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METHYL GALLATE AND RELATED POLYPHENOLS AS AUXIN PROTECTORS

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Key Word Index—*Castanea sativa*; Fagaceae; chestnut; IAA-oxidase inhibitors; methyl gallate; polyphenols.

Abstract—The systematic study of the effect of chestnut polyphenols (gallic acid, methyl gallate, (+)-catechin, (−)-epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate) on indole-3-acetic acid (IAA)-oxidase activity, proved that the tested polyphenols are much less inhibitory than their gallates. The inhibition caused by the methyl ester of gallic acid at 2 μM was about eight times stronger than that of gallic acid itself.

INTRODUCTION

In a systematic study of the chemical factors, if any, governing the rooting ability of chestnut (*Castanea sativa* × *C. crenata*) cuttings at juvenile stages, the patterns in the two dimensional chromatograms of adult and juvenile material when sprayed with diazotized benzidine showed that most of the phenolic compounds were present in both materials [1]. However, a spot giving a yellow colour with the reagent was more evident in juvenile chromatograms. This prompted us to examine that compound; thus, the crude juvenile extract was successively chromatographed and the isolate characterized as methyl gallate on the basis of its spectral data as well as by cochromatography with an authentic marker and cromogenic reactions. In this paper we examine its effect on indole-3-acetic acid (IAA) oxidation since its stimulating action on rhizogenesis (test *Phaseolus*) has already been proved [2] and the effects of phenolic compounds on rooting have been related with IAA enzymic oxidation [3, 4]. In addition, we compare its activity with that of other known chestnut constituents structurally related to the isolate.

RESULTS AND DISCUSSION

The isolate (R_f 0.64–0.78 in BEW; 0.55–0.60 in 15% HOAc) was tentatively identified as methyl gallate on the basis of its spectral data. The maximum at 275 nm in MeOH shifted to 236, 278, 317 nm when KOH was added. In the presence of $AlCl_3$ a bathochromic shift to 298 nm was nullified when acidified with HCl suggesting a free *ortho* diphenolic moiety. The paper chromatograms when sprayed either with diazotized benzidine or with *p*-nitroaniline gave a strong yellow colour. The compound cochromatographed in several solvents with an authentic specimen.

The low concentration of methyl gallate in the chestnut shoots precluded the study of its effect on the IAA oxidase activity. However, the product was commercially available and consequently the assays were carried out with synthetic compound. At the usual concentrations in this enzymic test (0.01–0.1 mM), methyl gallate exhibited a remarkable degree of activity. This prompted us to examine its action at much lower concentrations (1–4 μM) and, once more, a relevant inhibition on IAA oxidation was shown. Consequently, effort has been devoted to a comparative search on the activity of other chestnut constituents structurally related to the isolate: gallic acid, (+)-catechin, (−)-epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate.

The tested compounds exhibited a temporary inhibition, afterwards IAA oxidation resumed at the rate of the control. The length of lag in the oxidation of IAA was taken for comparing the inhibition activity. Relevant differences among the compounds in their effects on IAA oxidation were found. Whereas gallic acid itself has little effect at these very low concentrations (1–4 μM), its methyl ester is strongly active since at 4 μM it produces an 18 hr lag period (Fig. 1). In the cases of (−)-epicatechin and epigallocatechin, both were more active than gallic acid but less active than their gallates even though the differences were not so noticeable; at 4 μM epicatechin gallate is more than twice as active as epicatechin itself (Fig. 2) meanwhile epigallocatechin gallate is four times more active than epigallocatechin (Fig. 3). There were no detectable differences at the three lower concentrations, but the activity of (+)-catechin (Fig. 3) at 4 μM is two-fold that of (−)-epicatechin (Fig. 2). Discrepancies in the

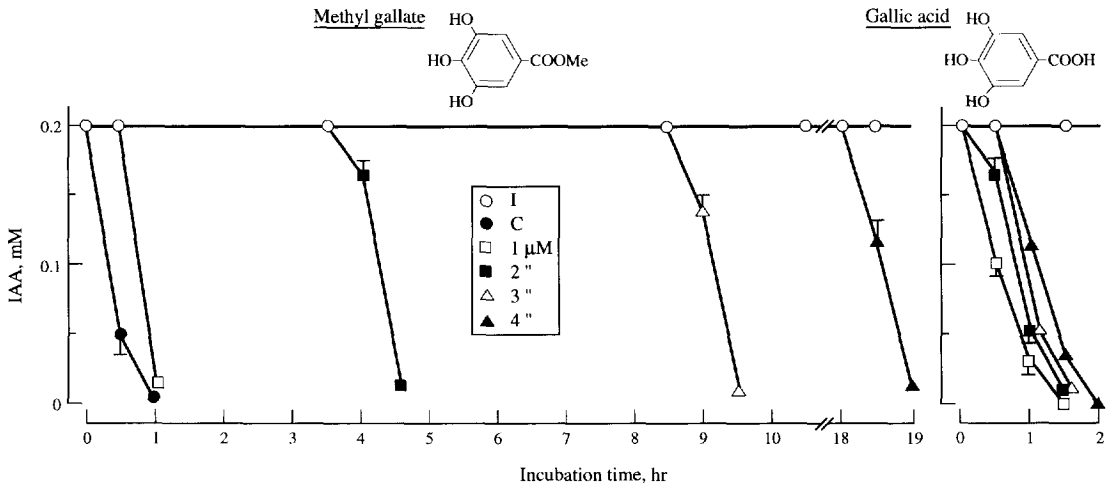

Fig. 1. Inhibition of horseradish peroxidase-catalysed IAA oxidation by gallic acid and its methyl ester at different concentrations. Data represent IAA (mM) remaining in the mixtures after different times of incubation. I, IAA initial concentration; C, control mixtures without polyphenol. The bars indicate S.D. of three experiments.

activity of (+)-catechin between the present values and those published in figure 4 of ref. [5] are only apparent since, due to an arithmetical error, those concentrations were shown 100 times higher than the tested ones. Namely, instead of mM, 0.2, 0.4 and 0.6 should read μM, 2, 4 and 6.

Retarding the IAA-oxidation catalysed by peroxidases, the tested phenolics exerted a protective role towards the auxin [6]. This means that they would preferentially be degraded by peroxidases and the auxin would be oxidized later, as stated in ref. [3].

EXPERIMENTAL

Chestnut shoots (262 g), 3 months old and 30–35 cm long, were collected in June. They were frozen in liquid N_2, homogenized and exhaustively extracted (7×) with 70% MeOH. The combined extracts were chromatographed on 3MM paper with *n*-BuOH–EtOH–H_2O (40:10:22) (BEW) and then in 15% acetic acid (HOAc). The eluate was characterized by spectrometry and cochromatography in several solvents.

Auxin protector assay was performed as previously described [5] and the concn of IAA estimated by reference to a calibration curve using a Quant II linear program. The reaction mixtures (10 ml) have the following composition: IAA (0.2 mM), K-Pi buffer (50 mM, pH 6.1), 2,4-dichlorophenol and $MnCl_2$ (0.05 mM each), horseradish peroxidase Sigma, type II (0.5 μg, amount required for 100% IAA destruction in

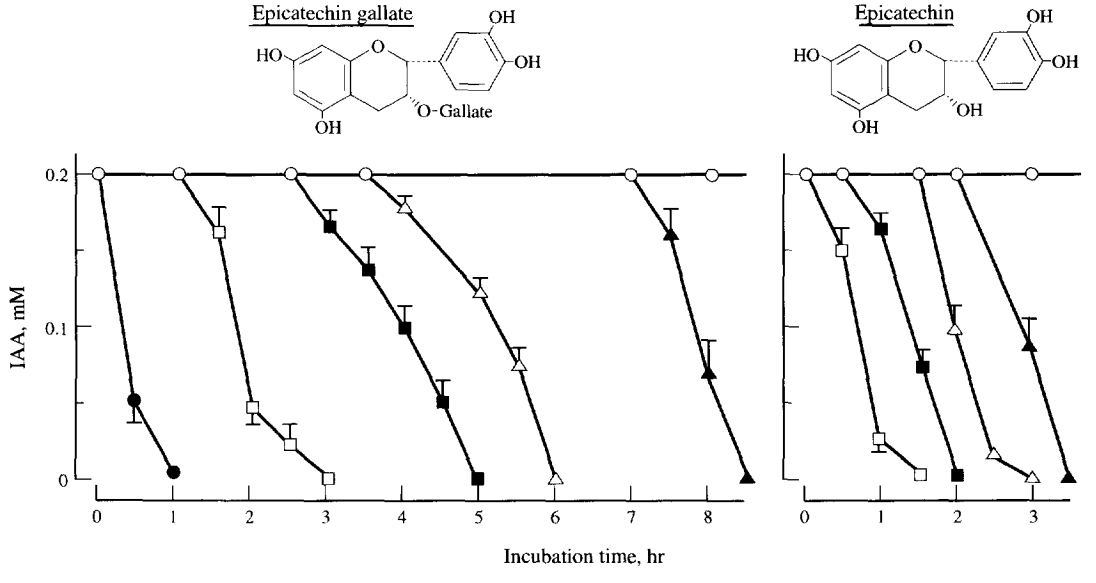

Fig. 2. Inhibitory effect of (−)-epicatechin and its gallate, at different concentrations, in the horseradish peroxidase-catalysed IAA-oxidation. Data as in Fig. 1.

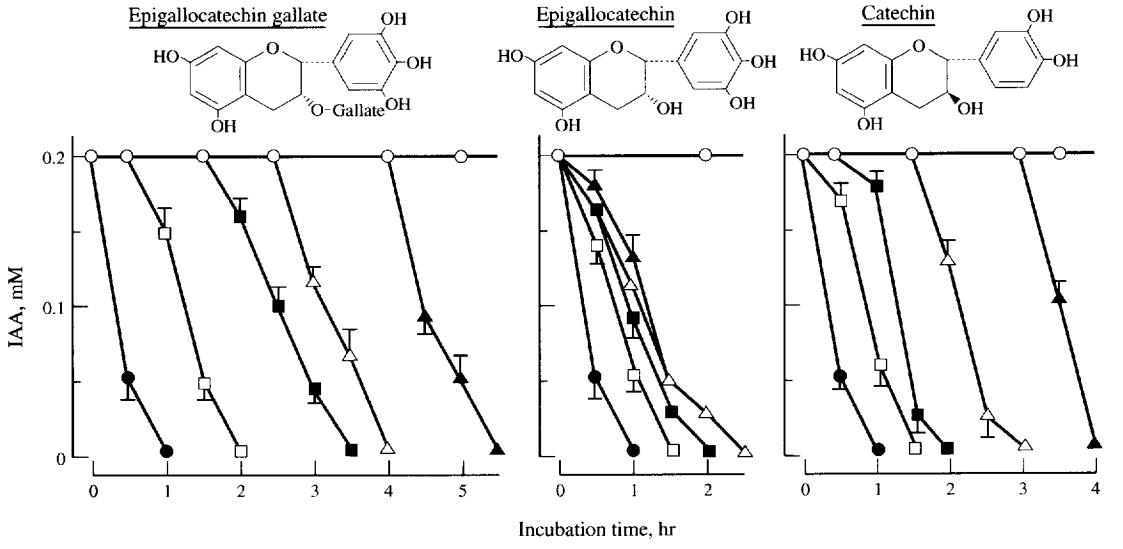

Fig. 3. Inhibition of peroxidase-catalysed IAA oxidation by (+)-catechin, epigallocatechin and its gallate, at different concentrations. Data as in Fig. 1.

1 hr) and polyphenols tested at various concns as indicated in figures.

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