Journal of Chromatography, 109 (1975) 172–174
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 8205

Note

Rapid determination of ellagic acids by gas–liquid chromatography

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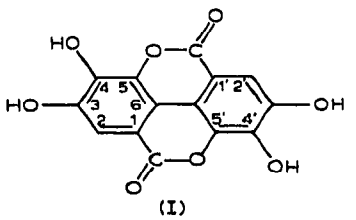
(Received January 20th, 1975)

Ellagic acid (I), in the free or combined form or both, is present in all tissues of eucalypts, in oak, chestnut and several other species. The amount present varies within and between trees of the same species and between species. Ellagic acid causes costly difficulties in the manufacture of pulp and its products from wood (see refs. 1 and 2 and references cited therein). It affects adversely the wet strength of bonds of phenol–formaldehyde adhesives with wood[3]. In the combined form as ellagitannins, it conveys resistance to wood-destroying fungi[4].



(I)

In view of the influence of ellagic acid on wood quality, a rapid and accurate method for its determination is needed to assist quality assessment. Moreover, it is desirable to be able to follow the fate of ellagic acid during the utilisation and processing of the wood. Paper chromatographic (PC)[5], gravimetric and UV absorption methods have been tried but the accuracy of the methods is limited. Ellagitannins can affect UV absorption data on ellagic acid and also some hydrolyse readily under mild acid conditions and thus can affect the data obtained from PC studies. Also pure ellagic acid is very insoluble in neutral solvents but the solubility is affected by similar components (*e.g.*, ellagitannins).

This communication reports a gas–liquid chromatographic (GLC) method which has yielded results rapidly and accurately in the examination of a range of samples. It is suitable also for pure methylellagic acids but these could not be resolved accurately from ellagic acid with the columns used. Gallic acid also could be determined but at a lower column temperature.

EXPERIMENTAL

Gas chromatography

The analyses were carried out on a Varian Aerograph Model 2100 gas chromatograph with dual flame ionisation detectors using glass columns (2 m × 3 mm I.D.). The nitrogen carrier gas flow-rate was 60 ml/min, the oven temperature was maintained at 250°, and the injector and detector temperatures were both 275°. The detector sensitivity ranged from 32×10^{-11} to 256×10^{-11} A/mV at full scale. The liquid phase, 3% SE-30 (methylphenylvinylsilicone gum (Varian, N. Springvale, Australia) was coated on acid-washed, silylated, 80–100 mesh Chromosorb W (Varian).

Silylation

The silylating reagent contained: (a) 2 ml of hexamethyldisilizane (HMDS) and 1 ml of trimethylchlorosilane (TMCS) (Sigma, St. Louis, Mo., U.S.A.) in 10 ml of dry pyridine (distilled over potassium hydroxide) and (b) an equal volume of N,O-bis(trimethylsilyl)acetamide (BSA; obtained in 1-ml glass ampoules of specially purified grade from Pierce, Rockford, Ill., U.S.A.). All reagents were kept in a refrigerated desiccator when not in use.

Silylation was carried out in glass Durham tubes (5 cm × 3 mm I.D.) and sealed with corks free from surface holes. To the sample equal volumes of the silylating reagents (a) and (b) were added and the tube was immediately corked. The mixture was momentarily boiled over a very small flame and then placed in an oven at 40° for 10 min. Trials were made to ensure that an excess of the silylating reagent was present so that a stable reaction resulted and no precipitation occurred. After the initial reaction, the tubes were kept (for about 2 h) in a desiccator until the contents were analysed. Long-term storage in a refrigerator at 0° showed a loss of only 5–10% in 24 h.

Momentary boiling was found to be essential for consistent reaction. Subsequent storage at 40° for 10 min was a convenient but not a critical storage period. It was also found essential that the reagents were pure and kept dry, and that an excess of silylating reagent was present during reaction. If the solution was diluted with pyridine, precipitation frequently occurred.

Retention times

With the system used the retention time for silylated ellagic acid was 12.0 min. Under the same conditions silylated 3,3'-di-O-methyl- and 3,3',4-tri-O-methylellagic acids had retention times of 13.8 and 12.6 min, respectively. Tetramethylellagic acid had a retention time of 12.0 min.

Silylated gallic acid had a retention time of 7.2 min when the oven temperature was 175°.

Analysis

Calibration curves based on peak areas were prepared using 1–10 mg of pure ellagic acid reacted with varying quantities of up to 300 μl of the silylating mixture injected in portions of 1–5 μl. The results from over 100 dual injections gave a linear curve over the range of $1–8 \times 10^{-3}$ mg of injected ellagic acid but between 8–15 ×

10^{-3} mg the linearity decreased. In the samples tested, the amount taken contained between 1–8 $\times$ 10^{-3} mg ellagic acid.

Dried, ground (less than 200 mesh), or sectioned (20-μ thick) wood samples (20 mg) were soaked overnight in dry pyridine (100 μl) and aliquots (20 μl) were silylated with 40 μl of the combined reagent. Between 5–10 mg of evaporated extracts obtained by extracting with methanol for 8 h, or deposits from pulping processes were mixed in pyridine (50 μl) and then silylated with 40 μl of the combined reagent. The total time for an analysis was about 30 min. The averages of two, three or four analyses on one sample showed variations of $\pm$ 5% for extracts and $\pm$ 8% for wood and non-soluble samples. Over 175 analyses have been made. The analysis of *Eucalyptus pilularis* wood and the methanol extract from the same sample gave the same content of ellagic acid.

RESULTS

The amount of ellagic and methylellagic acids in samples varied considerably within a species. The following averaged percentage results illustrate the variations in content of total ellagic acids (calculated on dry wood basis): *Eucalyptus cypellocarpa*, wood analysed 0.37 and 0.64, methanol extract from outer heartwood 0.77, 0.71, and 0.72 and methanol extract from inner heartwood 0.38; *E. pilularis*, wood analysed 0.51, 0.10, 0.12, and less than 0.05, and wood and extract of same sample 1.60; *E. dives*, wood 0.25; *E. camaldulensis*, slow-grown wood 0.43, 0.25, and 0.16, fast-grown wood less than 0.05, and extract 1.00, 0.57, and less than 0.05; *E. sideroxylon*, heartwood 0.14, and 0.13, and sapwood 0; *E. platypus*, less than 0.05.

Deposits collected from commercial pulping operations contained 0.12, 0.04 and 1.5% ellagic acid. After boiling with acid and washing, the deposits contained 6.4, 5.1 and 100% ellagic acid, respectively.

As the retention times of ellagic acid and its methyl derivatives are close, a two-dimensional PC examination using *n*-butanol–acetic acid–water (6:1:2) and 6% acetic acid was necessary to show the presence of the derivatives. Consequently, the above values represent total ellagic acids, and PC indicates methylellagic acids are present in varying amounts in *E. camaldulensis*, *E. sideroxylon* and *E. platypus*. It should be noted, however, that methylellagic acids are relatively more fluorescent in UV light than ellagic acid and when present are there generally in relatively small amounts.

Stilbenes, leucoanthocyanins and ellagitannins can accompany ellagic acid in different eucalypt woods. These compounds were added to known amounts of ellagic acid but did not affect the determination.

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