

## Note

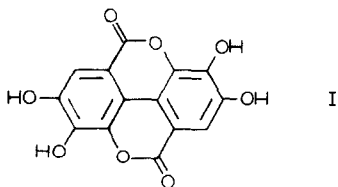
### Determination of free ellagic acid by reversed-phase high-performance liquid chromatography

BHIM S. DHINGRA\* and ARNOLD DAVIS

Department of Chemistry, Morgan State University, Baltimore, MD 21239 (U.S.A.)

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Ellagic acid (I), one of the polyphenol constituents of many plants, occurs widely in nature either in its free form or in the form of ellagitannins or glucosides. It is found in dicotyledonous plants in the genera of castanea, eucalyptus, eugenia, euphorbia, geranium, mangifera, platycarya, quercus, rhus and terminalia<sup>1</sup>. Its presence has also been reported in many fruits and vegetables, e.g. grapes, strawberries, guavas, mangoes, green tea and black walnuts<sup>1</sup>.



Ellagic acid has been found to be pharmacologically active as an antineoplastic, antioxidant, antimutagenic and anticarcinogenic agent<sup>1</sup>. These activities of ellagic acid have been ascribed to the ability of its four phenolic groups to complex readily with metals such as magnesium and calcium<sup>2</sup>. This ability of ellagic acid can create problems in the wood pulping process. For example, its metallic complexes can cause corrosion and scale formation and can adversely affect the wet strength of bonds of phenol-formaldehyde adhesives with wood. In view of the importance of ellagic acid, a direct and accurate method for its determination is required. Paper chromatography<sup>3</sup>, gravimetry and gas-liquid chromatography (GLC)<sup>4</sup> have been used for its determination. The sensitivity and accuracy of the first two methods is limited and in GLC, derivatization of the sample is required before its determination. In this paper, we present a direct method for the determination of ellagic acid by reversed-phase high-performance liquid chromatography (HPLC). Although ellagic acid was determined qualitatively by this method in all of the above-mentioned species, quantitative determinations were carried out only in *Eucalyptus cinerea*, green grapes and black grapes (*Vitis vinifera*).

## EXPERIMENTAL

*Extraction*

Dried, ground samples of *Eucalyptus cinerea*, green grapes or black grapes (*Vitis vinifera*) were extracted with 0.05 M triethanolamine (pH 8) in water. The extract was further washed with cyclohexane, toluene and methylene chloride to remove any non-polar impurities extracted in the amine solution. The amine extract was then concentrated for injection in the HPLC system.

*HPLC*

The analyses were carried out using a Perkin-Elmer Series 4 liquid chromatograph and LC-85B variable-wavelength spectrophotometric detector. A wavelength of 254 nm was used for detection. Separations were carried out using a Perkin-Elmer C<sub>18</sub> reversed-phase column (125 × 4.6 mm) containing 5- $\mu$ m particles. The chromatographic system was equipped with a Rheodyne 7010 injection system with a 20- $\mu$ l injection loop. The mobile phase used in all the separations was 0.05 M triethanolamine in water-acetonitrile-isopropanol (85:14:1).

*Reagents*

Triethanolamine, acetonitrile and isopropanol were purchased from Fisher Scientific. Ellagic acid was purchased from Aldrich. All solvents were of HPLC grade.

*Analysis*

Calibration curves were made by injecting standard solutions of ellagic acid in the mobile phase ranging from 0.1–1  $\mu$ g/ml. A 10- $\mu$ l volume of the amine extract of the grapes or wood samples was injected for each analysis. The retention time of the ellagic acid at the mobile phase flow-rate of 1.0 ml/min was 1.1 min (Fig. 1). The identity of the ellagic acid peak was confirmed by running the stop-flow spectroscopy on the peak and comparing it with the spectra of the standard. Ellagic acid solutions showed evidence of photochemical reaction. Over a period of time the solution changed from a clear to a yellow and eventually to a brown solution. When the solutions were kept in the dark, they remained clear for several days. New solutions had to be made after every few days.

## RESULTS AND DISCUSSION

The amount of total ellagic acid in the whole dry plant was as follows: *Eucalyptus cinerea*: 12.0 mg/g; black grapes: 7.0 mg/g; green grapes: 3.5 mg/g. The aver-

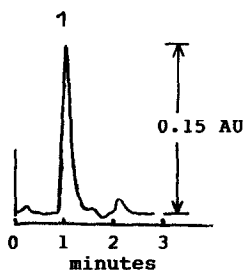


Fig. 1. Chromatogram of the extract of *Vitis vinifera*. Peak 1 = ellagic acid.

ages of five, six or seven samples showed a variation of  $\pm 2\%$ . The minimum quantity of the ellagic acid which could be detected with this method was 4 ng. The response of the detector was linear over a  $10^6$ -fold range of concentration. From the results described here, our method offers a fast, simple way of determining ellagic acid. With minor modifications, this method can also be used to keep track of ellagic acid in biological fluids. Results of extraction and quantitation in such fluids will be reported in a later paper.

#### REFERENCES

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