Volatile oxygenated constituents of hops

Identification by combined gas chromatography and mass spectrometry

The oxygenated constituents of hops have been studied by several authors[1-5] but identification has generally been limited to the major constituents. The present work reports a rather detailed study using the powerful analytical method of the direct combination of capillary gas–liquid chromatography and mass spectrometry (Cap–MS)[5-8].

Experimental

Authentic samples of compounds were obtained from reliable commercial sources or synthesized by well-established methods. They were purified by gas–liquid chromatography (GLC) separation before use.

Hop oil was obtained by the steam distillation of Brewers Gold hops. The oxygenated fraction was separated from the hydrocarbons on silica gel as described previously[4].

The capillary column used for the analysis was 192 ft. long by 0.01 in. I.D., stainless steel, coated with Silicone SF 96 (50)* containing the tail-reducing agents Carbowax 20 M and Alkaterge T (1 % of each in the silicone). The GLC conditions were: sample, 20 μl, injected into a 1/300 split stream injector having a temperature of 200°; carrier gas, helium at a velocity of 25.4 cm/sec for both mass spectral and flame ionization work; column temperature programmed non-linearly from 50–180°.

For the mass spectral analysis, approximately half of the capillary effluent was led into the ionization chamber of a Bendix Time-of-Flight mass spectrometer as described in previous work[5-8]. In order to obtain a chromatogram comparable to that obtained with a flame ionization detector and concurrent with the mass spectral analysis, the rest of the effluent was passed through a vacuum ionization gauge, which served as the GLC detector.

For retention time measurements the end of the capillary was led into a flame ionization detector. To determine accurately if an authentic sample had the same GLC retention time as a particular peak, it was mixed with a 20-fold amount of the oxygenated fraction and chromatographed, using the same GLC conditions as for Fig. 1. An appreciable increase in the particular peak would confirm that the retention time of the authentic compound was the same.

Results

Fig. 1 shows the capillary GLC analysis obtained using a flame ionization detector. Table I shows the results of the analysis; column 2 lists constituents whose mass spectral patterns and GLC retention times were identical with those of authentic samples. In several cases authentic samples were not available but the mass spectral pattern indicated the structure and molecular weight. Such identification can be only tentative but because it has some value it is listed in column 3.

A study using conventional packed column separation was carried along with the Cap–MS study and the identity of many of the constituents was confirmed by

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

comparison of their infrared absorption spectra with those of authentic samples (Table I).

TABLE I

IDENTIFICATION OF CONSTITUENTS IN OXYGENATED FRACTION OF BREWERS GOLD HOP OIL USING CAPILLARY GLC AND MASS-SPECTRAL ANALYSIS

Peak number in Fig. 1	*Identification confirmed by comparison of mass spectrum and GLC retention time with those of authentic sample*	*Mass spectral prediction where authentic sample not avaible*
4	2-Methylpropyl isobutyrate*	—
5	Methyl hexanoate	—
6	—	Methyl thio-2-methylbutyrate
7	Butyl isobutyrate	—
8	2-Methylbutyl propionate*	—
9	Methyl 5-methylhexanoate*	—
10	—	—
11	2-Methylpropyl 2-methylbutyrate	—
12	3-Methylbutyl isobutyrate	—
13	2-Methylbutyl isobutyrate*	—
14	Methyl heptanoate* and methyl 4-methylhex-2-enoate*	—
15	—	—
16	Pentyl isobutyrate	—
17	—	2-Methylpropyl branched pentenoate and methyl thiohexanoate (branched)
18	—	Pentenyl isobutyrate
19	—	Methyl 2,5-dimethylhexanoate
20	Methyl thiohexanoate*	and methyl 6-methylheptanoate**
20a	Ethyl heptanoate	—
21	2-Nonanone	and 2-methyl-5-pentenylfuran**
22	Hexyl propionate*	—
23	2-Methylbutyl isovalerate	—
24	2-Methylbutyl 2-methylbutyrate* and linalool*	—
25	Methyl octanoate*	—
26	Hexyl isobutyrate*	—
26c	—	Methyl thioisoheptanoate
27	—	Oxygenated terpene
28	Methyl thioheptanoate* and 2-decanone	—
29	—	Methyl nonanoate (branched)
30	Heptyl propionate*	—
31	Octyl acetate*	and methyl nonenoate**
32	Methyl nonanoate*	—
33	Heptyl isobutyrate	—
34	2-Methylbutyl hexanoate	—
35	—	Branched 2-undecanone
35a	—	Methyl 2-methylnonanoate
36	—	Mol. wt. 204
37	—	—
38	—	Methyl 8-methylnonanoate
39	2-Undecanone*	—
40	—	—
41	Methyl dec-4-enoate***	—
42	Methyl deca-4,8-dienoate***	—
43	Methyl geranate*	—
44	Methyl decanoate*	—
45	—	Methyl decenoate

(*continued on p. 401*)

TABLE I (*continued*)

Peak number in Fig. 1	*Identification confirmed by comparison of mass spectrum and GLC retention time with those of authentic sample*	*Mass spectral prediction where authentic sample not avaible*
46	Octyl isobutyrate	—
47	—	2-Methylbutyl heptanoate
48	—	2-Dodecanone (branched)
48a	Neryl acetate	—
48b	—	Branched nonanyl isobutyrate
48c	—	Methyl undecenoate (branched)
49	Geranyl acetate*	—
50	—	Methyl 9-methyldecanoate
51	—	—
52	—	Methyl undecenoate
53	—	Methyl undecadienoate
54	—	Methyl undecenoate
55	Methyl undecanoate	—
56	—	Methyl undecenoate
57	Neryl propionate	—
58	—	2-Tridecanone (branched)
59	—	—
60	Geranyl propionate*	—
61	—	—
62	Neryl isobutyrate	and methyl dodecanoate (branched)
63	2-Tridecanone*	—
64	—	Methyl dodec-8-enoate**,+ and methyl
65		dodecadienoate Mol. wt. 208
66	—	Linalyl propionate
67	Geranyl isobutyrate*	—
68	—	—
69	Methyl dodecanoate	—
70	—	Methyl dodecenoate
71	—	Branched 2-tetradecanone
72	—	Tetradec-9-en-2-one**,+
73	—	Sesquiterpene, mol. wt. 204
74	—	Mol. wt. 204
75	—	Terpene ester
76	—	—
77	2-Tetradecanone	
78	—	Methyl tridecenoate
79	—	Methyl tridecenoate
80	—	Sesquiterpenoid, Mol. wt. 220
81	—	Sesquiterpenoid, Mol. wt. 222
82	—	—
83	—	—
84	—	Pentadeca-6,9-dien-2-one**,+
85	—	Mol. wt. 220
86	—	Mol. wt. 222
87	2-Pentadecanone	—

* Infrared absorption spectrum identical with that of authentic sample.
** Identification supported by infrared absorption spectrum.
*** Identity previously proved by NMR and ozonolysis[4].
\+ Identification supported by ozonolysis.

Little information could be obtained from the mass spectral patterns of peaks 70–86. Many of these peaks appeared to be sesquiterpenoids but their mass spectral patterns did not match those of any of the limited number of sesquiterpenoids available. Peaks 75 and 80 had retention times corresponding to those of caryophyllene epoxide and humulene epoxide respectively[3].

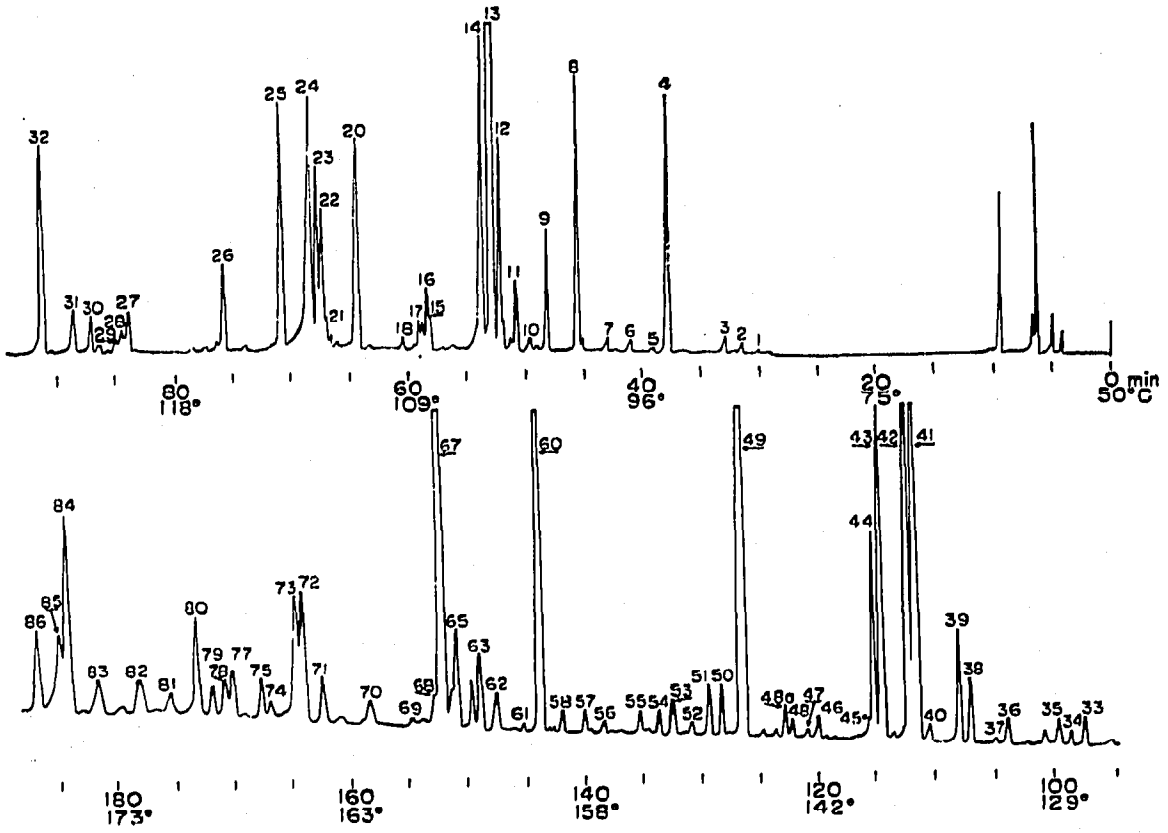

Fig. 1. Temperature programmed capillary column GLC analysis of the oxygenated fraction of hop oil (Brewers Gold hops). Column 192 ft. long × 0.01 in. I.D., coated with silicone SF 96 (50) plus tail reducers; helium carrier gas velocity 24.5 cm/sec; flame ionization detection.

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