrahydrofuran
16	*cis*-2-Methyl-2-vinyl-5-hydroxyisopropyl-tetrahydrofuran
17	4,4-Dimethylcrotonolactone
18	Linalool
20	β-Fenchol
21	Terpinen-4-ol
23	Methyl 8-methylnonanoate
23-1	Pinocarveol
23-2	2-Undecanone
24	4-Undecen-2-one
24	Methyl decanoate
25	Methyl 4-decenoate
25	Nonanol-1
26	α-Terpineol
26	Borneol
30	Methyl geranate
31	Benzyl alcohol
33-1	Nerol
36	Phenyl ethyl alcohol
38-1	Geraniol
46	2-Tridecanone
52	Caryophyllene-oxide
54	Humulene-epoxide-I
55	Humulene-epoxide-II
56	β-Caryophyllene alcohol
57	Nerolidol
61	T-cadinol
64	α-Bisabolol

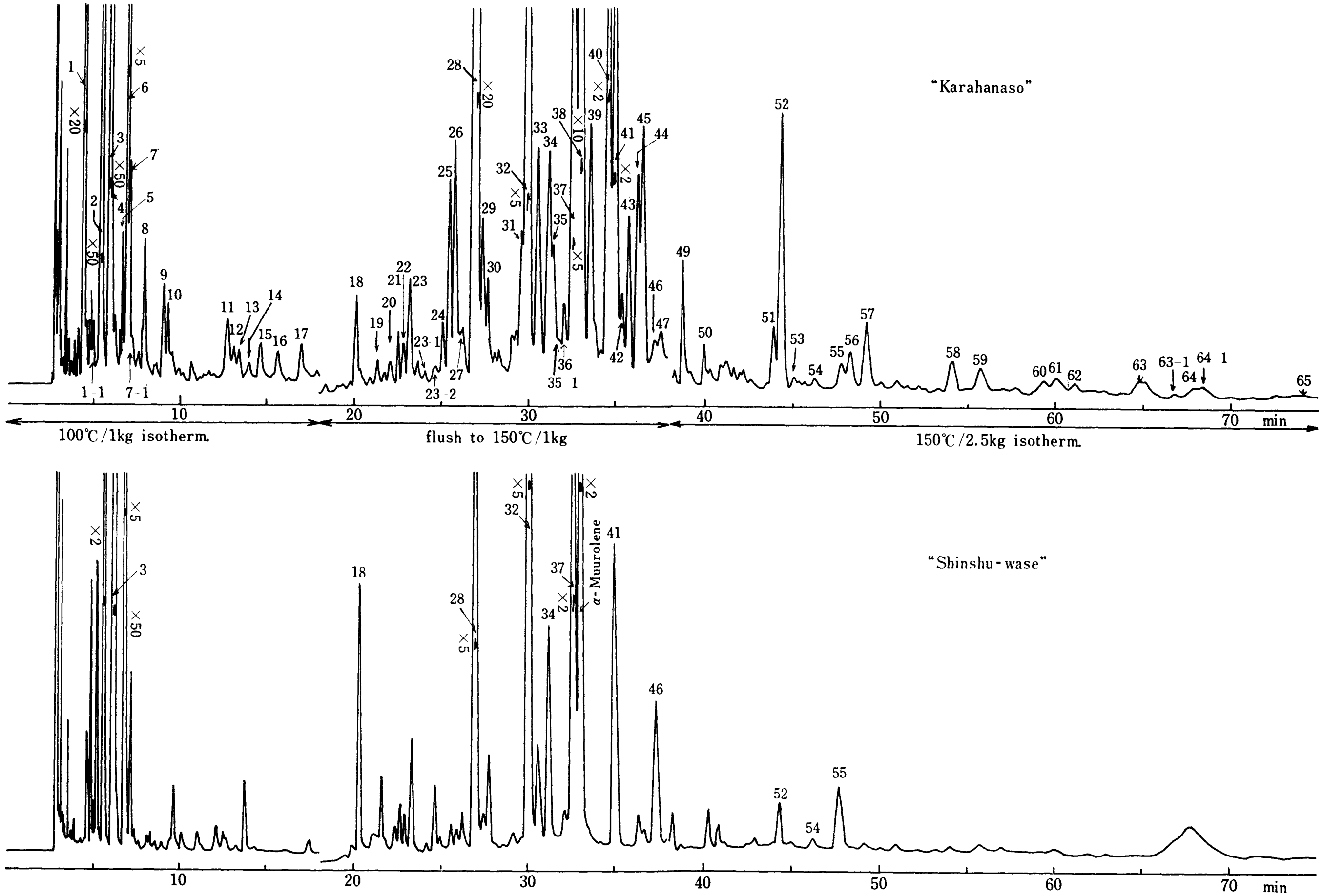

Fig. 1. Comparison of capillary gas-chromatograms obtained for hop oils from " Karahanaso " and " Shinshu-wase ".
Column 45 m×0.25 mm coated with HB 2000.

Table 3. Tentatively identified or unknown compounds

Peak No.	Compound	Molecular weight	Base peak
10-1	Karahana ether[2]	152	107
	Me-ester $C_{10}H_{20}O_2$	172	74
29	Me-ester $C_{11}H_{18}O_2$	182	85
	Me-ester $C_{12}H_{24}O_2$	200	74
34	Me-ester $C_{12}H_{22}O_2$	198	59
	Me-ester $C_{13}H_{24}O_2$	212	74
	Me-ester $C_{13}H_{22}O_2$	210	79
	Me-ester $C_{14}H_{26}O_2$	226	69
53	Aldehyde $C_{15}H_{24}O$	220	41
	Aldehyde $C_{16}H_{30}O$	238	69
	Aldehyde $C_{17}H_{32}O$	252	41
	Aldehyde $C_{18}H_{34}O$	266	41, 43
	Aldehyde $C_{19}H_{36}O$	280	41, 43
65	Ketone $C_{15}H_{24}O$	220	109
	Ketone $C_{15}H_{26}O$	222	43
	Ketone $C_{17}H_{30}O$	250	43
59	Alcohol $C_{15}H_{26}O$	222	161
63	Bulnesol $C_{15}H_{26}O$	222	59
63-1	Alcohol $C_{15}H_{26}O$	222	166
64	Alcohol $C_{15}H_{26}O$	222	43
64-1	Alcohol $C_{15}H_{24}O$	220	69
65-1	Alcohol $C_{15}H_{24}O$	220	41, 55, 93
32	α-Humulene	204	93
	trans-α-Farnesene	204	69
	Isocaryophyllene	204	93

GLC. Each separated compound was mixed with the original oil, and the peak on the programmed gas-chromatogram was confirmed by an appreciable increase in the height of the particular peak. Authentic compounds were obtained from reliable sources or were prepared by well-established methods. They were purified by GLC separation before use. Identifications are based on at least two physical methods (IR, MS, and NMR). Figure 1 shows a gas chromatogram of the steam volatile oil from "Karahanaso", together with that of the oil from the Japanese "Shinshu-wase," harvested in 1968,[*1] for the sake of comparison. Significant differences in the composition are seen in the fingerprinting of GLC analysis. Myrcene, α- and β-humulenes, caryophyllene, β-selinene, α-muurolene, and δ- and γ-cadinenes have been known[1–3] to be major components of " Shinshu wase." In "Karahanaso," myrcene, β-humulene, α- and β-selinenes, and δ- and γ-cadinenes occur in considerable amounts; bisabolane-type sesquiterpene (zingiberene, α-curcumene, together with β-bisabolene, and β-sesquiphellandrene) in an abnormally high concentration, and caryophyllene, in the highest abundance. Unlike the case of "Shinshu-wase", no α-muurolene was detected, whereas evidence was found for the presence of two other selinenes, selina-3,7(11)-diene and selina-4(14),7(11)-diene.

Oxygenated sesquiterpenes occur in relatively small quantities, and caryophyllene-oxide, nerolidol, and β-caryophyllene alcohol have been isolated in certain amounts. The isolation of many GLC peaks with long retention times has shown that each peak is composed of a mixture of several compounds. Most of these appear to be sesquiterpenic alcohols with a molecular weight of 220 or 222.

The authors are deeply indebted to Professor Tsunematsu Takemoto of Tohoku University for supplying "Karahanaso," harvested in 1969 at Miyagi, and to Professor Akira Yoshikoshi for supplying the IR spectrum of synthetic bulnesol.

[*1] Previous reports[1–3] have been concerned with "Shinshu-wase" harvested in 1963, the constituests of which were essentially the same as in that harvested in 1968.