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# ON THE STRUCTURE OF HUMULINONE

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**Abstract**—Two structures have been proposed for humulinone (II and III in Fig. 1). The high resolution NMR spectrum of this and related substances points to the five-membered ring structure II.

HUMULONE is the most important hops constituent. It has structure I. Humulinone is an oxidation product of humulone and was assigned structure II by two of us[1]; the oxidation being followed by an isomerization.

Howard and Slater[2] preferred a non-isomerized six-membered ring structure (III) for humulinone, and claimed to have proved this by preparing the tribenzoate of desoxytetrahydrohumulone† from tetrahydrohumulinone in 3 per cent yield. Although we could obtain the tribenzoate in 25 per cent yield starting from tetrahydrohumulone, all our attempts to obtain the tribenzoate from tetrahydrohumulinone failed. The oxidation of tetrahydrohumulone or of humulone to the corresponding tetrahydrohumulinone or humulinone respectively occurs in ether and saturated sodium bicarbonate solution. The sodium salt of the humulinone compounds being insoluble, these substances precipitate readily when formed.

We have found that humulone, when its concentration is high, can also precipitate as the solid sodium salt. A possible explanation of the result of Howard and Slater can be found in the contamination of their tetrahydrohumulinone by tetrahydrohumulone. The question of the humulinone structure (five or six-membered ring) was therefore still open.

High resolution NMR spectroscopy was used to study the problem. From this it became apparent that the hydroxyl hydrogens were of little use for the purpose. Rapid proton exchange results in excessive broadening of the bands, the positions of which were moreover dependent on concentration. Other portions of the spectra of humulone and humulinone were practically identical. Significant changes occurred only in those resonances that could be assigned to the $-CH_2$-groups of the sidechains. In order to be certain, about the assignments of given resonances to definite $CH_2$ groups, dihydrohumulone (IV) was studied. This derivative was obtained by partial reduction of humulone.[3] The spectra were recorded in various solvents and in varying concentrations. The best results were obtained in deuterated chloroform. A typical specimen of the relevant spectra is shown in Fig. 1.

A trace of tetramethylsilane was added as internal standard.[4] The distance from

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† The tetrahydroderivatives have saturated instead of unsaturated sidechains. The desoxy compound is a true phloroglucinol derivative, the tertiary OH-group of humulone being replaced by hydrogen.

[1] F. Alderweireldt and M. Verzele, *Bull. Soc. Chim. Belg.* **66**, 391 (1957).
[2] G. A. Howard and C. A. Slater, *J. Chem. Soc.* 1460 (1958).
[3] M. Verzele and M. Anteunis, *Bull. Soc. Chim. Belg.* **68**, 315 (1959).
[4] G. Van Dyke Tiers, *J. Phys. Chem.* **62**, 1151 (1958).

the tetramethylsilane peak to the chloroform peak (still present in trace quantities) corresponds to 438 cps at 60 mc. The resolution obtainable for acetaldehyde on the NMR spectrometer at the moment of recording of the spectra of Fig. 1 was of the order of 0·4 cps for the width of the quadruplet bands measured at hal height. That the complex high molecular weight molecules do not give the same resolution under those conditions is a normal phenomenon.[5]

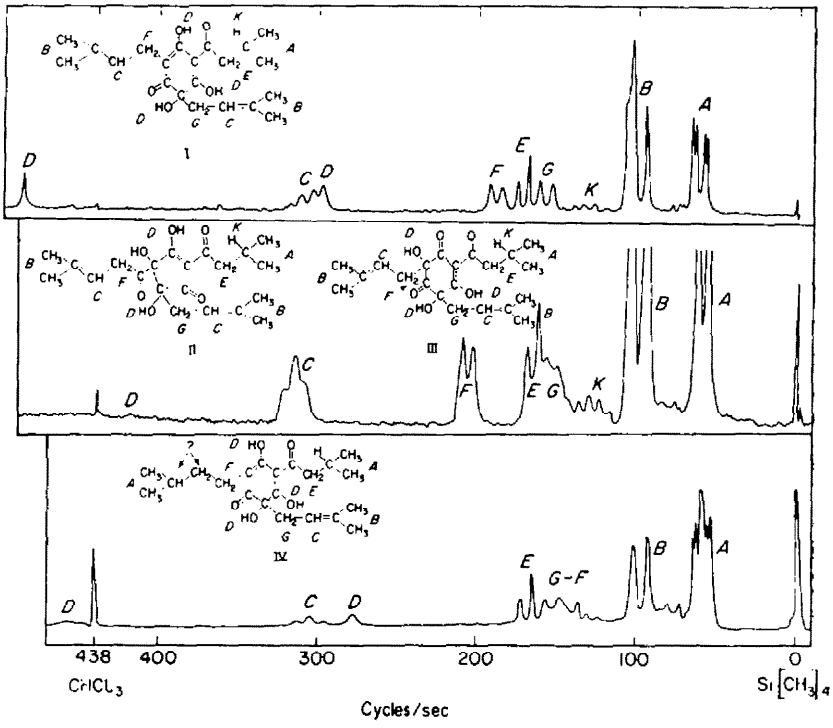


FIG. 1. Photographic reproduction on the same scale of the NMR spectra of humulone (upper), humulinone (middle) and dihydrohumulone (lower). The lettered resonances on the spectra derive from the identically labeled hydrogen atoms in the structural formulae.

As the protons are more depleted of their electrons, or as they are surrounded by more magnetically unshielding groups, their resonances fall at lower field values. This consideration together with a study of the relative surface area of the resonances corresponding with the number of hydrogen atoms that give rise to each particular band, permit assignment of the bands with reasonable certainty.

At about 60 cps from the tetramethylsilane peaks we find the resonances of the methyl groups labelled A; at about 100 cps the methyl groups labelled B; around 310 cps lies a triplet from the hydrogen atoms labelled C. Hydroxyl proton resonances are found at 294 and 480 cps in the humulone spectrum. An additional hydroxyl peak, found at very low field values (1150 cps) is not shown on the graph. The peak at 480 cps for humulone is not always sharp and is sometimes found as a low broad band stretching from 400 to 480 cps. The position of the peak at 294 cps in Fig. 1 is dependent on concentration. In the humulinone spectrum the broad band under the

[5] Varian Assoc., *Tech. Inf. Bull.* **2**, 1-n° 3 (1959).

chloroform peak is attributed to hydroxyl proton resonances. In the dihydrohumulone spectrum the OH peaks are found at 278 and 452 cps. The six resonances in humulone ranging from 152 to 190 cps must be ascribed to the $CH_2$ groups labelled E, F and G. Each group gives a doublet because of spin interaction with the one alpha hydrogen in each case. The central doublet at 166 and 173 cps has a characteristic shape which is found practically unchanged in humulinone (161–168 cps) and in dihydrohumulone (165–172 cps) and can therefore be attributed to the $CH_2$ group labelled E since it is the only one with unchanging environment in the three compounds. This is confirmed by studies in progress on other humulone derived substances. The humulone doublet at 183 and 190 cps has disappeared in dihydrohumulone and must belong to the $CH_2$ group labelled F. The remaining doublet at 152 and 159 cps is then caused by the $CH_2$ group G.

For a substance with structure III we would expect from the above considerations to obtain a NMR spectrum with a doublet of double intensity around 152–159 cps ($CH_2$ groups F and G in III), next to the unchanged doublet at about 166–173 cps. ($CH_2$ group E), but without a doublet at still lower field intensities. As seen in Fig. 1 this is not the case. A doublet about 50 cps lower than expected is found at values 203–208 in humulinone. This is in agreement with what we would expect for humulinone with structure II. The methylene group F attached to two double bonds in humulone has now become attached to one doubly bonded carbon and a carbonyl group. According to reference[5] the effective shielding constants of a doubly bonded carbon and a carbonyl group are 79 cps and 102 cps at 60 mc respectively (difference 23 cps). Counting on additivity of the shifts, which is very often the case[5] we would expect a downward resonance shift of about 23 cps going from the $CH_2$ group F in humulone to the same group in humulinone with structure II. This is in excellent agreement with the doublet found at 203–208 cps in humulinone (originally at 183–190 cps in humulone).

Taking into consideration the close reproducibility of the chemical shifts for all other $CH_2$ resonances in the three different compounds we think that the observed phenomena are significant and that NMR spectroscopy points quite definitely to a five-ring humulinone structure.

## EXPERIMENTAL

### 1. *Substances*

*Humulone* is obtained from hops extracts by precipitating the hop alpha acids as lead salts. Humulone is then separated from the other alpha acids (pre-, ad-, co- and posthumulone) by repeated crystallization of the *o*-phenylenediamine complex. The purity of the humulone was ascertained by partition chromatography, m.p. 70–71°.

*Humulinone* is obtained by oxidation (ether–sodium bicarbonate) of pure humulone[1], m.p. 72°.

*Dihydrohumulone.* Humulone was hydrogenated with 5% $PtO_2$ as catalyst in methanol brought to pH 5·0 with potassium-methylate. One mole of hydrogen is allowed to react. The product (2·5 g) was distributed (300 transfers) in a two-phase system of iso-octane as upper and a mixture with pH 7·6 of 15% triethanolamine; 0·6 N HCl; 25% ethyleneglycol as lower phase. Cells 140 to 170 afforded 510 mg dihydrohumulone. This was further purified by crystallization of the *o*-phenylenediamine complex until the m.p. of this reached 85°. The dihydrohumulone then obtained is a pale yellow oil and gives a single peak on re-distribution and in partition chromatographic purity assays.

### 2. *NMR Spectra*

All spectra were obtained from dilute solutions in deuterated chloroform. Each sample was diluted until broadening effects due to viscosity and intermolecular association were no longer observed.

The instrument used for the measurements was a Varian Associates Model V-4300 C High Resolution NMR spectrometer with a V-4012-SM 12″ Electromagnet and VK-3506 Flux Stabilizer. Samples were placed in 5 mm thin-walled (0·37 mm) glass tubes and rotated at several hundred rpm by a small air turbine during the recording of the spectra. Calibration of peaks relative to the internal references $(CH_3)_4Si$ was accomplished by the standard audio-frequency sideband method, using a Hewlett–Packard 200-CD oscillator monitored continuously with a Hewlett–Packard 521 C Electronic Frequency counter.

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