381. *The Chemistry of Hop Constituents. Part I. Humulinone, a New Constituent of Hops.*

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Extraction of the cones of *Humulus lupulus* (the common English hop) led to the isolation of *humulinone*, a new constituent of the petroleum-soluble hop resins.

In general, extraction of the cones or lupulin glands of a number of varieties of hops with organic solvents has in the past yielded two well-known crystalline compounds, humulone, $C_{21}H_{30}O_5$, and lupulone, $C_{26}H_{38}O_4$, to which structures were assigned by Wieland *et al.* after preliminary work on their constitution by Wöllmer (*Ber.*, 1916, **49**, 780; 1925, **58**, 672; cf. Barth and Lintner, *Ber.*, 1898, **31**, 2022; Bungener, *Bull. Soc. chim.*, 1886, **45**, 487; Hayduck, *Wochenschr. Brau.*, 1888, 937; Lintner and Schnell, *Z. ges. Brauwesen*, 1904, **27**, 666; Wieland, Schneider, Martz, and Hoek, *Ber.*, 1925, **58**, 102, 1012; 1926, **59**, 2352). During the working up of methanolic extracts of the cones of the Worcester Fuggle or Kent Golding varieties of hops, incidental to a wider examination of hop constituents, a new compound was obtained. This compound, termed *humulinone*, was isolated as the crystalline *sodium* salt, $C_{21}H_{29}O_6Na$, by treatment of the petroleum-soluble material with sodium hydrogen carbonate and was present to the extent of 1·0—2·0% by weight of the cones. Under the conditions used and in the presence or absence of air, both humulone and lupulone were shown to be stable, as was *iso*humulone which has been regarded as an isomer of humulone and is obtained from humulone by short treatment with hot alkali (Windisch, Kolbach, and Schleicher, *Wochenschr. Brau.*, 1927, **44**, 453, 473, 485, 497; Verzele and Govaert, *Congr. Intern. Inds. Fermentation, Confs. et Communs.*, 1947, 297). Little doubt remained, therefore, that humulinone was present in the hop cones as such and was not formed from other compounds.

The new compound was isolated directly from a light-petroleum solution of the hop constituents by treatment with aqueous sodium hydrogen carbonate or, more economically, together with humulone as a mixture of lead salts from 90—95% methanol. In this way, lupulone was obtained from the filtrate resulting on removal of the lead salt, by direct crystallisation of the residue from light petroleum. Humulinone was obtained from the lead salts by regeneration with sulphuric acid and treatment with sodium hydrogen carbonate. Acidification of the sodium salt gave free humulinone, $C_{21}H_{30}O_6$, a white crystalline substance which did not crystallise from solvents. This and its sodium salt appeared from their ultra-violet absorption spectra to possess tautomeric structures; the significant data together with the absorption characteristics of humulone, lupulone, and *iso*humulone (all in ethanol) are given in Table I.

TABLE I.

	$\lambda_{max.}$, A.	$E^{1\%}_{1\,cm.}$		$\lambda_{max.}$, A.	$E^{1\%}_{1\,cm.}$
Humulinone	2290	330	Lupulone	2280	340
	2680	250		2800	150
	2800	300		3380	250
Sodium salt of humulinone	2500	470	*iso*Humulone	2230 2280	350
	2590	500			
	2670	470		2680	280
Humulone	2370	380		2800	250
	2820	230			
	3300	220			
	3400 *	180			

* Inflexion.

The absorption curves of humulinone and *iso*humulone were almost superposable, and both substances gave a purple colour with alkaline sodium nitroprusside. Humulinone resembled humulone and *iso*humulone on potentiometric titration with alkali, behaving as a monobasic acid, and back-titration with acid indicated that it had not suffered any change. It was a strong acid of pK_a 2·7, as compared with pK_a values of 5·5 and 3·4 for humulone and *iso*humulone respectively. At $2{\cdot}1 \times 10^{-3}$ mm. pressure, humulinone distilled with decomposition at 125°. It contained two double bonds (iodine value), five active hydrogen atoms (Zerewitinoff), and one *gem*-dimethyl group (Kuhn–Roth oxidation).

Hydrolysis of humulinone with hot aqueous sodium hydroxide yielded acetone and *iso*hex-3-enoic acid,* together with a mixture of unidentified acidic products. It thus seemed that this acid might be that isolated on alkaline hydrolysis of humulone, and not the 2-enoic acid as generally supposed. This proved to be the case. The other products isolated from humulone in this reaction were *iso*butyraldehyde, humulinic acid (cf. Wöllmer and Wieland *et al., locc. cit.*), and acetone, of which the last had not previously been obtained. The structure of humulinone, as of humulone and lupulone, is being investigated.

TABLE II.

Activities (Dilutions, w/v).

	M. phlei.	*B. coli.*	*Staph. aureus.*	*Lactobacillus β.*†	*Lactobacillus plantarum.*
Lupulone	1 : 100,000	Inactive	1 : 400,000	Inactive	1 : 40,000
Humulone	1 : 10,000	,,	1 : 50,000	1 : 20,000	1 : 100,000
*iso*Humulone	1 : 10,000	,,	Inactive	1 : 20,000	1 : 200,000
Humulinone	1 : 10,000	,,	,,	1 : 10,000	1 : 20,000

† The strains of lactobacilli, kindly supplied by Dr. T. K. Walker, are common sources of infection in beers.

In view of the recent interest in lupulone as a tuberculostatic agent *in vivo* in mice (Yin-Ch'ang Chin, Anderson, Alderton, and Lewis, *Proc. Soc. Exp. Med.*, 1949, **70**, 158; Chin and Anderson, *Fed. Proc.*, 1949, **8**, 281), humulinone was compared with lupulone, humulone, and *iso*humulone as inhibitors of *Mycobacterium phlei* and other organisms (Table II) (cf. Blakebrough, M.Sc. Thesis, Manchester, 1948). It may be seen that humulinone was not comparable with lupulone in activity against *M. phlei.*

EXPERIMENTAL.

Isolation of Humulinone.—(*a*) The cones of Worcester Fuggle hops (500 g.) were kept in methanol (5 l.) under nitrogen for 20 hours. Filtration and evaporation of the methanolic extract in a vacuum gave a green oil (95 g.), which was extracted with warm light petroleum (b. p. 40—60°; 700, 250 c.c.). Evaporation of the light petroleum extracts gave an oil (*A*) (28 g.). A part (15 g.) was dissolved in methanol (220 c.c.) and water (11 c.c.), and the total lead salt precipitated with 90% methanolic lead

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* Geneva nomenclature.

acetate using aqueous sodium sulphide as external indicator. After 20 hours the salt (8·75 g.) was collected, finely ground, and suspended in methanol (85 c.c.), and 30% sulphuric acid (12 c.c.) was added to the ice-cold suspension. The mixture was well shaken, lead sulphate removed, and the filtrate diluted with water (250 c.c.) and light petroleum (90 c.c.). After re-extraction of the aqueous layer, the light petroleum extracts were washed and dried. Evaporation gave an oil (5·55 g.), which was set aside with saturated aqueous sodium hydrogen carbonate (30 c.c.) and ether (30 c.c.) for 2—3 days. The *sodium* salt of humulinone separated, and concentration of the ethereal filtrate and addition of light petroleum (25 c.c.) gave a second crop (total yield, 1·15 g.). Recrystallised from methanol–ether, it separated as colourless wedge-shaped prisms, m. p. 227° (decomp.) (Found : C, 63·4, 62·5, 62·7; H, 7·5, 7·2, 7·5; Na, 5·8, 5·75, 6·05; OMe, 0. $C_{21}H_{29}O_6Na$ requires C, 63·0; H, 7·3; Na, 5·75; OMe, 0%). With ethanolic ferric chloride, it gave an orange colour.

(*b*) The oil (*A*) (0·5 g.) was set aside with ether (10 c.c.) and saturated aqueous sodium hydrogen carbonate (10 c.c.) for 7 days. The ether was separated, rapidly dried, and allowed to evaporate after the addition of a little light petroleum. Yield : 0·114 g. of material, m. p. 224° (decomp.).

(*c*) The cones of Kent Golding hops (250 g.) were kept as above with methanol (3 l.) for 25 hours. Evaporation, extraction of the residue with warm light petroleum (150, 100, 50 c.c.), and evaporation of the petroleum solution gave an oil (26·5 g.). This oil was treated with ether (250 c.c.) and saturated aqueous sodium hydrogen carbonate (250 c.c.). After a few days, the product (0·95 g.) was filtered off. Drying and addition of light petroleum (200 c.c.) to the ethereal filtrate brought the total yield to 3·25 g. (1·7% of the wt. of cones)

Humulinone.—The sodium salt (0·109 g.) was shaken with ether (20 c.c.) and 2N-hydrochloric acid until the solid had completely disappeared. Washing, drying, and evaporation of the ether gave *humulinone* as a white solid (0·103 g.), m. p. 74° (Found : C, 66·8, 66·8; H, 8·0, 8·1; active H, 1·33, 1·43; Kuhn–Roth oxidation gave 1·05, 1·09 mol. of acetic acid; I value, 73·6, 76·8%; equiv., 367. $C_{21}H_{30}O_6$ requires C, 66·65; H, 8·0; active H for 5H, 1·33; I value for 2 double bonds, 67·1%; equiv., 378·5). With bromine in carbon tetrachloride, hydrogen bromide was evolved. Humulinone was soluble in all organic solvents, but insoluble in water.

Stability of Humulone to Sodium Hydrogen Carbonate.—Humulone (0·85 g.) was kept in ether (10 c.c.) and saturated aqueous sodium hydrogen carbonate (15 c.c.) for 6 days under nitrogen. A similar experiment was conducted with the omission of nitrogen. In each case, separation, washing, and drying the ethereal layer gave a yellow, hygroscopic solid, insoluble in light petroleum, which presumably was the sodium salt of humulone since treatment with methanolic lead acetate gave the lead salt (1·2 g.). Decomposition of the salt as above gave humulone, m. p. 60—65° (0·6 g.), identified as the *o*-phenylenediamine complex (0·65 g.), m. p. 111°, undepressed on admixture with authentic material (m. p. 117°).

Similar treatment of lupulone or *iso*humulone gave unchanged starting materials; the former had m. p. 93°, after crystallisation from light petroleum (b. p. 40—60°); *iso*humulone had b. p. 100°/2 × 10^{-4} mm. (Found : C, 69·4; H, 8·6. Calc. for $C_{21}H_{30}O_5$: C, 69·6; H, 8·3%). With ethanolic ferric chloride, *iso*humulone gave a carmine-red colour and with alkaline sodium nitroprusside a strong red colour.

Hydrolysis of Humulinone.—The sodium salt of humulinone (0·400 g.) was added to 0·1N-sodium hydroxide (50 c.c.), which had been refluxed for 1·5 hours in a current of nitrogen. The solution was distilled slowly in a current of nitrogen, the volume being made up as necessary with boiled-out water. The distillate was collected under 6N-hydrochloric acid containing 2 : 4-dinitrophenylhydrazine (*A*). After 40 minutes the solution was acidified with 2N-sulphuric acid, and the acid solution distilled (40 c.c.). The distillate was extracted with chloroform (4 × 20 c.c.) (*B*). Solution *A* was extracted with benzene (4 × 20 c.c.), and the washed, dried benzene extract evaporated. The residue in benzene (3 c.c.) was placed on a column of alumina (Spence, Type H, 21·5 × 1·2 cm.) and the broad yellow band eluted with benzene. Evaporation of the eluate yielded orange needles (98 mg.), m. p. 126°, undepressed on admixture with authentic acetone 2 : 4-dinitrophenylhydrazone, after recrystallisation from methanol (5 c.c.) (Found : N, 23·1. Calc. for $C_9H_{10}O_4N_4$: N, 23·5%). Solution *B* was evaporated to give an oil (121 mg.) and neutralised with 0·59 c.c. of 0·987N-potassium hydroxide in water (0·5 c.c.). After addition of 2N-hydrochloric acid (0·05 c.c.) and *p*-bromophenacyl bromide (120 mg.) in ethanol (2·5 c.c.), the mixture was heated under reflux for 1 hour. On cooling the crystalline product had m. p. 97—98°, and, recrystallised from aqueous ethanol, p-*bromophenacyl* iso*hex*-3-*enoate* separated as rectangular plates, m. p. 97—98° (Found : C, 53·9, 54·1; H, 4·9, 4·9; Br, 25·65, 25·2. $C_{14}H_{15}O_3Br$ requires C, 54·0; H, 4·9; Br, 25·7%).

iso*Hex*-2-*enoic Acid*.—This was prepared by the method of Goldberg and Linstead (*J.*, 1928, 2353) and had b. p. 116—117°/19 mm. The *p*-bromophenacyl ester was prepared as above and separated as colourless prisms, m. p. 74—75°, from benzene–light petroleum (lit., m. p. 71—72°) (Found : C, 54·0; H, 4·9; Br, 25·5%). The m. p. was depressed on admixture with the derivative above.

iso*Hex*-3-*enoic Acid*.—Prepared by the method of Goldberg and Linstead (*loc. cit.*), this had b. p. 107—108°/13·5 mm. The *p*-bromophenacyl ester, prepared as above and recrystallised from aqueous ethanol, had m. p. 97—98°, undepressed on admixture with the above derivative (Found : C, 54·0; H, 4·9; Br, 25·6%).

Hydrolysis of Humulone.—Humulone (1·0 g.) was treated by the above method with 0·1N-sodium hydroxide (100 c.c.). The acid distillate gave an oily acid (158 mg.), which gave *p*-bromophenacyl *iso*hex-3-enoate, undepressed by the authentic material above. The distillate from the alkaline solution gave *iso*butyraldehyde 2 : 4-dinitrophenylhydrazone (370 mg.). The filtrate from the latter was extracted with benzene as above and chromatographed on alumina, giving acetone 2 : 4-dinitrophenylhydrazone (128 mg.), m. p. 125—126°, undepressed on admixture with authentic material. The residue from the distillation of the *iso*hexenoic acid was extracted with chloroform and identified as humulinic acid, m. p. 93—94°. Light absorption (ethanol) : Max. at 2600, 2680, and 2800 A. with $E^{1\%}_{1\,\text{cm.}}$ = 530, 530, and 460 respectively.

Biological Testing.—The usual " Lab-Lemco "-peptone medium was used for culturing *Staph. aureus*, *M. phlei*, and *B. coli*, and for the lactobacilli standard maltwort of s.g. 1·040 was used (*cf.* Blakebrough, M.Sc. Thesis, Manchester, 1948). For tests against all organisms, the compounds were dissolved in ethanol (5—10 mg./c.c.) and the required volumes of the solutions added to standard amounts of the media in the usual " series dilution " tests. The *Staphylococcus* and *B. coli* strains were incubated for 18 hours at 36—37°, and *M. phlei* for 48 hours at the same temperature. Incubation of the lactobacilli was effected during 18 hours at 37°, and then at room temperature (25°) for 48 hours.

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[*Received, March* 30*th*, 1950.]