

group determination by a modified Kuhn-Roth method on the acid as received gave 3.33 equiv. of acetic acid, indicating the presence of four methyl groups<sup>9</sup>. The acid (887 mg) was converted into methyl ester by means of diazomethane, and the ester purified by chromatography on neutralized and slightly deactivated aluminium oxide. The ester fractions with the same refractive index ( $n_D^{25}$  1.4520) were combined (589 mg), and distilled at 0.2 mm pressure: b. p. (air-bath temp.) 220—225°. The distilled methyl ester had m. p. 20.8—21.4°,  $[\alpha]_D^{21.5}$  —7.8° (chloroform; c, 7.7);  $n_D^{21.5}$  1.4535;  $d_4^{21.5}$  0.8550.

The infra-red spectrum in the sodium chloride region was identical with that of methyl mycoceranate recently isolated by one of us (J.A.) from the *Test* strain of *M. tuberculosis* and with that of a specimen of methyl mycoceranate obtained from Dr. N. Polgar, Dyson Perrins Laboratory, Oxford, in 1953.

The purified methyl mycocerosate gave an excellent mass spectrum with a parent peak at  $m/e = 494$  corresponding to the empirical formula  $C_{33}H_{66}O_3$ . There was no indication of the presence of homologous impurity. The empirical formula of the acid is thus  $C_{32}H_{64}O_2$ . In order to determine the nature and positions of the side chains the relevant parts of the mass spectrum were examined (Fig. 1 f). The low peak at  $m/e = 74$  and strong peak at  $m/e = 88$  show the presence of a methyl group in position 2. The low peak at  $m/e = 115$  and the strong peaks at  $m/e = 101$  and 129 indicate a further methyl side-chain in position 4 (cf. Fig. 1 e). Finally, the peak at  $m/e = 157$  is very weak compared with those at  $m/e = 143$  and 171, indicating the presence of a third methyl group in position 6. The methyl group positions derived for mycocerosic acid are thus the same as found for mycoceranic acid by Polgar<sup>9</sup>.

A direct comparison with methyl mycoceranate from the *Test* strain was unfortunately not possible as the original sample had been converted into alcohol ("mycoceranol", m. p. 35.3—36.0°,  $[\alpha]_D^{17}$  + 3.5° (chloroform)) before we had found that the methyl esters were especially suited to structure analysis. A mass-spectrometric study of the alcohol indicates the empirical formula  $C_{32}H_{66}O$ , and a comparison with the spectra of synthetic alcohols, including 2,4-dimethyleicosanol-1, shows that mycoceranol has methyl side-chains in positions 2, 4, and 6.

The results reported above make us believe that, apart from possible stereochem-

ical differences (which cannot be studied by mass-spectrometric methods), mycocerosic, mycoceranic, and  $C_{31}$ -mycosanoic acids have the same structure, viz. that of 2,4,6-trimethylnonacosanoic acid.

We are indebted to Professor R. J. Anderson, Yale University, for the specimen of mycocerosic acid, and to Mrs. Stina Stållberg-Stenhagen for synthetic branched-chain acids. We also wish to thank Professor Einar Hammarsten for making possible the construction of a mass-spectrometer suitable for the study of structure problems in organic chemistry. Details of the instrument and details of the methyl ester and alcohol spectra will be given in forthcoming papers. The expenses of this work have been defrayed in part by grants from the Swedish Medical Research Council.

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## Gradient Elution Analysis of Hop Bitter Substances

L. O. SPETSIG and MAJ STENINGER

*Forskningslaboratoriet LKB, Äppelvikens, Sweden*

Studies in this laboratory<sup>1</sup> have shown recently that the humulones may be separated by reversed-phase partition chromatography with carbon tetrachloride as the stationary phase and 61 % (w/w) aqueous methanol as eluant. In the same manner the lupulones were resolved using 69 % (w/w) methanol as the moving phase.

Subsequent work has shown that both humulones and lupulones can be separated

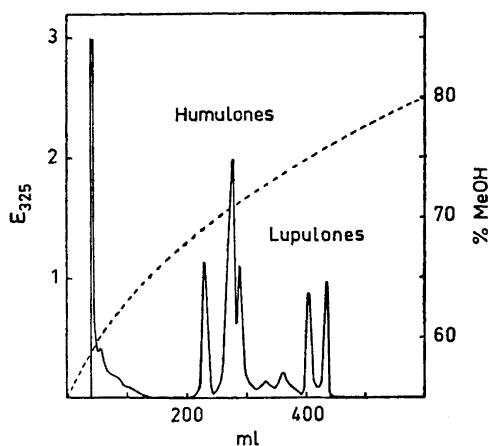


Fig. 1. Gradient elution analysis of humulones and lupulones from Hallertau hops. Column  $800 \times 8$  mm.

in one single operation by means of gradient elution. If the analysis of the eluate is performed spectrophotometrically at  $325 \text{ m}\mu$ , where the contributions from other hop substances is small, a solvent extract of hops can be used without purification. Fig. 1 shows the result of an analysis of the humulone and lupulone groups of Hallertau hops. The concentration gradient of the eluant was accomplished by letting 90% (w/w) methanol flow into a constant volume mixing chamber loaded with 55% (w/w) methanol, the effluent of which was fed to the column. The resolution was satisfactory but somewhat less complete than with normal elution. Thus lupulone and adlupulone were not separated.

In other experiments it has been possible to vary the methanol concentration over still wider ranges. Carbon tetrachloride extracts of hops have been shown to be very heterogeneous mixtures containing substances which may be eluted at methanol percentages varying from 0 to 85% or still higher.

The collection of eluate fractions from a chromatographic column and the determination of the ultraviolet absorption of each fraction are inherently subject to systematic errors, which may be of a considerable magnitude, especially when working with substances which are unstable to air. The column effluent was therefore passed through a 5 mm absorption cell in

a Hilger Uvispek spectrophotometer and the optical density at 280 or  $325 \text{ m}\mu$  recorded automatically by a Brown recorder.

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## On the Production of Magnetic Centres in Glycine by X-Rays

A. EHRENBERG, L. EHRENBERG  
and K. G. ZIMMER

*Biochemical Department, Nobel Medical Institute; Institute for Organic Chemistry and Biochemistry, University; and Genetics Department, Forest Research Institute, Stockholm, Sweden*

The demonstration of the production of magnetic centres in a variety of chemicals by ionizing radiation might well lead to a better understanding of the mechanisms by which a relatively small amount of energy absorbed in the form of radiation causes marked biological effects<sup>1-4</sup>. The paramagnetic resonance spectra obtained have been tentatively explained by assuming that organic free radicals constitute the magnetic centres<sup>5-8</sup>. These results are of great interest to radiation biology when considered in relation to other evidence concerning aftereffects of irradiation and protection against radiation damage<sup>9</sup>. We thought it worth while to try and obtain data in order to evaluate, more quantitatively, the importance of the earlier observations for radiation biology. As a first step we investigated the relation between the dose of radiation administered and the number of magnetic centres produced in an amino acid, glycine.

For irradiation we used X-rays produced at 175 kV and filtered by 2 mm of aluminium (about  $800 \text{ r} \cdot \text{min}^{-1}$ ). The dose was measured during the irradiation by means of a wavelength independent thimble chamber connected to a valve electrometer and integrating counter (*Duplex-Dosemeter*) carefully calibrated in Roentgens. The glycine, in amounts of about 150 mg, in the form of small crystals, was irradiated in evacuated and sealed quartz tubes, which were only half-filled. This en-