

LVII.—*Essential Oil of Hops.*

By ALFRED C. CHAPMAN.

In former communications to the Society (Proc., 1893, 9, 177; Trans., 1895, 67, 54, and 780), I have dealt with the results obtained by distilling the oil of hops fractionally under reduced pressure, and have described the preparation and properties of the sesquiterpene, humulene, and some of its more important derivatives.

At the time when an account of the above work was given, the hydrocarbon of lower boiling point had not been identified, and no conclusion was arrived at with regard to the nature of the small fraction or fractions boiling at temperatures intermediate between the boiling points of that hydrocarbon and humulene. Circumstances, which compelled me to relinquish the work temporarily, occasioned the delay which has occurred in presenting an account of the complete investigation to the Society. In this work I have made use of three additional samples of oil, which I shall refer to by the numbers 5, 6, and 7, in order to bring them into line with the four samples already investigated. The first was distilled by Messrs. Schimmel and Co., of Miltitz, near Leipzig, from Bavarian hops grown in 1894. The second was distilled for me by Messrs. Schimmel from Californian hops (yield = 0.53 per cent.) grown in 1899, and purchased in this country. No. 7 was distilled by Messrs. Schimmel & Co. from Bavarian hops grown in 1901, and the genuineness of the three samples is consequently entirely above suspicion. I desire to take this opportunity of expressing to this firm my best thanks for their kindness and courtesy.

The three samples had the following specific gravities and specific rotatory powers:

	Sp. gr. 15°/15°.	Sp. gr. 20°/20°.	$[\alpha]_D^{20}$.
No. 5	0.8676	0.8645	+ 0.30°
No. 6	0.8639	0.8610	- 0.20°
No. 7	0.8403	0.8357	- 0.08°

No. 5 was almost entirely used for the study of the oxidation products of the oil, and need not be more particularly referred to at present.

The Californian oil (No. 6), after a long series of fractional distillations under 46 mm. pressure, was resolved into the following six fractions which had the sp. gr. (15°/15°) indicated.

(1) 82—84° sp. gr. 0.8046	(2) 85—92° sp. gr. 0.8108
(3) 95—120° „ 0.8298	(4) 120—130° „ 0.8638
(5) 135—150° „ 0.8925	(6) 155—160°.

The third sample of oil (No. 7) was similarly fractionated under 33 mm. pressure, and gave the following five fractions:—(1) 76—78°, (2) 80—85°, (3) 115—130°, (4) 130—140°, (5) 140—150°.

In both cases the first and the last fractions were by far the largest, amounting together to more than 80 per cent. of the original oil. The last fractions (No. 6 of the second oil and No. 5 of the third) consisted of nearly pure humulene, and need not be further referred to, except to say that after purification they were used in the oxidation experiments of which an account is given in the latter part of this paper.

The first fraction in the case of each oil amounted to nearly 50 per cent. of the sample taken, and as these were found to be identical in composition, one description of the experimental investigation will suffice.

The Fraction of Lowest Boiling Point.

At the time when my first paper on this subject was published, I had obtained similar fractions from the oils then examined, and had arrived at the conclusion that they consisted of a mixture of an "olefinic terpene" with a hydrocarbon having the formula $C_{10}H_{18}$, the statement in regard to the existence of the latter substance being based on the percentage of hydrogen found on analysis.

Having obtained a much larger quantity of this fraction from the two samples of oil above referred to, I was anxious to purify it as completely as possible with the object of ascertaining whether I could obtain more definite evidence of the existence of the hydrocarbon $C_{10}H_{18}$. To this end it was submitted to repeated distillation over bright sodium under reduced pressure until the metal remained quite unaltered. In this way a considerable quantity of liquid of practically constant boiling point was obtained, although it was not found possible to remove the last trace of oxygen.

The following are the results of two combustions :

0.2147 gave 0.6908 CO_2 and 0.2263 H_2O . C = 87.75, H = 11.71.

0.2222 „ 0.7125 CO_2 „ 0.2357 H_2O . C = 87.45; H = 11.77.

$C_{10}H_{16}$ requires C = 88.23; H = 11.77 per cent.

It will be observed that in both analyses the percentage of carbon is slightly lower than that demanded by theory, but this is unquestionably due to the rapidity with which the hydrocarbon oxidises in air, and to the impossibility of removing the last trace of oxygen by distillation over sodium. Other hydrogen estimations gave 11.80, 11.82, 11.74, and 11.83, so that there can be no doubt that the fraction consists of one compound having the formula $C_{10}H_{16}$.

The hydrocarbon has sp. gr. 0.8046 at 15°/15° and 0.8020 at 20°/20°; it boiled at 74—75° (33 mm.) and at 166—168° under atmospheric pressure (774 mm.), but in the latter case there was some slight decomposition towards the end of the distillation, a small quantity of very viscous liquid remaining in the flask. It was optically inactive and a determination of its index of refraction at 20° gave the value $N_D = 1.4645$, whence the molecular refractive energy is 46.8, the calculated number being 46.78 for $C_{10}H_{16}$ with 3 ethylene linkings. This compound is therefore an aliphatic hydrocarbon, and its properties are almost identical with those of myrcene, which was first obtained from bay oil by Power and Kleber, and which has since been identified in the oil of sassafras leaves.

A comparison of the constants of these two substances will make this clearer:

	Hydrocarbon from hop oil.	Myrcene from bay oil.
Specific gravity at 15°	0.8046	0.8023
Boiling point (atmospheric pressure)...	166—168°	167°
Molecular refractive energy	46.8	47.1
Action on polarised light	Inactive	Inactive

It has a very characteristic odour, which Dr. Power, to whom I sent a specimen, informs me is very similar to that of the myrcene from bay oil, and oxidises with great readiness when exposed to the air, becoming ultimately converted into a colourless resin.

From basil oil, van Romburgh has recently (Schimmel and Co., *Report*, April—May, 1901, 12) isolated an "olefinic terpene" closely resembling myrcene in its general properties, but which is stated to oxidise more readily in the air, and to which the name *ocimene* has been given. Van Romburgh states that when a small quantity is placed in a tube with oxygen over mercury "the latter rises rapidly, and after a short time the oxygen has disappeared and the tube is completely filled with mercury." A similar experiment was made with the freshly distilled hydrocarbon from hop oil, 1 c.c. being introduced into a graduated tube standing over mercury and containing 20 c.c. of oxygen. Absorption took place slowly, and at the end of three days at the ordinary room temperature, 16 c.c. of oxygen had united with the hydrocarbon, and the action was still proceeding. The words "rises rapidly" could scarcely be employed in referring to the rise of the mercury due to oxygen absorption in this experiment, and on the other hand, van Romburgh's statement that he had kept myrcene prepared from bay oil for many months without being able to detect any change requires to be supplemented by a statement of the conditions under which the hydrocarbon was kept. The hydrocarbon

from hop oil, when sealed up in a tube from which the air has been exhausted, can apparently be kept for a long time without appreciable alteration, but, as I have already stated, it rapidly changes when exposed to air. Van Romburgh's statement as to the stability of myrcene in air is not only opposed to Power and Kleber's observations, and to the recent experiments of Harries (*Ber.*, 1903, 35, 3259), but is scarcely in accordance with the well-known diminution of solubility which bay oil undergoes on storage. The existing accounts of the oxidisability and tendency to polymerisation of myrcene are discrepant, and no well-defined differences between the two unsaturated hydrocarbons obtained respectively from bay oil and from basil oil, either in regard to their physical constants or to their chemical properties (other than the degree of readiness with which they are said to unite with oxygen), have yet, so far as I can ascertain, been published. The investigation of ocimene is being continued, and pending the publication of further results, it would be wiser, I think, to reserve judgment in regard to the chemical individuality of that hydrocarbon.

When hydrated by means of acetic and sulphuric acids (Bertram's method), the hop oil fraction behaved like myrcene, yielding an alcohol (? myrcenol), an oil having an odour of lavender, and a hydrocarbon, which could not, however, be identified with dipentene. When oxidised with alkaline potassium permanganate solution, it gave acetic and oxalic acids and carbon dioxide, whilst with chromic acid mixture, succinic acid was obtained, together with a small quantity of an oily acid having an odour strongly resembling that of valeric acid. Myrcene was present in the last three samples of oil to the extent of from 40 to 50 per cent., but the actual proportion in any sample is doubtless dependent on the climatic and storage conditions to which the hops had been subjected, and more particularly to the manner in which they were distilled. Old samples of oil will naturally contain less myrcene than newer ones, and the solubility and specific gravity of the oil, which are so greatly influenced by the amount of this constituent present, cannot consequently be regarded as of much importance as analytical "constants" in arriving at an opinion as to the genuineness of any sample. Oil No. 1, for example, had at 15° a sp. gr. 0.880, whilst that of No. 7 was at the same temperature only 0.840. That the resinification of myrcene is very largely due to oxidation and not to simple polymerisation is shown by the fact that it retained its mobility at the end of two years when sealed up in a tube from which all the air had been exhausted.

Attempts to prepare compounds with hydrogen chloride, bromine, nitrosyl chloride, and other reagents were in all cases unsuccessful. It may be mentioned that in no case does the amount of bromine which unites with the hydrocarbon correspond with the presence of

three double linkages, but this may well be due to the polymerisation which myrcene appears to undergo so readily.

Fractions 2 and 3 (Oil No. 6) and Fraction 2 (Oil No. 7) consisted of mixtures of myrcene with a small quantity of the fractions of higher boiling point, but were too small to be further separated by distillation.

Fraction 4 (Oil No. 6) and Fraction 3 (Oil No. 7), which were found to be practically identical in composition, formed a colourless, mobile liquid possessing a powerful and fragrant odour, but only a few c.c. were obtained.

(1) 0.1333 gave 0.3819 CO₂ and 0.1380 H₂O. C = 78.13 ; H = 11.50.

(2) 0.1702 ,, 0.4860 CO₂ ,, 0.1790 H₂O. C = 77.87 ; H = 11.69.

(3) 0.1900 ,, 0.5344 CO₂ ,, 0.2023 H₂O. C = 76.70 ; H = 11.84.

C₁₀H₁₈O requires C = 77.92 ; H = 11.68 per cent.

1 and 2 show the composition of fraction 4 from oil No. 6, and 3 that of fraction 3 from oil No. 7.

The product, which had a sp. gr. 0.8571 at 20°/20°, produced a rotation $[\alpha]_D = -0^{\circ}40'$, and united readily with bromine. The quantity of this fraction obtained, even when working with 500 c.c. of the hop oil, was so small that further experiments were impossible, but there can be very little doubt that it consisted of inactive linalool mixed with a little of some active substance.

The next higher fraction was also very small, and had a less fragrant odour than the preceding one. A preliminary experiment having shown that the oil consisted of, or contained, an ester, it was saponified by boiling with alcoholic potash and then distilled in steam. The residue, after acidification with sulphuric acid and extraction with ether, yielded a small quantity of an oily acid practically insoluble in water, and possessing a powerful odour recalling that of valeric and pelargonic acids. The ethereal solution, when dried over calcium chloride and distilled from a very small fractionating flask under a pressure of 11 mm., yielded about 2 c.c. boiling near 130° :

0.1545 gave 0.3813 CO₂ and 0.1444 H₂O. C = 67.31 ; H = 10.38.

C₉H₁₈O₂ requires C = 68.35 ; H = 11.39 per cent.

It gave a white, curdy silver salt, which, after drying over sulphuric acid in an exhausted desiccator, gave the following results with two different preparations :

(1) 0.2588 gave 0.1024 Ag. Ag = 39.57.

(2) 0.2240 ,, 0.0920 Ag. Ag = 41.07.

C₉H₁₇O₂Ag requires Ag = 40.52 per cent.

Sodium hydroxide (prepared from sodium) containing 0.0025

gram Na per c.c. was run into a solution of the acid in dilute alcohol, phenolphthalein being used as indicator. 0.1659 gram acid required for neutralisation 9.4 c.c. sodium hydroxide solution (= 0.0235 gram Na). For a monobasic acid of the formula $C_9H_{18}O_2$, 0.0241 gram Na would be required.

The crystalline sodium salt dried at 100° was analysed with the following result :

0.2687 gave 0.1109 Na_2SO_4 . Na = 13.3.

$C_9H_{17}O_2Na$ requires Na = 12.78 per cent.

This acid does not solidify, even when cooled in a freezing mixture of salt and ice, and is evidently *isononoic acid*, which is said to remain liquid at -11° (Kullhem, *Annalen*, 1874, 173, 319).

The distillate obtained in the preparation of the above acid was thoroughly shaken with ether, and this together with the extracted ethyl alcohol was evaporated. A small quantity of a colourless mobile liquid remained, having a strong odour of linalool. Well-formed crystals slowly separated when powdered calcium chloride was added to the cooled product. Dry ether was then added, and the crystalline substance with the excess of calcium chloride collected and treated with hot water, when a very small quantity of an oil separated ; this product, which had a strong odour of geraniol, could not be further examined. After distilling off the ether from the filtrate, some liquid remained which had a strong odour of linalool, and which yielded crystals of terpin hydrate on shaking for some days with dilute sulphuric acid. The ester would, therefore, appear to be the linalyl *isononoate*. These alcohols and esters occur in the essential oil of hops in very small proportions, and their separation in a state of even moderate purity is consequently an exceedingly difficult and expensive matter. From both samples of oil, nearly pure humulene was obtained by fractional distillation, to the extent of about 40 per cent. It will be observed that the proportion of humulene obtained from the earlier samples of oil with which I worked was considerably higher than the above, but this is due to the fact that those samples were not examined immediately after their distillation from the plant, as was the case with Nos. 6 and 7, and consequently much polymerisation of the myrcene had occurred. Since all were distilled in steam immediately prior to fractionation, and the altered myrcene thus eliminated, the proportion of humulene would obviously be much increased.

Oxidation of the Oil.

In my earlier paper (*Trans.*, 1895, 67, 54), a few oxidation experiments with some of the fractionated constituents of the oil were

referred to, but the action of oxidising agents on the oil itself was not discussed.

Oxidation with Chromic Acid Mixture.—Ten c.c. of the oil were added to a mixture of 60 grams of potassium bichromate, 45 c.c. of sulphuric acid, and 200 c.c. of water, and the whole was boiled under a reflux condenser until all the chromic acid was reduced. A further quantity of chromic acid mixture was then added, and the oxidation continued for about 24 hours. The product was subsequently filtered through muslin to remove a small quantity of unattacked resin, the slight excess of chromic acid reduced by the cautious addition of sulphurous acid, the resulting solution then distilled in steam until practically the whole of the volatile acids had distilled over, and the residue in the flask after dilution was thoroughly extracted with ether, which on evaporation left a small quantity of an oily substance from which crystals separated on standing. The acid distillate was neutralised with potassium carbonate and evaporated to dryness in order to obtain the mixed potassium salts.

Numerous oxidation experiments were made, the composition of the oxidising mixture being occasionally slightly varied. In this way the potassium salts (representing the volatile acids) and the residues from the ethereal extracts of the fixed oxidation products were thus accumulated in sufficient quantity for examination. The potassium salts were dissolved in water, the acids liberated by the addition of sulphuric acid, and extracted from the solution by means of ether; after removing the solvent, the acids were distilled from a small flask under reduced pressure. The first and larger fraction consisted of nearly pure acetic acid, yielding a silver salt containing 64.1 per cent. of silver (silver acetate requires $Ag = 64.46$); the second was evidently a mixture, whilst the third, which was very small, had a strong odour of valeric acid, and gave a silver salt containing 49.4 per cent. of the metal (silver valerate requires $Ag = 51.44$). Several other salts were prepared from this fraction, the properties of which closely resembled those of the corresponding valerates.

Another quantity of the purified potassium salts was fractionally precipitated with silver nitrate, and 5 silver salts were obtained, containing from 64.42 (acetate) to 44.9 per cent. of silver. From this it is evident that an acid (probably isononoic) was present, having a salt containing a smaller percentage of silver than the corresponding valerate.

Although the united residues obtained after evaporating off the ether used for the extraction of the non-volatile oxidation products from the chromic acid mixture, partially crystallised on long standing, yet, as it was found impossible to recrystallise without serious loss owing to the considerable quantity of uncrystallisable oil present, it was thought better to attempt to distil the whole under reduced

pressure. From 130° to 160° (under 30 mm. pressure) an oily liquid distilled over, but the distillate obtained at 165° commenced to solidify in the condenser tube, and the receiver was consequently changed. At 200°, all the crystalline substance appeared to have distilled, and only a small quantity of a red decomposition product having a strong empyreumatic odour came over. The first oily fraction slowly solidified completely to a crystalline mass, and both acids were purified by repeated crystallisation from water. These are referred to as No. 1 and No. 2.

No. 1 consisted of well-formed, needle-shaped crystals, easily soluble in water, acetone, alcohol, and ether, and somewhat less soluble in hot benzene, from which it crystallised readily in small, colourless needles.

After two crystallisations from benzene, it melted sharply at 140°.

0.1749 gave 0.3140 CO₂ and 0.1092 H₂O. C = 48.96; H = 6.93.

0.1611 ,, 0.2893 CO₂ ,, 0.0960 H₂O. C = 48.97; H = 6.63.

C₆H₁₀O₄ requires C = 49.31; H = 6.85 per cent.

Titration of the Acid.—Sodium hydroxide (prepared from sodium) containing 0.004669 gram Na per c.c. was run into an aqueous solution of the acid, phenolphthalein being used as indicator. 0.2015 gram acid required for neutralisation 13.7 c.c. sodium hydroxide solution (= 0.0639 gram Na). For a dibasic acid of the formula C₆H₁₀O₄, 0.0634 gram Na would be required.

When solutions of the metallic salts indicated below were added to an aqueous solution of the sodium salt, the following results were obtained:

Magnesium sulphate.	No reaction	Lead acetate	On warm-
Calcium chloride.....	No reaction			ing, a dense white precipitate.
Barium chloride.....	No reaction	Copper sulphate	On warm-
Silver nitrate	White gran-			ing, a green precipitate.
	ular precipitate			
	slightly soluble			
	in water.			

Two preparations of the silver salt, dried at 100°, gave 59.5 and 60.0 per cent. Ag respectively. C₆H₈O₄Ag₂ requires Ag = 59.78. The iron salt dried at 100° was found to contain 21.0 per cent. Fe. (C₆H₈O₄)₃Fe₂ requires Fe = 20.58. All the above results show that this acid is unsymmetrical dimethylsuccinic acid, CO₂H·CH₂·C(Me)₂·CO₂H.

No. 2 Acid.—This acid crystallised well from water or alcohol, and gave all the reactions of succinic acid; the crystals from the latter solvent melted at 184°.

0.161 gave 0.2425 CO₂ and 0.0756 H₂O. C = 41.06; H = 5.22.

C₄H₆O₄ requires C = 40.68; H = 5.08 per cent.

The oxidation products of the oil obtained by the action of chromic acid mixture are, therefore, acetic, valeric, dimethylsuccinic, and succinic acids, with probably *isononoic* acid from the linalyl ester.

When an alkaline solution of potassium permanganate was used instead of chromic acid mixture for the oxidation, all the above oxidation products were identified with the exception of the dimethylsuccinic acid, and oxalic acid was found in considerable quantity when dilute nitric acid was employed.

It may be well to correct a very common belief that valeric acid is a product of the aerial oxidation of hop oil. I have made many experiments in connection with this point, and in no instance was even a trace of valeric acid formed.

The dimethylsuccinic acid is an oxidation product of humulene, as was proved by submitting the purified sesquiterpene to the action of the chromic acid mixture, and isolating the products in the same manner as in the hop oil experiments. Along with that acid (m. p. = 140°), acetic acid was found in considerable quantity, together with a little valeric acid, mixed apparently with some higher homologue.

Myrcene, on oxidation, yielded chiefly acetic acid with some succinic acid; levulinic acid could not be identified.

It is interesting to note that dimethylsuccinic acid, to the extent of about 1.5 per cent., was obtained by Levy and Engländer (*Ber.*, 1885, 18, 3209) by the oxidation of caryophyllene from copaiba balsam by means of chromic acid solution. Owing to the small yield, the above-named authors doubted whether this acid was to be regarded as an oxidation product of the caryophyllene itself, or whether it might not have been due to the presence of some unrecognised impurity. The fact that this acid has also been obtained from carefully purified humulene supports the former view, and it will be interesting to note whether it is an oxidation product of sesquiterpenes in general. On this point, I propose to make further experiments.

The essential oil of hops, therefore, contains the following compounds: myrcene, humulene, linalool, linalyl *isononoate*, probable traces of some ester of geraniol, and a small amount of a diterpene.

In all the freshly distilled samples of oil examined by me, the hydrocarbons, myrcene and humulene, were present to the extent of from 80 to 90 per cent.

In conclusion, I desire to offer my sincere thanks to my assistants, Messrs. P. H. Carpenter and Theo. C. Thomas, for much valuable help in connection with this work.
