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IDENTIFICATION OF VOLATILE, WATER-SOLUBLE COMPOUNDS FROM HOPS (*HUMULUS LUPULUS* L.)

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Abstract—The separation and identification of nine previously undetected volatile, moderately water-soluble compounds in hops are described. The occurrence of the *gem*-dimethyl group in every structure strongly suggests isoprenoid participation in their origin. Although the ketone, two acids, and four alcohols identified also occur in certain fruits, the 3-methylbut-2-en-1-al and 2,2-dimethyl-5-oxo-2,5-dihydrofuran appear to be characteristic of hops.

INTRODUCTION

IN RECENT years many workers have been concerned with the identification of the constituents in the essential oil of hops. Hop oil is a complex mixture consisting mainly of terpenes and oxygenated substances. Most of the major constituents of steam-distilled hop oil have now been identified, and an excellent review of the literature has recently been published.[1] Although the likelihood of a volatile, water-soluble fraction has been postulated, this has remained undetected because it is not readily isolated by normal steam distillation methods.[2,3] In the brewing process, hops are normally boiled in the wort for about 2 hr. It is also common practice to add hops to barrels of new beer. The hops are steeped for about 3 weeks. This process is known by experience to give added hop character to beer. Hence the volatile, water-soluble fraction of hops may be of considerable importance in beer flavour and aroma.[4]

A special distillation–extraction apparatus and technique for the examination of worts and beers has been described in which the steam distillate was continuously extracted with a suitable solvent.[5] This method was used in our current work to provide an extract containing volatile constituents possessing moderate water-solubility. A preliminary communication described the isolation and identification of 2-methylbut-3-en-2-ol as the major constituent of the extract examined.[6]

In this study eight more compounds which appear to be the most important in the extract from a quantitative viewpoint have now been identified after separation by gas–liquid chromatography (GLC).

RESULTS AND DISCUSSION

Hops (variety Bullion) which had been stored for approximately 6 months, were employed to provide various extracts. The normal steam distillation method[3] was used to obtain an

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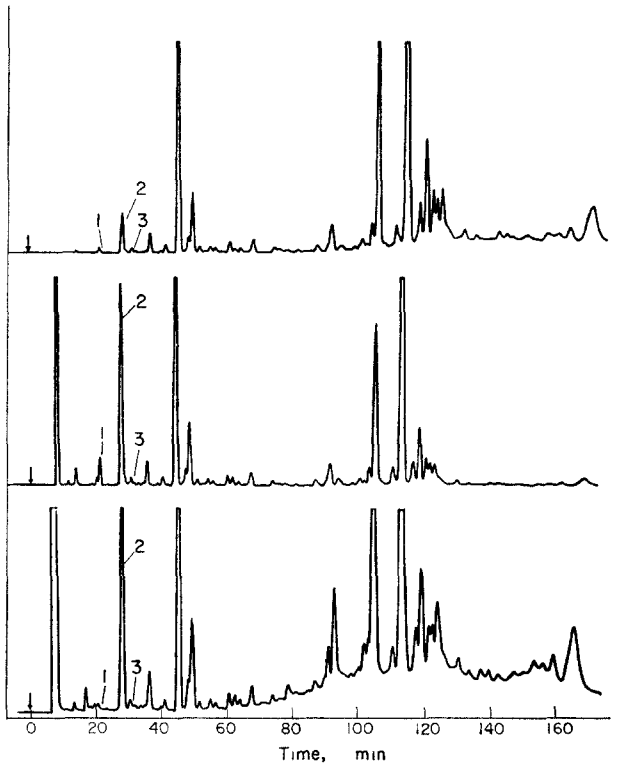

FIG. 1. CHROMATOGRAMS (FROM TOP TO BOTTOM) OF MATERIALS A, B AND C OBTAINED FROM HOPS BY (*a*) NORMAL STEAM DISTILLATION, (*b*) DISTILLATION–EXTRACTION AND (*c*) EXTRACTION AT ROOM TEMPERATURE.

Peak Nos. 1–3 correspond to compounds listed in Table 1. Conditions of chromatography are given in the text. The solvent peak nearest the start in (*b*) and (*c*) is due to ether.

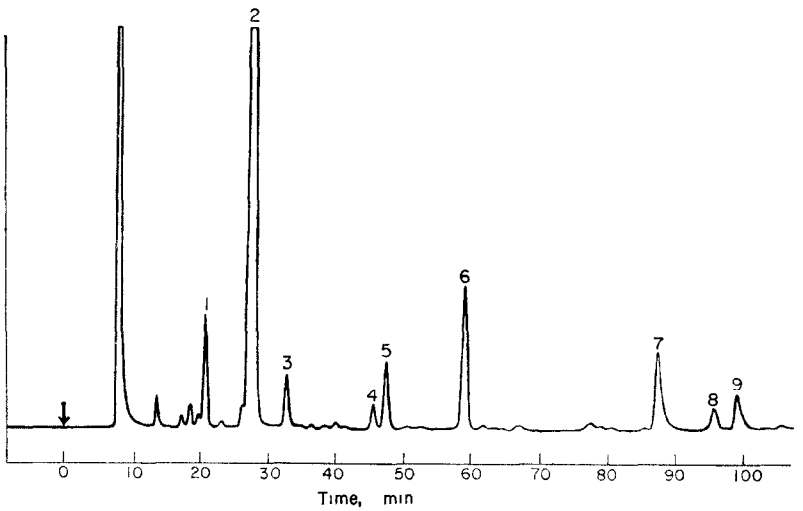

FIG. 2. CHROMATOGRAM OF EXTRACT D. THIS WAS OBTAINED BY EXTRACTION OF MATERIAL B WITH WATER AND THEN EXTRACTION OF THE AQUEOUS PHASE WITH ETHER.

Peak Nos. correspond to compounds listed in Table 1. Conditions of chromatography are given in the text. The solvent peak nearest the start is due to ether.

essential oil extract (A). An extract (B) was prepared from the same pocket of hops by the distillation–extraction technique[5] using ether as solvent. A third extract (C) was obtained by grinding similar hops with ether at room temperature. Aliquots of fractions A, B and C were examined by analytical GLC (see Figs. 1(*a*), 1(*b*) and 1(*c*)). A comparison of Figs. 1(*a*) and 1(*b*) reveals that the fraction B isolated by the distillation–extraction technique contained proportionately more of several compounds with low retention time. Figure 1(*c*) also shows appreciable quantities of these compounds indicating that they were not artifacts produced during the distillation process.

Fraction B was further extracted with water and the aqueous phase extracted with ether. This extract (D) was examined analytically by GLC and the results are shown in Fig. 2. The major constituents of this fraction were isolated by preparative GLC and identified mainly by physical methods (see Tables 1 and 2). Each compound had a characteristic

TABLE 1. COMPOUNDS ISOLATED FROM HOPS

Peak no.*	Compound	Relative amount†	GLC relative retention‡	Methods of identification
1	3-Methylbutan-2-one	6·9	0·41	GLC, i.r., PMR, u.v.
2	2-Methylbut-3-en-2-ol	100	0·57	GLC, i.r., PMR, u.v., MS, D
3	2-Methylpropan-1-ol	3·3	0·69	GLC, i.r.
4	3-Methylbutan-1-ol	1·8	1·00	GLC, i.r.
5	3-Methylbut-2-en-1-al	5·7	1·05	GLC, i.r., PMR
6	3-Methylbut-2-en-1-ol	13·1	1·31	GLC, i.r., PMR
7	2-Methylpropanoic acid	9·6	1·97	GLC, i.r.
8	2,2-Dimethyl-5-oxo-2,5-dihydrofuran	2·2	2·17	GLC, i.r., PMR
9	3-Methylbutanoic acid	4·0	2·26	GLC, i.r.

* Peak No. in chromatogram Fig. 2.
† Based on peak areas in Fig. 2.
‡ 3-Methylbutan-1-ol eluted after 45 min.
GLC = Gas–liquid chromatography—authentic and natural compounds had the same retention time.
i.r. = Infra-red absorption spectroscopy—authentic and natural compounds had identical spectra.
PMR = Proton magnetic resonance spectroscopy—spectrum of natural compound agrees with structure.
u.v. = Ultra violet absorption spectroscopy—authentic and natural compounds had identical spectra.
MS = Mass spectrometry—authentic and natural compounds had identical spectra.
D = Derivative (see Ref. 6).

odour. The most abundant compound was 2-methylbut-3-en-2-ol which represented at least 0·02 per cent of the kiln-dried hops,[6] compared with a total essential oil content by the Wright and Connery technique[3] of 0·6 per cent. Of the compounds found, none has been shown previously to be present in hops. According to a recent review,[7] the ketone, two acids, and four alcohols identified have been detected in various fruit aromas. 3-Methylbut-2-en-1-al and 2,2-dimethyl-5-oxo-2,5-dihydrofuran, however, have not been found in any other natural product and so may well prove to be characteristic of hops. Every compound isolated contained the *gem*-dimethyl group and six of the nine constituents had the isoprene skeleton.

Consideration has been given to the interrelationship of these compounds. It has been shown that 2-methylbut-3-en-2-ol is converted to 3-methylbut-2-en-1-ol under acidic conditions[8] and therefore it is possible that the latter compound was formed in this way. This

[7] H. E. NURSTEN and A. A. WILLIAMS, *Chem. Ind.* 486 (1967).
[8] I. N. NAZAROV, I. N. AZERBAYEV and V. N. RAKCHEEVA, *Izvestia Akad. Nauk SSR* 419 (1946).

TABLE 2. SPECTRAL DATA. I.R. AND U.V. SPECTRA FOR NATURAL AND AUTHENTIC COMPOUNDS. PMR SPECTRA FOR NATURAL COMPOUNDS

Compound	Position of bands in spectra: i.r. (cm^{-1})	PMR: τ (ppm)	PMR: Assignment	u.v. (nm)	Source of authentic sample
3-Methylbutan-2-one	2980, 1725, 1477, 1364 1198, 1150, 1105	8·94d (7) 7·96	CMe_2 COMe	190	EK
2-Methylbut-3-en-2-ol	3470, 3250, 2880, 1660 1405, 1380, 1160, 920	8·74 8·56 4·04q; 4·88, 5 10m ($J_1=17, J_2=10$)*	CMe_2 OH $H_2C=CH$	192	KL
2-Methylpropan-1-ol	3680, 3350, 2980, 1480 1400, 1375, 1042, 942	—	—	—	BDH
3-Methylbutan-1-ol	3600, 3300, 2920, 1467 1387, 1370, 1058, 1005	—	—	—	BDH
3-Methylbut-2-en-1-al	1680, 1636, 1450, 1380 1200, 1172, 1128, 1047	6 03d (~1), 5 82d (~1) 2·13m (9) 0·06d (8)	$=CMe_2$ C=CH CHO	—	Synthesized†
3-Methylbut-2-en-1-ol	3680, 3380, 2950, 1680 1456, 1387, 1040, 990	8·88 8 29; 8·33 6·01d (7) 4 65t (7)	OH $=CMe_2$ CH_2 C=CH	—	Synthesized†
2-Methylpropanoic acid	2960, 1715, 1485, 1427 1298, 1245, 945	—	—	—	BDH
2,2-Dimethyl-5-oxo-2,5-dihydrofuran	2980, 1760, 1610, 1280 1135, 972, 950, 705	8·52 4 08d (6); 2·72d (6)	CMe_2 HC=CH	—	Synthesized†
3-Methylbutanoic acid	2950, 1710, 1475, 1417 1308, 1220, 1175, 940	—	—	—	BDH

EK = Eastman Kodak.
KL = Koch-Light.
BDH = British Drug Houses.
i.r. = Infra-red spectra (clear film or carbon tetrachloride solvent).
PMR = Proton magnetic resonance spectra (60 Mc/s, carbon tetrachloride solvent, tetramethylsilane internal reference).
Coupling constant (J, c/s) in parenthesis d = doublet, t = triplet, q = quadruplet, m = multiplet.
* Approx. first-order analysis only.
u.v. = Ultra violet spectra (water solvent).
† Details given in text.

alcohol could also arise from 3-methylbut-2-en-1-al by dismutation, but it was shown by GLC that this reaction did not in fact occur since 3-methylcrotonic acid, the other product which would be formed in this reaction, was absent from the extract.

As the materials isolated from hops are soluble in water it is likely that the efficiency of the extraction technique was not very high. Hence the amounts of the compounds available for identification could be smaller than those present in wort produced in a brewing process using the same quantity of hops.

Fresh green Bullion hops from the bine were extracted (to give fraction E) in the same manner as that used to obtain fraction C from hops which had been kilned and stored. Fraction E was shown by GLC to contain a constituent with the retention time of 2-methylbut-3-en-2-ol. The amount of this compound present in the fresh hops was very small compared with the stored material. Examination of the extract (F) from fresh kilned hops gave a similar result to the fresh unkilned sample, indicating that 2-methylbut-3-en-2-ol was not produced during the drying process. It seems likely therefore that this compound mainly arises during storage after kilning.

The compounds found could have been formed by oxidation in air of the terpenoid compounds present in hop resin (e.g. the α-acids, i.e. humulone and analogues, or β-acids, i.e. lupulone and analogues) or those in hop oil (e.g. the sesquiterpenes). The α-acids are responsible for the characteristic bitterness of beers.

It is well known that hops lose α- and β-acids and essential oil during storage. At harvest, kiln-dried Bullion hops normally contain approximately 8 per cent of α-acid and 1 per cent of oil but after 4 months' storage at room temperature about one-tenth of each of these is lost by oxidation.[9] This variety has the highest α-acid and essential oil content of any English hop and also loses α-acid at the fastest rate.[9] Many brewers are unable to use this variety directly in the copper due to the production of an off-flavour. 2-Methylpropanoic and 3-methylbutanoic acids, with their rank aromas, might contribute to this. Work is at present in progress to examine this possibility.

Bullion is a commercial hop variety known to have a noticeable blackcurrant aroma and it is therefore of interest that five of the nine compounds isolated from extract D in the present work have been found in distillates of blackcurrant.[7] Investigations are continuing to identify the highly polar volatile compounds that remain in the aqueous extract after ether extraction.

EXPERIMENTAL

Preparation of Materials A–C, E and F from Hops (var. Bullion) for Separation by Analytical GLC

Material A was obtained by the method of Wright and Connery[3] using ground kiln-dried hops (100 g) that had been stored for 6 months.

Material B. An aliquot was taken of the ether solution from the distillation–extraction carried out as described below (see preparation of material D for separation by preparative GLC).

Materials C, E and F. Hops (two cones each time, approx. 0·4 g kiln-dried or 2 g green) were extracted with redistilled ether (10 ml for kiln-dried cones, 100 ml for green cones) by grinding in a pestle and mortar. After filtration the ethereal solution was dried over anhydrous magnesium sulphate and concentrated to a small volume (approx. 0·5 ml) in a stream of nitrogen.

Material D. An aliquot was taken from the material prepared for preparative GLC (see below).

Conditions of Analytical GLC

A Pye series 104 Chromatograph having a flame ionization detector was employed with an 18-ft glass column (i.d. 4 mm) containing 10 per cent Carbowax 20 M on 100–120 mesh acid-washed celite. The rate

[9] R. D. HARTLEY, *J. Inst. Brewing* **73**, 538 (1967).

of flow of argon carrier gas was 15 ml/min and the gauge pressure 70 p.s.i. The column oven was programmed from 80–210° at 1°/min and held at the upper limit. The sample sizes and amplifier attenuations were as follows: Material A, 0·4 μl, 2×10^{-9}; B, 4 μl, 10^{-8}; C, E and F, 15 μl, 5×10^{-9}; D, 4 μl, 5×10^{-9}.

Preparation of Material D for Separation by Preparative GLC

Ground hops (600 g var. Bullion) were added to water (5 l.) and distilled-extracted by the method previously described.[6] The ether extract was removed after 3 hr and distillation–extraction continued for a further 3 hr to provide a second extract. The combined ethereal fractions (B) from eight batches (total volume 80 ml) were extracted with an equal volume of water. The centrifuged aqueous solution was then extracted with an equal volume of ether. The ethereal solution was concentrated to a small volume (10 ml) in a stream of nitrogen and dried over anhydrous magnesium sulphate.

Preparative GLC

The dried ethereal solution from above was separated in batches (0·5–1·0 ml each time) using a Pye Series 104 chromatograph fitted with a manual-preparative kit and flame ionization detector. GLC conditions were as follows: 18 ft glass column (i.d. 4 mm) containing 10 per cent Carbowax 20M on 100–120 mesh acid-washed celite, rate of flow of argon carrier gas 15 ml/min, gauge pressure 70 p.s.i. and amplifier attenuations 2×10^{-9} or 5×10^{-9}. The temperature programmes were 60–210° (for isolation of peak Nos. 1, 4 and 5), 70–210° (for isolation of peak Nos. 2 and 3) and 80–210° (for isolation of peak Nos. 6–9) at a rate of increase of 1°/min.

Examination and isolation of the authentic compounds by GLC was carried out using similar conditions to those employed for the natural compounds. Sources of authentic compounds are given in Table 2; the methods of preparation for the synthesized compounds are given below

Synthesis of Authentic Compounds

3-*Methylbut-2-en*-1-*al*. 3-Methylbut-2-en-1-ol (25 mg) was dissolved in chloroform (1 ml), freshly precipitated manganese dioxide (200 mg) was added, and the mixture shaken then allowed to stand. After 16 hr GLC indicated a 90 per cent conversion to 3-methylbut-2-en-1-al. The reaction solution was decanted from manganese dioxide, dried over anhydrous magnesium sulphate and 3-methylbut-2-en-1-al isolated by preparative GLC.

3-*Methylbut-2-en*-1-*ol*. This was prepared by the method already reported[8] and isolated by preparative GLC.

2,2-*Dimethyl*-5-*oxo*-2,5-*dihydrofuran*. Methylmagnesium iodide was prepared in dry ether (30 ml) from magnesium (1·0 g) and methyl iodide (6·6 g). This solution was added dropwise at 0 to 5° with stirring to maleic anhydride (2·0 g) in dry ether (30 ml). After stirring for 30 min, N sulphuric acid (50 ml) was added. After drying the ethereal layer and concentrating it tenfold, the yield (1·2 g) was estimated by analytical GLC. The product was isolated by preparative GLC. The i.r. and PMR spectra were identical with those obtained using the natural compound.

Natural and authentic compounds were shown to be identical using the methods listed in Table 1. The main spectral peaks are given in Table 2. The instruments used to determine the i.r., PMR, u.v., and mass spectra were a Parkin Elmer 21, Varian A-60A, Unicam SP.700 and A.E.I. MS9 respectively.

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