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Note

High-performance liquid chromatography of α- and β-acids in beer

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Gradient elution high-performance liquid chromatography (HPLC) of an isooctane beer extract with detection at 336 nm produces a very complex chromatogram. None of the observed peaks is from an iso-α-acid (the main beer bitter compounds), as these do not absorb at 336 nm. In this paper we show that some of the observed peaks are due to α- and β-acids. The concentration of these α- and β-acids varies considerably from beer to beer, but we found some in all beers analysed so far. For this study we developed a specific method to determine α- and β-acids in beer.

The α- and β-acids in hops and in hop-derived products can be determined by HPLC on a reversed-phase column[1–6]. As a result of the complexity of beer extracts and the large difference in concentration between the α- and β-acids, the use of this method for beer is excluded. In particular, isocratic elution is not applicable; due to peak broadening at the end of the chromatographic analysis, the small amount of β-acids cannot be detected. A methanol–water gradient elution of the beer extract has to be used.

EXPERIMENTAL

A Varian 5020 LC liquid chromatograph, a 10-μl Valco 7000-p.s.i. injector and a Varian UV-50 variable-wavelength detector operated at 336 nm were employed. Integration and plotting was carried out with a Varian Vista Data System. The column was a 25 × 0.46 cm Lichroma tube provided with Valco fittings. It was slurry packed with 5-μm ROSIL-C_{18}-HL-D material, an octadecylated silica gel from Alltech-RSL.

The distilled water and methanol were from Burdick and Jackson and the phosphoric a cid (85%) was from Merck. The *meta*-nitroanilide of palmitic acid was found to be a suitable internal standard for the analysis of α- and β-acids in beer. It was obtained by standard procedures from the acid chloride and *m*-nitroaniline. It was recrystallized four times from isooctane–methylene chloride (60:40) and then had a m.p. of 95–96°C and an absorbance of 35.2 at 336 nm. Humulone was obtained by decomposition of its many times recrystallized *o*-phenylenediamine complex, with

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hydrochloric acid in contact with freshly distilled diethyl ether. It was crystallized from acetic acid, and had a melting point of 71°C. Colupulone was recrystallized several times, first from hexane and then from methanol–water to a constant m.p. of 96–97°C.

Analytical procedure

In a 1/3-l beer bottle, 100 μl *n*-octanol or another anti-foaming agent were introduced and then 250 g beer in such a way as to avoid foaming. A 2-ml volume of the internal standard solution (≈ 3 mg *meta*-nitroanilide of palmitic acid in 100 ml methylene chloride) was injected, followed by 60 ml of isooctane. The bottle was then filled as completely as possible with water. The bottle was rotated slowly at one turn per second for 1/2 h.

The isooctane layer, isolated in a separating funnel, was drawn off into a suitable flask. The residual emulsion was destroyed by magnetic stirring. A 50-ml volume of the isooctane layer could be recovered and was evaporated on a Rotavapor with the temperature of the water-bath not exceeding 35–40°C. The residual isooctane solution was rinsed in a small pear-shaped flask or in a test-tube with 3 × 1 ml isooctane and the isooctane then completely evaporated with a nitrogen stream. The

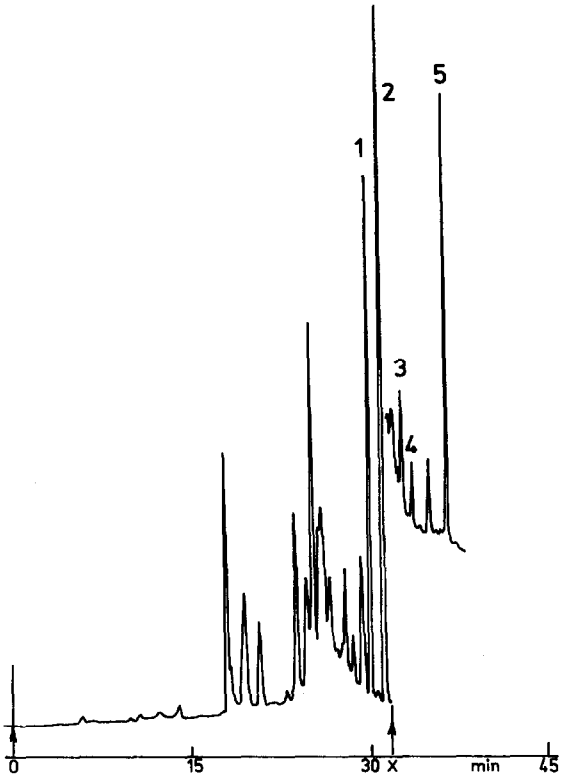

Fig. 1. Chromatogram of isooctane beer extract with UV detection at 336 nm. Conditions as in the text. At point X just after the humulone–adhumulone peak, the attenuation was changed from 0.5 to 0.05. Identity of peaks as in Fig. 2.

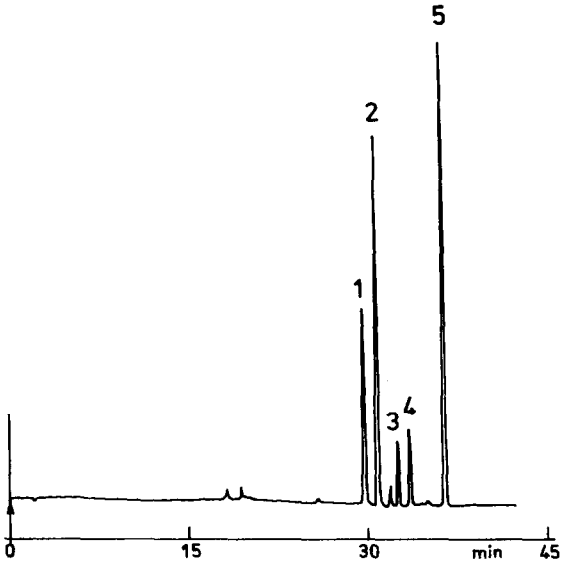

Fig. 2. Chromatogram of a hop extract with UV detection at 336 nm to establish retention data for the peaks of interest in Fig. 1. Main peaks in order of appearance: 1 = cohumulone; 2 = humulone and adhumulone; 3 = colupulone; 4 = lupulone; 5 = the internal standard, *m*-nitroanilide of palmitic acid.

residue was dissolved in 200 μl methanol. A 10-μl volume of this solution was injected and eluted at 1 ml/min with a methanol–water gradient, 50:50 to 100:0 in 30 min. To both methanol and water, 0.5% phosphoric acid was added. The β-acids are observed only as small peaks even at high sensitivity. The injection of a large sample, *e.g.*, 25 μl instead of 10 μl, causes peak broadening and insufficient separation.

Using standard solutions in methanol of humulone, colupulone and the *m*-nitroanilide of palmitic acid, calibration equations were obtained. For humulone, $x = 0.184y$ and for colupulone, $x = 0.169y$; x is the ratio of the concentrations and y is the ratio of the peak areas (measured compound to internal standard). These equations are used to calculate the amounts of all α- and β-acids in beer. We assume therefore that the absorbancies of humulone, cohumulone and adhumulone are the same at 336 nm. The same applies to the β-acids.

Identification of α- and β-acids

A typical chromatogram of an isooctane beer extract under the conditions

TABLE I

CONCENTRATIONS (μg/kg) OF α- AND β-ACIDS IN SOME COMMERCIAL BEERS

Beer	*Cohumulone*	*Humulone + adhumulone*	*Total α*	*Colupulone*	*Lupulone*	*Total β*
1	97.2	87.3	184.5	1.9	3.9	5.8
2	56.3	76.2	132.5	6.9	8.5	15.4
3	74.8	81.7	156.5	2.3	1.2	3.5
4	154.9	176.6	331.5	7.6	8.9	16.5
5	403.0	580.0	983.0	7.0	3.0	10.0

TABLE II

CONCENTRATIONS (μg/kg) OF α- AND β-ACIDS IN SIX BOTTLES OF LAGER BEER FROM THE SAME SIX-PACK

No.	*Cohumulone*	*Humulone Adhumulone*	*Total α*	*Colupulone*	*Lupulone Adlupulone*	*Total β*
1	292.5	261.4	543.9	1.3	2.3	3.6
2	273.9	233.3	507.3	1.5	2.7	4.2
3	291.4	249.3	540.7	1.7	2.9	4.7
4	286.4	244.6	531.0	1.8	2.9	4.8
5	282.1	238.5	520.6	1.6	2.1	3.8
6	276.6	237.4	514.1	1.6	3.3	5.0

described is shown in Fig. 1. Cochromatography with the purified humulone and colupulone, obtained as described above, results in coincidence of retention times of peaks 2 and 4 respectively. Comparison of retention times with the other α- and β-acids can be made by chromatographing a hop extract under the conditions of Fig. 1. The result of such an experiment is shown in Fig. 2. Peak 1 of chromatogram 1 is therefore cohumulone and peak 3 is colupulone. Peak 5 is the internal standard. Coincidence of retention times was also observed on different batches of octadecylated silica gel and on columns which had been used for a long time. Last but not least, the UV characteristics of the collected peaks are in accord with those of α-acids for peaks 1 and 2 and with those of β-acids for peaks 3 and 4.

ANALYTICAL RESULTS AND DISCUSSION

The contents of α- and β-acids in six major West European lager beers are presented in Table I. It is seen that a high α-acid content is not necessarily accompanied by a high β-acid content and *vice versa*. This is most curious. The possible reproducibility of the method is shown by the analysis results for six bottles from the same six-pack (Table II).

Considering that the analysis determines ppb (10^9) amounts of surface-active compounds, the results are good. A number of precautions have to be taken. The sample must include a complete bottle and the glassware used must be lipid-free. The importance of the presence of α- and β-acids in beer and of their variable concentration will probably only become apparent by systematic analysis coupled to organoleptic evaluations. This has now become possible.

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