REPORT ON THE PRESERVATIVE PRINCIPLES OF HOPS.

PART I.

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This investigation was instituted by the Hop Sub-Committee of the Research Fund Scheme of the Institute of Brewing, with the ultimate object of isolating and identifying the constituent, or constituents, of the hop, on which its preservative and antiseptic properties depend.

Numerous previous chemical investigations of hops have been carried out, and certain groups of substituents have been studied in detail. Thus, A. C. Chapman (Trans. Chem. Soc., 1903, 83, 505) has shown that the essential oil of hops consists to the extent of some 80—90 per cent. of the hydrocarbons myrcene and humulene, the remainder consisting of linalool, linalyl acetate, a small amount of a diterpene, and probable traces of some ester of geraniol. The same author (Proc. Chem. Soc., 1913, 29, 182; Trans. Chem. Soc., 1914, 105, 1895) subjected the soluble nitrogenous constituents of hops to a close investigation, and recorded the presence therein of histidine (and probably arginine), betaine, adenine, and hypoxanthine, besides confirming the presence of asparagine and choline, which had been identified previously as constituents of hops by Bungener and Fries (Zeitschr. ges. Brauw., 1885, 8, 267), and by Griess and Harrow (Ber., 1885, 18, 717), respectively. Neither the essential oil (compare A. C. Chapman, 'The Hop,' Brewing Trade Review, London, 1905, p. 67) nor the nitrogenous constituents, however, are likely to contribute largely to the antiseptic value of the hop, which is believed to be occasioned mainly by the so-called "soft resins," the portion soluble in light petroleum, for the soft resin content of a hop is regarded as some indication of its preservative value in practical experience.

Now the soft resins have been examined by many previous authors, who have isolated two characteristic feebly acidic compounds, termed respectively α-hop-bitter acid, or humulon, and β-hop-bitter acid, also known as lupulinic acid, lupulinic acid and lupulon, which have been shown to possess antiseptic properties (compare Hayduck, Wochenschr. f. Brauerei, 1888, 5, 937; H. Bungener, Bull. Soc. Chim., 1886, (2), 45, 487; A. J. Brown and D. Clubb, J. Inst. Brew., 1913, 19, 261).
is not our intention, on this occasion, to present a detailed discussion of the previous work on these compounds, but only to give a brief summary of their properties.

_Humulon_ (the a-acid) is characterised by the fact that it may be precipitated from alcoholic solution as the lead salt, and is stated to melt in the neighbourhood of 56° C. by Lintner and A. Bungener (Zeitschr. ges. Brauw., 1891, 14, 357), Barth (ibid., 1900, 23, 509), Bamberger and Landsiedl (ibid., 1902, 25, 461), and Siller (Zeitschr. Unters. Nahr. Genussmittel, 1909, 18, 241), whilst the probable formula, judged from the work of these authors, was C_{30}H_{50}O_{10}, or C_{25}H_{32}O_{3}. More recent careful work by Wollmer (Ber., 1916, 49, 780), however, has shown that the preparations of previous workers were impure, and that the melting point of the compound may be raised to 65—66.5° C. by suitable purification. Analysis of the purified acid led to the formula C_{21}H_{30}O_{6}, which received confirmation from the fact that the compound gave, on hydrolysis, humulin (humulinic acid), C_{15}H_{26}O_{4}, together with a hexenoic acid, C_{6}H_{10}O_{2}, iso-butyraldehyde and acetic acid.

\[
C_{21}H_{30}O_{6} + H_{2}O = C_{15}H_{26}O_{4} + C_{6}H_{10}O_{2}
\]
\[
C_{21}H_{30}O_{5} + 2H_{2}O = C_{15}H_{26}O_{4} + C_{6}H_{10}O_{2} + CH_{3}COOH.
\]

_Lupulon_ (the β-acid), which is not precipitated from alcoholic solution as lead salt, is stated to melt at 92—93° C. and to have the formula C_{25}H_{32}O_{4} or C_{26}H_{32}O_{4} (H. Bungener, Bull. Soc. Chim., 1886, 2, 45, 487; Barth and Lintner, Ber., 1898, 34, 2022; Barth, Zeitschr. ges. Brauw., 1900, 23, 509).

Both humulon and lupulon are insoluble in water, but soluble in all organic solvents, feebly acidic in character, unsaturated, and easily resinified.

Some doubt has been thrown on the individuality of these compounds by Power, Tutin and Rogerson (Trans. Chem. Soc., 1913, 103, 1267), who failed to isolate them in the course of an extended and systematic investigation of the constituents of hops, but the methods employed by these authors—extraction with hot alcohol and distillation of the extract with steam, followed by hydrolysis of the portion soluble in light petroleum with alcoholic potash—were unsuitable for the isolation of compounds which, according to previous authors, were readily resinified and decomposed. The compounds which Power and his collaborators isolated from hops were mainly substances common to other plant materials such as hentriacontane, ceryl alcohol, a phytosterol, and a number of saturated and unsaturated fatty acids, but two new phenolic compounds—humulol, C_{17}H_{16}O_{4}, and xanthohumol, C_{18}H_{14}O_{3}—were
isolated, the first in a yield of 0.022 per cent., and the second (apparently) in traces only. One very important result, however, was achieved by these authors in the isolation of a hexenoic acid from the products of saponification of the resins soluble in light petroleum and its identification as \( \beta \)-iso-propylacrylic acid. It seems to us probable that this acid is identical with Wöllmer's hexenoic acid, and, like this, arose from the hydrolysis of humulon, for Wöllmer obtained as by-products iso-butyraldehyde and acetic acid, which might well result from the further hydrolysis of \( \beta \)-iso-propylacrylic acid.

\[
\text{CH}_3\text{CH}:\text{CH}-\text{COOH} + \text{H}_2\text{O} = \text{CH}_3\text{CHO} + \text{CH}_2\text{COOH}.
\]

For the purpose of the present investigation, ground hops were extracted completely with light petroleum, and the acidic constituents were removed fractionally by systematic extraction with alkalis of increasing strength. Attempts were then made to crystallise the various fractions or to obtain crystalline salts or derivatives from them, but the results have been disappointing. Lupulon was isolated but only in small yield. No humulon was obtained by this method, although we have since been able to isolate it from the original light petroleum extract through the lead salt. The only other crystalline compounds isolated—all in small quantity—were the constituents of the wax, hentriacontane, ceryl alcohol and cerotic acid, previously shown to be present in hops by Power and his collaborators (loc. cit.) and lactaric acid, \( \text{C}_{18}\text{H}_{36}\text{O}_2 \), a saturated fatty acid previously found only in the fungus \( \text{Agaricus Integrus} \) (Thoerner, \textit{Ber.}, 1879, 12, 1636; Chuit, \textit{Bull. Soc. Chim.}, 1889 (3), 2, 155). This method of attacking the problem has consequently been abandoned and others are now under investigation.

\textit{Experimental.}

The hops employed in this investigation were Canterbury Goldings (1920) dried with brimstone without an open fire. They were ground in a ball mill and completely extracted in the autumn of 1920 with light petroleum (b.p. 65—75° C.; \( d = 0.895 \)), and gave 16.7 per cent. of extract in the form of a dark brown fat, having the consistency of butter.

432 grms. of this extract were dissolved in 1500 c.c. of ether and kept for some hours, when 10 grms. (2.31 per cent.) of an insoluble solid separated.

\textit{Examination of the Solid.}—Preliminary experiments indicated that this material was a mixture of a hydrocarbon with ceryl ceroate. It was consequently hydrolysed by means of alcoholic potash and...
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separated into a neutral and acidic portion. The latter was insufficient in quantity for complete characterisation, but after purification melted at 81.5° C., and was doubtless ceric acid. The neutral portion was separated (by treatment with phthalic anhydride, removal of the hydrocarbon from the acid phthalic ester, and hydrolysis of the latter) into ceryl alcohol, which crystallised from alcohol in glistening leaves, melting at 78° C. (found: C = 81.6; H = 14.0; calc.: C = 81.8; H = 14.1 per cent.), and hentriacontane, which melted at 67.5° C. (found: C = 85.0; H = 14.9; calc.: C = 85.3; H = 14.7 per cent.).

Fractional Extraction of the Etheral Solution.—The filtrate from the solid matter was extracted first with 2.5 per cent. aqueous hydrochloric acid, which removed only traces of material giving no reaction with Meyer’s reagent, whence no alkaloid was present. The etheral solution was then washed with water and extracted with aqueous ammonium carbonate. On acidifying the extract and extracting the solution with ether, 2.0 grms (0.46 per cent.) of a dark red fluid extract were obtained. On then extracting the ethereal solution with water, acidifying, and extracting with ether, a further 8 grms (1.84 per cent.) of very dark syrupy extract were obtained. The ethereal solution was next extracted with aqueous sodium carbonate, and this was acidified and extracted with ether, when 2 grms (0.46 per cent.) of a dark red fluid extract were obtained.

Isolation of Lactaric Acid.—After extracting with aqueous sodium carbonate, the ethereal solution was next thoroughly shaken with water until nothing further was removed. In this case a very dark coloured viscid extract was obtained, amounting to 47 grms. (10.88 per cent.). On keeping it for a short time, small colourless crystals were observed in the gum, and on the addition of a little light petroleum, both the gum and crystals dissolved, giving a homogeneous liquid; but on the further addition of light petroleum (about 800 c.c. in all) a dark syrup was precipitated, leaving a pale yellow, clear, supernatant liquid. This was decanted and concentrated to about 50 c.c., when, on cooling, a crystalline substance was deposited in the form of laminae, melting at 65—67° C. After several alternate crystallisations from alcohol and ethyl acetate, it melted at 69.5—70° C. On analysis it proved to have the formula C_{15}H_{30}O_{2}. (Found: C = 74.5, 74.2; H = 12.9, 12.9; C_{15}H_{30}O_{2} requires C = 74.4; H = 12.4 per cent.). On titration against N/50 alcoholic potassium hydroxide, using phenolphthalein as indicator, the equivalent found was 259, whereas C_{15}H_{30}O_{2} requires the equivalent 242. It was very easily soluble in ether, benzene, chloroform, or in hot alcohol, but sparingly soluble in cold alcohol and almost insoluble in
water or light petroleum. It was tasteless, optically inactive (in benzene solution), gave no colour with ferric chloride in alcoholic solution, and dissolved only slowly in aqueous alkaline carbonates. In its formula, melting point, and behaviour with solvents this acid agrees completely with lactaric acid, a compound first isolated, together with mannitol, from the fungus *Agaricus integer* by Thoerner (*loc. cit.*), and also studied by Chuit (*loc. cit.*). For further evidence of its identity with this acid, the acid amide was prepared by the action of thionyl chloride and subsequently of strong ammonia, and was found to melt at 108° C. and to be sparingly soluble in ether, thus agreeing completely with Chuit's description of the amide of lactaric acid.

**Examination of the Barium Hydroxide Extract.**—The ethereal solution was next extracted with saturated aqueous barium hydroxide and subsequently with water, when the united extracts after acidification and extraction with ether gave 27 grms. (6.25 per cent.) of dark red syrup, which showed no tendency to crystallise even on long keeping. It did not appear to contain humulon, for it gave no precipitate with lead acetate in methyl alcoholic solution. A lead salt was prepared, however, by double decomposition of the potassium salt with lead acetate in methyl alcoholic solution, but neither this nor the acid regenerated from it could be obtained in crystalline form. Attempts to obtain crystalline substances from it by means of the copper salt and by esterification (to remove fatty acids) followed by extraction with alkalis, were also unsuccessful.

**Examination of the 5 per cent. Aqueous Potassium Hydroxide Extract**

**Isolation of Lupulon.**—After extraction with baryta and water, after baryta, the original ethereal solution was perfectly clear, and appeared to contain no solid in suspension. Special mention is made of this, because all subsequent treatment with alkalis resulted in the isolation of small amounts of solid substances containing barium. The ethereal solution was next extracted repeatedly with a solution of 5 per cent. aqueous potassium hydroxide. The first six extractions gave three layers: (a) aqueous; (b) viscous mass; (c) top ethereal layer. The succeeding extractions resulted in the usual two layers. Fraction (b) was a dark greenish brown plastic mass (4.5 grms.), and contained a quantity of barium; no crystalline organic compounds were obtained from it. Fraction (a), on acidification and extraction with ether, gave a dark red syrup, amounting to 47 grms. (10.88 per cent.). No crystals were observed in it after keeping (out of contact with air) for ten weeks, but, after a further period of eight weeks, it was found to contain a small quantity of large crystals. These were separated
from the viscous mother liquor by the addition of a mixture of ethyl alcohol and light petroleum, in the proportions of 1 to 3 by volume, and filtration, when 0·75 grm. of the crude crystalline material was obtained. It was purified by dissolving it in light petroleum at 60° C. (higher temperatures caused resinification) and quickly cooling the solution, and finally by crystallisation from 90 per cent. aqueous methyl alcohol, when it formed colourless prisms which melted at 94·5—95·5° C. (corr.) ; found: C = 75·4; H = 9·0; and on another specimen which had been crystallised from light petroleum, but not from methyl alcohol, and melted at 94—95° C., C = 75·6; H = 9·0. C_{25}H_{30}O_{4} requires C = 75·0; H = 9·0 per cent. The substance is thus identical with the lupulon of H. Bungener (loc. cit.) and Barth (loc. cit.), who give as the melting point 92—93° C. and 92—93·5° C. respectively. It also agrees with the lupulon of these authors in its solubilities, in resinsifying easily, in yielding a copper salt soluble in ether, and in reducing ammoniacal silver nitrate only in the presence of a small quantity of sodium hydroxide. The mother liquor from the lupulon did not yield a precipitate with methyl alcoholic lead acetate, and thus did not appear to contain humulon. Attempts to obtain crystalline substances from it through the lead salt, through the copper salt, after removal of fatty acids as esters, and by benzoylation, all failed.

Examination of subsequent Extracts.—After extraction with 5 per cent. aqueous potassium hydroxide, the ethereal solution was extracted with the following solvents, which were then acidified and extracted with ether: (1) with water, which gave 15 grms. (3·46 per cent.) of reddish brown syrup; (2) with 20 per cent. aqueous potassium hydroxide, which removed 3·5 grms. (0·81 per cent.) of dark syrup; (3) with water, which removed 114·5 grms. of dark green extract; (4) with 40 per cent. aqueous potassium hydroxide, which gave a precipitate of 2·5 grms. (0·68 per cent.) of the potassium salt of a higher fatty acid, which was not examined further; (5) with water, which removed 13 grms. (3·01 per cent.) of a dark red viscid syrup. None of these extracts gave crystalline derivatives, and all—as well as those described above—were more or less bitter in taste.

The final ethereal solution—after drying with calcium chloride and removing the solvent—left 108 grms. (25·0 per cent.) of a thick brown syrup, which was not bitter in taste.

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