

VIII.—*Essential Oil of Hops.*

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THE essential oil of hops has been submitted to investigation at different times by several chemists who, according to their published statements, obtained from it terpenes boiling at 160° and 175° , and an oxidised constituent to which the formula $C_{10}H_{16}O$ (or $C_{10}H_{18}O$) has been assigned, but which does not appear to have been isolated in a state of purity.

Payen and Chevallier examined the oil in 1822, and separated it into two fractions by distillation. Wagner, in 1853, obtained a terpene boiling at 175° and an oxygenated compound which yielded valeric acid on oxidation. Personne, in an admirable paper entitled "*Histoire chimique et naturelle de lupulin*" (*J. Pharm.*, [3], **26**, 241, 329; **27**, 22), obtained a fraction, boiling between 150 — 160° , of a light yellow colour, and sp. gr. of 0.8887. It rotated a ray of polarised light 2.7° to the right in a tube 80 mm. long. When acted on by fused caustic potash, potassic valerate was formed and hydrogen evolved.

The essential oil of hops has also been examined by J. Ossipow (*J. Pr. Chem.*, [2], **28**, 448), who paid particular attention to the products obtained on oxidising the oil by means of chromic mixture. In his first paper, entitled "*Vorläufige Notiz über das Hopfenöl aus käuflichem Lupulin*," published in 1883, he refers to the action of bromine, and of chromic mixture on the oil, and in his second paper, published in 1886, he practically confines his attention to the fractionation of the volatile acids obtained on its oxidation. Of these acids, he was able to identify acetic acid and isovaleric acid. These various researches, however, throw but little light on the real nature of the constituents of this essential oil.

The important researches of Wallach and others on the terpenes and essential oils has enormously enlarged our knowledge of these substances during the last few years, and the investigation described in this paper was undertaken with the object of studying the essential oil of hops in the light of this extended knowledge. In a preliminary notice communicated to the Society (*Proc.*, 1893, 177) I published an account of a partial examination of one sample of oil, and briefly described a sesquiterpene obtained from it by fractional distillation. Since then I have submitted to examination three other samples of the oil, and have obtained results of a more definite character. Owing to unexpected difficulties in connection with two of the constituents, I have not as yet been able to complete the investigation,

but having obtained a large quantity of the oil, I hope to be able to examine these constituents more thoroughly.

In this research I have examined four samples of the essential oil, which will be referred to as Nos. 1, 2, 3, and 4 respectively.

Sample No. 1.—This sample I prepared myself by the steam distillation of about 80 kilos. of hops, some of which had been grown in Burgundy, some in Alsace, and the remainder in Kent and Sussex. The oil so obtained was twice distilled over water to free it from resin, and was dried over chloride of calcium and anhydrous sodium sulphate. About 140 c.c. of nearly colourless oil was finally obtained.

Sample No. 2.—This sample I obtained through the kindness of Messrs. Wright, Layman, and Umney, of Southwark. It had been distilled in May, 1893, from German hops of 1892 growth, by Messrs. Schimmel and Co., of Leipzig, and was guaranteed genuine by that firm. The oil was of a pale yellow colour and perfectly bright.

Sample No. 3.—This was the largest sample with which I worked. For it I am indebted to Messrs. Schimmel and Co., of Leipzig, who kindly distilled it for me from the best Bavarian hops of 1893 growth.

Sample No. 4.—This sample I prepared myself by the steam distillation of about 100 kilos. of English and foreign hops of 1894 growth, obtaining about 150 c.c. of the purified and dried oil.

Of the genuineness of all these samples, therefore, there can be no doubt whatever.

The above four samples of oil had the following relative densities and specific rotatory powers.

	$d\ 15^{\circ}/15^{\circ}$.	$d\ 20^{\circ}/20^{\circ}$.	$[\alpha]_D^{*}$
No. 1.....	0.8802	0.8776	+0.41°
No. 2.....	0.8662	0.8636	+0.58°
No. 3.....	0.8771	0.8739	+0.50°
No. 4.....	0.8743	0.8716	—

All of these samples were neutral to litmus, and free from sulphur. On cooling to -20° they became more viscous, but did not yield any crystalline substance. The oil dissolved in cold concentrated sulphuric acid, giving a blood-red solution, from which water precipitated a reddish oil, having a smell somewhat resembling that of valeric acid. Shaken up with a saturated solution of sodium hydrogen sulphite, the oil gave no crystalline compound, and there was no noticeable development of heat. On shaking with a strong solution of caustic potash, it experienced no appreciable diminution of volume, and it did not reduce an ammoniacal solution of silver nitrate.

* The determinations of circular rotation mentioned in this paper were made with a Laurent instrument, using sodium light.

Fractionation of the Oil.

The first sample of oil was distilled under atmospheric pressure. It commenced to boil at 170°, but the temperature rose rapidly to 230°, and the greater part of the oil distilled over between 230° and 270°. After several fractionations three fractions were obtained, the first boiling at 170—255°, the second (corresponding to about four-fifths of the whole oil) at 255—260°, and a third small fraction at 260—270°. The first and third fractions were very small. The second fraction, after purification by distillation over sodium, was found to consist of a sesquiterpene, which is described fully in this paper, and which has also been obtained in considerable quantity from all the other three samples.

The three samples (representing the great bulk of the oil with which I worked) were fractionally distilled under a pressure of 60 mm., a current of carbon dioxide being passed into the liquid to ensure regular ebullition; each sample was distilled separately, the fractions of corresponding boiling points obtained from the different samples being mixed after their identity had been ascertained.

After prolonged fractionation under these conditions, the following fractions were obtained.

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|--------------|--------------|
| (1) 89—91° | (2) 145—150° |
| (3) 163—168° | (4) 168—173° |

Nos. 1 and 4 were the main fractions, the remaining two being small. No. 4 fraction corresponded to nearly two-thirds of the oil used.

Fraction No. 1 (b. p. 89—91°).—This was a colourless, very mobile liquid, of characteristic smell. When boiled with a small piece of sodium, it was found to be practically unacted on, and, on analysis, was found to consist almost entirely of carbon and hydrogen. It was purified by repeated distillation over sodium under a pressure of 50 mm. until the metal remained bright to the end of the operation; thus purified, it boiled at 86—89° (50 mm.), and further fractionation failed to split it up into two distinct fractions.

The greater part of this liquid boiled between 166° and 171° under atmospheric pressure, but towards the end the boiling point suddenly rose to about 250°; on stopping the distillation, a small quantity of a yellowish, viscous oil remained in the flask. On continuing the distillation of this viscous oil, the thermometer rose to 320°, and a solid resin remained. A portion of this fraction, therefore, undergoes polymerisation, and oxidation change, when boiled under ordinary atmospheric pressure. On exposure to the air, it absorbs oxygen with great rapidity, becoming first very viscous and ultimately changing into a colourless, hard resin. I found $d_{20}^{20} = 0.799$.

Being extremely surprised at this low number, I made many density determinations of the corresponding fractions obtained from all three samples of oil, and found in all cases almost identical numbers, namely, 0.798, 0.797, and 0.799.

When examined in a tube 100 mm. long, at 15°, it produced a rotation of 0.56° to the left. It has, therefore, practically no action on polarised light.

On combustion, it gave the following numbers as the mean of two experiments which were in close agreement.

	Found.	Calculated for $C_{10}H_{17}$.
C.....	87.75	87.59
H.....	12.30	12.41

It will be seen that the carbon is lower (and the hydrogen, of course, proportionately higher) than would be required for a terpene or mixture of terpenes. The formula, $C_{10}H_{16}$, requires C = 88.23, H = 11.77. The differences are greater than can be accounted for by experimental error, more than ordinary care having been taken to ensure the accuracy of the hydrogen determinations.

A vapour density determination, by Hofmann's method, in toluene vapour (b. p. 111°), gave 5.0, $C_{10}H_{17}$ requiring 4.75.

On passing hydrogen chloride into a cooled solution of the liquid in ether, the gas was readily absorbed, and, when saturated, the solution was reddish-purple. The yellow oil left on evaporation of the ether, after having been kept for some days in a desiccator over caustic potash, was submitted to distillation under a pressure of 40 mm. Between 95° and 110° a colourless mobile oil distilled over containing 30.5 per cent. HCl. A dihydrochloride of the formula $C_{10}H_{16}, 2HCl$ requires 34.9 per cent. HCl. This result points either to a decomposition of the compound or (which is more probable in view of the other ascertained facts) to the liquid being a mixture of two distinct hydrochlorides. The quantity was too small to distil fractionally with any chance of success.

On adding bromine or a solution of bromine in chloroform, drop by drop, to a well-cooled solution of this fraction in ether or chloroform, the colour disappeared immediately at first, but when 3 to 4 atoms of bromine had been added for every molecule of the substance, it remained permanently coloured. Up to this point, there was practically no disengagement of hydrogen bromide, and, on allowing the solvent to evaporate, a dense yellow oil was left; this could not be obtained in a crystalline condition. When this oil was treated with aniline, action occurred at once, the mixture became very hot, and aniline hydrobromide separated. On adding hydrochloric acid and distilling with steam, no volatile hydrocarbon passed over, but a

resinous mass remained in the distilling flask. The removal of hydrogen bromide in this way is therefore accompanied by the polymerisation of the original hydrocarbons.

Many attempts were made by well-known methods to prepare a nitrosochloride, but, although nitrosyl chloride was readily absorbed, no crystalline compound could be obtained. Attempts to prepare a nitrosite and a nitrosate were equally unsuccessful.

In the next place, the action of oxidizing agents on this fraction was investigated. A cold aqueous solution of potassium permanganate, containing 40 grams of the salt per litre, was added little by little to 3 grams of the oil. At first the colour of the permanganate disappeared at once, then somewhat more slowly, and finally remained tolerably permanent when an amount of the solution equivalent to 21 grams of the salt had been added. In this solution, carbonic acid, acetic acid, and oxalic acid were easily recognised. There was also present a very small quantity of some volatile acid having an odour resembling that of valeric acid. The oxalic acid was identified by its calcium salt, which was analysed. Boiling with dilute nitric acid of various strengths was also tried, but in all the experiments the only solid oxidation product which could be recognised with certainty was oxalic acid, the acid crystallising fairly readily from the different liquids after concentration. No evidence of the formation of any terephthalic acid was obtained. The low specific gravity of this fraction and the impossibility of obtaining any crystalline compounds from it show that it is not any of the well-known terpenes. These terpenes have relative densities varying between 0.84 and 0.86 at 20°; this fraction is 0.799 at that temperature. It seems extremely probable, judging from the experimental results, that this fraction consists of two hydrocarbons, one having the formula $C_{10}H_{16}$ and the other $C_{10}H_{18}$. The latter is probably tetrahydrocymene (b. p. 167°), whilst the former may possibly be one of the "olefinic terpenes" described by Semmler (*Ber.*, 1891, **24**, 682). So little, however, is known at present of the latter that until more work has been done in connection with them, it will be impossible to arrive at more definite conclusions. One thing, at least, is certain, the great bulk, if not the whole of the lower boiling point fraction of the essential oil of hops consists of hydrocarbons other than the ordinary terpenes. The general statement made in this connection by previous observers is to the effect that the lower fraction consists of "a terpene" boiling at about 170°. I may add that these hydrocarbons did not undergo any polymeric changes when sealed up in an exhausted tube and allowed to stand for six months at ordinary temperatures. At the time when this experimental work was done, I thought that I was the first to recognise

the presence of a hydrocarbon of the formula $C_{10}H_{18}$ in an essential oil, but I have quite recently become acquainted with a paper by Andres and Andréeff (*Ber.*, 1892, **25**, 609) on Russian peppermint oil. In this paper, they state that the lower fractions of the oil consist of a mixture of two hydrocarbons, $C_{10}H_{16}$ and $C_{10}H_{18}$, and they consider the latter to be identical with the "dekanaphthylene" of Markownikoff and Ogloblin. They conclude by remarking, "Soweit uns bekannt, ist das der erste Fall, dass ein derartiger Kohlenwasserstoff in einem ätherischen Oele gefunden wird."

Fraction No. 2 (b. p. 145—150°).—Owing to the very small proportion in which this constituent occurred in all the samples of oil examined, I was unfortunately unable to obtain sufficient to permit of its satisfactory identification. The small fraction obtained was a colourless oil having a pleasant smell of oil of geranium with an after-smell of oil of rue. It did not solidify on being cooled to -10° . $d_{15}^{15} = 0.885$.

0.121 gave 0.348 CO_2 and 0.1275 H_2O . $C = 78.43$; $H = 11.71$.

$C_{10}H_{18}O$ requires $C = 77.92$; $H = 11.69$ per cent.

The compound unites readily with bromine, and in many of its properties appears to bear some resemblance to geraniol. I am now engaged in fractionating a large quantity of the essential oil and hope to be able ere long to complete the identification of this oxygenated constituent.

Fraction No. 3 (b. p. 163—168°) was a small fraction, having very little smell and containing 3.4 per cent. oxygen. It was found to consist of a mixture of the next higher fraction with a little of the fraction just described.

The Sesquiterpene.

Fraction No. 4 (b. p. 168—173°).—This fraction, which was by far the largest obtained, representing, in fact, nearly two-thirds of the whole of the oil used, was found to be identical with the fraction boiling between 256° and 261° (760 mm.), obtained from the first sample of oil, and of which a preliminary account has already been given to the Society. The substance was purified by repeated distillation over sodium under 60 mm. pressure, until the metal remained bright throughout the whole distillation. Thus purified, it boiled at 166 — 170° (60 mm.), and on combustion gave the following results.

I. 0.1065 gave 0.345 CO_2 and 0.1145 H_2O . $C = 88.26$; $H = 11.92$.

II. 0.1400 „ 0.454 „ „ 0.1480 „ $C = 88.42$; $H = 11.71$.

III. 0.1540 „ 0.498 „ „ 0.1610 „ $C = 88.20$; $H = 11.69$.

$C_{15}H_{24}$ requires $C = 88.23$; $H = 11.77$ per cent.

In determining its vapour density, it was found that Victor Meyer's method, using diphenylamine (b. p. 310°), did not give satisfactory results, since the vaporisation was very slow, and the substance underwent some decomposition. Two determinations, therefore, were made by Hofmann's method, using quinoline (b. p. 237°). The two results obtained were (1) 6.91 and (2) 7.1. The formula $C_{15}H_{24}$ requires 7.1. This fraction, therefore, consists of a sesquiterpene. The boiling point of this compound, corrected for the emergent mercurial column, is $263\text{--}266^{\circ}$. Its density determinations gave $d_{15^{\circ}/15^{\circ}} = 0.9001$, $d_{20^{\circ}/20^{\circ}} = 0.8977$.

The liquid when examined in a tube 100 mm. long, at 20° , produced a rotation of 0.5° to the left.

The sesquiterpene prepared from the first sample of essential oil (which was probably not quite so pure as this second and larger quantity) produced, under the same conditions, a rotation of 1.5° to the right. It is, therefore, evident that the sesquiterpene is optically inactive, the slight levorotation observed in this case being due to a trace of some active impurity.

The following are the results of determinations of the index of refraction for the red hydrogen line and the sodium line (C and D).^{*} The observations were made at 19° , and gave the values $\mu_D = 1.5021$, and $\mu_{H\alpha} = 1.4978$.

The specific refractive energy of the compound calculated by means of the $\frac{n^2 - 1}{(n^2 + 2)d}$ formula is, therefore, 0.3245, and the molecular refractive energy 66.2. Taking Brühl's numbers for the atomic refractions of carbon and hydrogen, the calculated number for $C_{15}H_{24} = 2$ is 65.7. This evidence of the presence of two pairs of "doubly linked" carbon atoms is borne out by the determinations of the amount of bromine with which one molecule of the sesquiterpene can directly combine, and by the preparation of a dihydrochloride described below.

Action of Bromine.—A solution of bromine in chloroform was run drop by drop into a solution of the sesquiterpene in five times its volume of the same solvent, kept cool by means of ice. The colour disappeared rapidly at first, and did not become permanent until 4 atoms of bromine had been added for every molecule of the compound present. At this stage, the evolution of hydrogen bromide, which had previously been slight, increased considerably. On allowing the chloroform to evaporate spontaneously, a viscous, yellow oil was left; it could not be induced to crystallise. Other experiments,

^{*} My best thanks are due to Mr. G. Finch, working in the Physical Laboratory of University College, London, for having been kind enough to make these determinations for me.

using both chloroform and carbon bisulphide as solvents, showed that 1 molecule of the compound was capable of uniting directly with 4 atoms of bromine.

Action of Hydrogen Chloride.—Hydrogen chloride was passed to saturation into a well-cooled solution of the sesquiterpene in four times its volume of ether. The gas was readily absorbed, and the solution finally became purple. After allowing it to remain for some hours, and then evaporating the ether, a yellow oil was left; this, after being washed with small quantities of cold alcohol (in which it is somewhat soluble), was allowed to remain for several days in a vacuum over caustic potash and sulphuric acid. The clear yellow oil thus obtained, on being cooled to -15° , became more viscous, but did not show any tendency to crystallise even after the lapse of some hours. Distillation under reduced pressure (60 mm.) was then tried; at first there was a very considerable disengagement of gas, and then a colourless liquid distilled over between 165° and 185° . After two more distillations under the same pressure, the liquid boiled at $168-175^{\circ}$. A chlorine determination gave 3.7 per cent. HCl, $C_{15}H_{24}, 2HCl$ requiring 26.3 per cent. HCl. It is evident, therefore, that even under a pressure of 60 mm. this hydrochloride is almost completely decomposed, so that it is impossible to purify it by this means. Another quantity of the yellow oil, prepared and partially purified as above described, gave on analysis numbers approximately agreeing with those required by the formula $C_{15}H_{24}, 2HCl$. The relative density of this impure hydrochloride was 1.063 at $15^{\circ}/15^{\circ}$.

On oxidation with cold aqueous solution of potassium permanganate, the sesquiterpene yielded considerable quantities of carbonic acid and acetic acid, together with a non-volatile acid, which could not be identified with certainty.

Action of Nitrosyl Chloride.—One volume of the sesquiterpene was dissolved in three volumes of chloroform, and the solution cooled in a freezing mixture to -15° ; on passing nitrosyl chloride into it slowly, care being taken not to allow the temperature to rise, the liquid became green, and after a time a white crystalline substance separated, the quantity of which was increased when the product was poured into cold alcohol. The crystalline precipitate was quickly collected on a filter, washed with cold alcohol, and dried in a vacuum over sulphuric acid. The nitrosochloride obtained in this way is a white, finely crystalline substance, fairly soluble in chloroform; it can be obtained from this solution in a more distinctly crystalline condition by the addition of alcohol. Compared with the nitrosochlorides of the terpenes, it is very stable, since it can be heated to 140° without the slightest change of colour. At $164-165^{\circ}$, it melts to a brownish-black liquid, which then undergoes considerable decomposition. It

was found to contain 12.90 per cent. chlorine instead of 13.16, the calculated amount.

When this nitrosochloride was heated with an excess of piperidine, almost to the boiling point of the latter, a vigorous action occurred resulting in the formation of a nitrolpiperide. Sufficient alcohol was added to completely dissolve the products formed, and then a little water; on standing, the nitrolpiperide crystallised out. It was purified by crystallisation from hot alcohol, from which it separates in the form of small, white, glistening plates. It is but slightly soluble in cold, and not very readily in hot alcohol. When purified by several recrystallisations, it melted at 153°. It was found to contain 8.72 per cent. nitrogen, instead of 8.80, the calculated amount.

It now became necessary to ascertain whether this sesquiterpene was identical with any of the well-known and properly characterised sesquiterpenes obtained from other sources. In the first place, its boiling point, relative density, and optical inactivity, as also its inability to furnish a crystalline dihydrochloride, show that it is not cubebene (cadinene). On obtaining a crystalline nitrosochloride, melting at 164—165°, I was struck by the fact that Wallach (*Annalen*, **271**, 295) had obtained from caryophyllene (the sesquiterpene which occurs in oil of cloves) a nitrosochloride melting at nearly the same temperature, and which appeared from his statements to resemble somewhat closely the one I had obtained. I thought, therefore, that the hop oil sesquiterpene might possibly be inactive caryophyllene. Wallach, in the paper above referred to, has shown that caryophyllene, when boiled in glacial acetic acid solution with dilute sulphuric acid, readily forms a well-crystallised, characteristic alcohol of the formula $C_{15}H_{25}\cdot OH$. Attempts to prepare a similar compound from the hop-oil sesquiterpene failed, nothing but dense, oily products being formed, showing not the slightest tendency to crystallise. The conditions prescribed by Wallach were closely followed, a parallel experiment with caryophyllene itself readily yielding the crystalline alcohol. Wallach, moreover, states that the nitrolpiperide obtained from caryophyllene nitrosochloride could not be obtained in the crystalline condition, whereas the corresponding compound from the hop-oil sesquiterpene crystallises readily. There can be no doubt, therefore, that this compound is not caryophyllene. Its relative density and the formation of a nitrosochloride prove that it is not identical with clovene, whilst it differs in several important respects from cedrene.

These are the only sesquiterpenes of which we possess any knowledge sufficiently definite to admit of their identification. The evidence I have given as to its chemical individuality justifies me, I think, in adding to this list the sesquiterpene of the essential oil of hops, for which I venture to propose the name *humulene*.

In three out of the four samples of oil with which I have worked, humulene was, as regards quantity, the main constituent. Since these samples have been prepared in different years from hops grown in several countries under various climatic conditions, the essential oil of hops must be regarded, I think, as one of those oils in which a sesquiterpene figures as an important constituent.

I hope, before long, to be able to render the examination of this essential oil more complete.
