

ALPHA-ACID—1,2-DIAMINOBENZENE COMPLEX: A CONVENIENT STANDARD FOR ALPHA-ACID ANALYSIS

BY COLIN P. GREEN and PETER OSBORNE

Horticulture Research International, Department of Hop Research, Wye College, Ashford, Kent, TN25 5AH

Received 13 October 1992

A convenient preparation of the complex of alpha-acid and 1,2-diaminobenzene (o-phenylene diamine) is given. Use of the complex as an external standard for HPLC analysis of hops is described. Pure alpha-acid can be recovered from the complex by treatment with ion-exchange resin. The complex is stable in the absence of light and can easily be made in sufficient quantity and purity for distribution as a primary alpha-acid standard.

Key Words: *Hop analysis, high performance liquid chromatography, alpha-acid standard*

INTRODUCTION

Most methods of hop analysis for resin components do not use standards. The polarimetric method for alpha-acid relies on well established optical rotations of alpha-acid in several solvents. The spectroscopic method estimates alpha-acids and beta-acids by measuring the UV absorbance at three wavelengths and entering the values into two regression equations. The conductometric method is the only one that uses a standard. This uses sulphuric acid solution to standardise the titrant, but the method is not checked against alpha-acid.

Analysis of hops by high performance liquid chromatography (HPLC) introduces new problems. Differences between instruments and between runs mean that daily calibration is required. This is done in this laboratory by analysing solutions containing known amounts of alpha-acid and beta-acid and either drawing a graph or calculating the calibration equation by regression analysis. Ideally, pure alpha-acid and beta-acid should be used for calibration because the use of impure standards leads to an over-estimation of the alpha-acid and beta-acid content of the hop sample. Large quantities of pure beta-acid are easily obtained but it is difficult to prepare pure alpha-acid. Hop extract of known composition can be used as an alternative, but extract is often inhomogeneous and must still be standardised. The major problem of HPLC analysis is the absence of a suitable alpha-acid standard.

When HPLC analysis was introduced at Wye, alpha-acid regenerated from the complex of alpha-acid and 1,2-diaminobenzene was used for calibration. It has always been found difficult to obtain crystalline alpha-acid. The product from the lead salt and 1,2-diaminobenzene route did not crystallise. A laboratory scale adaptation of the elution method of Bishop, Whitear and Brown² gave good yields of crystalline beta-acid but alpha-acid was obtained as an oil which crystallised poorly after several weeks at low temperature.

The crystalline complex of alpha-acid and 1,2-diaminobenzene can be easily obtained pure and in good yield and during chromatography its components are separated. This suggests that it would be a better and more convenient standard than alpha-acid.

EXPERIMENTAL

Preparation of 1,2-diaminobenzene complex from CO₂ hop extract

Method 1

Hop extract (20 g) in toluene (25 cm³) was treated with a hot solution of 1,2-diaminobenzene (2.6 g) in toluene

(25 cm³). The complex appeared on cooling and it was filtered under suction through a sintered glass funnel and washed with petrol (b.p. < 40°C) to give yellow needles (8.8 g, m.p. 112.0–114.3°C). After three recrystallisations from iso-propanol/iso-octane the complex was obtained as fine pale yellow needles (2.0 g, m.p. 119.6–120.1°C).

Method 2

Hop extract (20 g) and 1,2-diaminobenzene (2.6 g) were dissolved in hot toluene (55 cm³). The complex appeared on cooling and was recrystallised three times as in method 1 to give fine pale yellow needles (2.7 g, m.p. 117.8–118.1°C).

Preparation of Alpha-acid

The 1,2-diaminobenzene complex (2.35 g) in methanol (50 cm³) was passed through a 13 mm I.D. column containing Zeolit 225 resin (H⁺ form, 20 g) and the column was washed with methanol (50 cm³). The combined eluates were evaporated to dryness under reduced pressure. Residual solvent was removed in a vacuum oven overnight at 35°C to give crystalline alpha-acid (1.83 g, m.p. 67.1–67.7°C).

HPLC Analysis

HPLC was as previously described.³

RESULTS AND DISCUSSION

Preparation of complex

The usual route to the 1,2-diaminobenzene complex of alpha-acid is from the lead salt and this was the method used first. Although the yield of crude complex from this method was quite good (68% based on the weight of lead salt), the final yield from first and second crop of crystals was only 14%. The procedure was also expensive in time and materials.

Hop extract is a readily available, alternative source of alpha-acid. It is manufactured by extracting hops with organic solvents, liquid CO₂ or supercritical CO₂. Hop extract prepared by organic solvent extraction contains fats, waxes and chlorophylls in addition to alpha-acids and beta-acids. Attempts to make the complex of alpha-acid and 1,2-diaminobenzene from solvent extract were unsuccessful. However, liquid CO₂ extract gave the complex in good yield when treated with 1,2-diaminobenzene in hot toluene solution. The crystals from the reactions were contaminated with brown residues, probably from decomposition of the 1,2-diaminobenzene which is sensitive to light. These were removed by washing with petrol (b.p. < 40°C), in which the complex is only slightly soluble.

Since this work was completed a method for the preparation of alpha-acid–1,2-diaminobenzene complex from hop extract has been published.⁴ After reacting a large quantity

