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Oxidation of pentagalloylglucose to the ellagitannin, tellimagrandin II, by a phenol oxidase from *Tellima grandiflora* leaves

Ruth Niemetz, Georg G. Gross*

Molekulare Botanik, Universität Ulm, D-89069 Ulm, Germany

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Dedicated to Meinhart H. Zenk on the occasion of his 70th birthday

Abstract

A new enzyme has been isolated from leaves of the weed *Tellima grandiflora* (fringe cups, Saxifragaceae) that catalyzed the O_2-dependent oxidation of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose to tellimagrandin II, the first intermediate in the $^4C_1$-glucose derived series of ellagitannins. CD-spectra revealed that the 4,6-*O*-HHDP-residue of the in vitro product had the (*S*)-stereoconfiguration characteristic of tellimagrandin II from natural sources. The enzyme, for which a M_r of ca. 60,000 was determined, was purified to apparent homogeneity. It had a pH-optimum at pH 5.0, an isoelectric point at pH 6.3 and was most stable at pH 4.2. Inhibition studies suggested that this new enzyme, for which the systematic name 'pentagalloylglucose: O_2 oxidoreductase' is proposed, belongs to the vast group of laccase-type phenol oxidases (EC 1.10.3.2).

Keywords: *Tellima grandiflora*; Saxifragaceae; Fringe cups; Ellagitannin biosynthesis; Laccase; Pentagalloylglucose; Phenol oxidase; Tellimagrandin II

1. Introduction

Hydrolyzable plant tannins are usually divided into gallotannins and ellagitannins that both are derivatives of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (cf. compound **1** in Fig. 3). In the gallotannin subclass, this pivotal precursor has been recognized to be esterified with additional *meta*-depsidically linked galloyl units. Recent studies have led to the detection of several enzymes that catalyzed these reactions, allowing the presentation of a scheme that summarizes the major routes to hexa- and heptagalloylglucoses (Fröhlich et al., 2002). Our knowledge of the biosynthesis of ellagitannins, in contrast, is still in its infancy. Already many decades ago, oxidation reactions have been postulated which were thought to connect suitably orientated galloyl residues of the pentagalloylglucose core to form characteristic 3,4,5,3′,4′,5′-hexahydroxydiphenoyl (HHDP) moieties which, after eventual hydrolytical release, spontaneously rearrange to the name-giving dilactone, ellagic acid (Erdtman, 1935; Schmidt and Mayer, 1956). This view was later refined by Haslam and coworkers, proposing that the energetically preferred $^4C_1$ conformer of pentagalloylglucose was sequentially oxidized to tellimagrandin II (Fig. 3, **2**) and casuarictin (bearing an additional 2,3-*O*-HHDP group), while the less stable $^1C_4$ analogue provided the basis for various 1,6-, 3,6- and 2,4-*O*-HHDP derivatives (reviewed in detail by Haslam, 1989, 1998). In both cases, subsequent intermolecular oxidations yield the widespread dimeric and oligomeric ellagitannnins.

Earlier attempts to unravel the biochemical mechanisms involved in the transitions of pentagalloylglucose to ellagitannins, employing the enzyme systems laccase/O_2 (Hathway, 1957), peroxidase/H_2O_2 (Mayer et al., 1984) or unspecified crude extracts from *Cornus capitata* (Cornaceae; Tanaka et al., 2001), resulted only in the formation of free ellagic acid, while true ellagitannins, characterized by glucose-bound HHDP-residues, never could be isolated. Quite recent screening experiments, utilizing [*U*-^{14}C]pentagalloylglucose as substrate to increase both sensivity and specificity of the enzyme assays, provided first evidence of the long sought in vitro oxidation of pentagalloylglucose to ellagitannins. Partially purified extracts from leaves of the weed *Tellima grandiflora* (fringe cups, Saxifragaceae) were found

* *Corresponding author. Tel.: +49-731-50-22624; fax: +49-731-50-22809.

E-mail address: georg.gross@biologie.uni-ulm.de (G.G. Gross).

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to form tellimagrandin II, together with several not fully characterized structurally related ellagitannins (Niemetz et al., 2001). Here, we report the purification and characterization of a laccase (EC 1.10.3.2)-type phenol oxidase that specifically catalyzes the O_2-dependent linkage of the 4,6-*O*-galloyl groups of pentagalloylglucose to yield tellimagrandin II, the primary metabolite in the biosynthesis of $^4C_1$-pentagalloylglucose derived ellagitannins.

2. Results and discussion

2.1. Enzyme purification

The oxidase was extracted from leaves of the weed *T. grandiflora* (fringe cups, Saxifragaceae) in the presence of borate that is known to form complexes with inhibitory endogenous plant phenolics. Residual contaminants were removed by stirring with Amberlite XAD resin, followed by ammonium sulfate fractionation of the crude extract. The desalted enzyme solution was further purified by gel-filtration on Sephacryl S-300 which also efficiently removed a pentagalloylglucose degrading esterase ('plant tannase'; Niehaus and Gross, 1997). The active fractions were further purified by hydrophobic-interaction chromatography (HIC) on butyl-sepharose, column chromatography on hydroxyapatite and a final chromatofocussing step on a Mono P column. The enzyme had thus been purified 566-fold with 43% yield to apparent homogeneity, as shown by PAGE under non-denaturing conditions (Fig. 1). The results of a representative purification protocol are summarized in Table 1. The pure enzyme could be stored at 0–4 °C for several weeks without significant loss of activity.

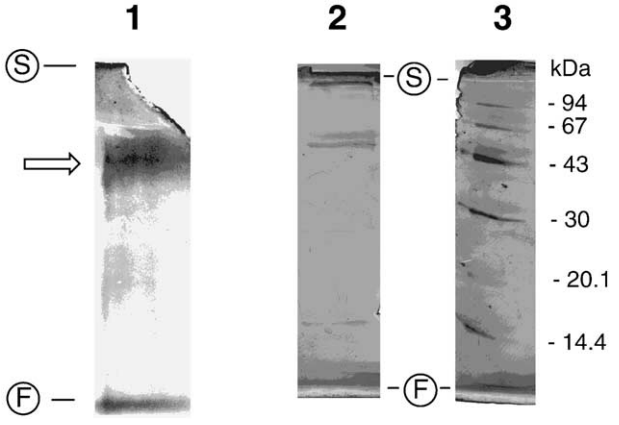

Fig. 1. PAGE of pentagalloylglucose: O_2 oxidoreductase from *Tellima grandiflora* leaves. Lane 1: Native PAGE of the pure enzyme; the arrow, coinciding with the sole detectable protein band, indicates the position of enzyme activity as determined by cutting a parallel gel lane into 1 mm segments, extraction with chilled buffer, and analysis of extracts in the standard enzyme assay (cf. Experimental). Lane 2: SDS-PAGE of the pure enzyme. Lane 3, molecular weight marker proteins. Ⓢ, Start (top of separating gel), Ⓕ, migration front.

2.2. General properties of the enzyme and reaction product identification

The enzyme reaction proceeded linearly for 30 min under standard assay conditions. The substrate dependence curve displayed a lag phase from 0 to ca. 30 μM pentagalloylglucose, followed by normal Michaelis–Menten kinetics up to saturation at ca. 250 μM; no substrate inhibition was observed at higher concentrations. Replotting the data according to Lineweaver and Burk (1934) revealed a K_m value of 80 μM and a v_{max} of 75 μmol s^{-1}. No reaction occurred with heat or acid denatured protein (cf. Fig. 2). Upon reversed-phase HPLC, the sole reaction product coincided with authentic tellimagrandin II that had been identified by chemical degradation, negative FAB-MS and NMR spectroscopy in earlier experiments with crude enzyme preparations (Niemetz et al., 2001). Tellimagrandin II was again isolated now from scaled-up enzyme assay mixtures that utilized pure enzyme as catalyst. The product was subjected to circular dichroism (CD) spectroscopy where it displayed a strong positive Cotton effect at 234 nm (+19.6; in methanol) and two weaker peaks at 261 nm (−6.1) and 283 nm (+5.0). These values are in excellent agreement to recently published data of Feldman and Sahasrabudhe (1999) and prove that the 4,6-*O*-HHDP residue of the enzyme reaction product has the same (*S*)-stereoconfiguration as it is known for this compound from natural sources; the (*R*)-conformer would display an inverse CD spectrum (cf., eg., Khanbabaee and van Ree, 2001). It is evident from these results that stereospecific phenolic coupling in the biosynthesis of tellimagrandin II does not require the assistance of auxiliary 'dirigent' proteins analogous to those involved in the biosynthesis of chiral lignans (Davin et al., 1997; Lewis and Davin, 2000).

The enzyme was inactive below pH 3.5 and above pH 7.0, with a maximum at pH 5.0; half-maximal activities were at pH 4 and 5.8. After preincubation at 45 °C for 3 h, the oxidase was found most stable at pH 4.2 (half-maxima at pH 3.3 and 5.3); rapid inactivation occurred below pH 3 and above pH 6. An isoelectric point of pH 6.3 was determined for the protein by chromatofocussing on a Pharmacia Mono P column. The enzyme reaction proceeded optimally at 45 °C, with half-maximal activities at 30 and 58 °C. No reaction occurred above 72 °C, while a relative activity of 14% was observed even at 10 °C. After preincubation for 3 h at different temperatures, the phenol oxidase was stable between 0 and 55 °C, but was rapidly inactivated above 70 °C. From the above data, an average activation energy of 54.8 kJ mol^{-1}, corresponding to a Q_{10} value of 2.0, was calculated for temperatures between 30 and 40 °C. Gel-

Table 1
Purification of pentagalloylglucose: O_2 oxidoreductase from *Tellima grandiflora* leaves[a]

Step	Total protein (mg)	Total activity (pkat)	Specific activity (pkat mg^{-1})	Purification (-fold)	Recovery (%)
$(NH_4)_2SO_4$, 30–80% ppt.	40	29.2	0.73	–	100
Desalted $(NH_4)_2SO_4$ pellet	27	101	3.7	5.1	346
Sephacryl S-300	24.3	108	4.5	6.2	370
Butyl-sepharose	4	161	40	55	551
Hydroxyapatite	0.14	18.6	133	182	64
Mono P chromatofocussing	0.03	12.4	413	566	43

[a] Crude extracts were not assayed because of excess contaminants preventing HPLC analysis of reaction products.

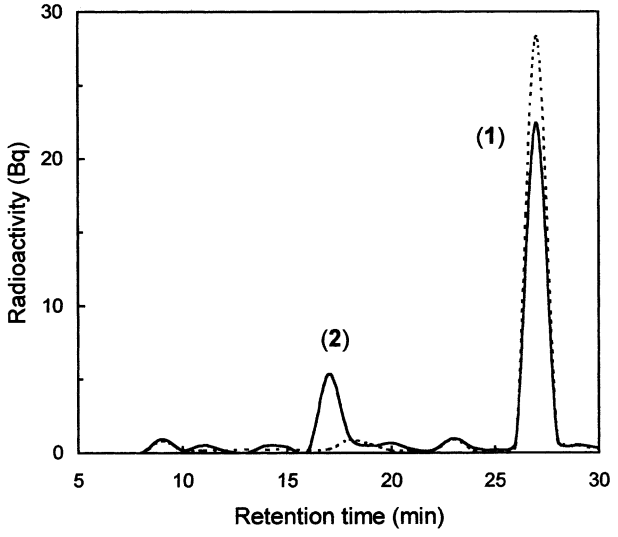

Fig. 2. Reversed-phase HPLC of an enzyme assay with pentagalloylglucose: O_2 oxidoreductase from *Tellima grandiflora* and [*U*-^{14}C]pentagalloylglucose as substrate. (——), Enzyme assay; (. . .), blank with acid-denatured enzyme. (**1**), pentagalloylglucose; (**2**) tellimagrandin II. For HPLC conditions, see Experimental.

filtration experiments with a calibrated Sephacryl S-300 column revealed an apparent M_r value of ca. 60,000 for the native enzyme. An additional minor activity peak corresponding to ca. M_r 125,000 suggests that the enzyme has a certain tendency to form dimeric aggregates. After denaturing SDS-PAGE of the pure oxidase, only one protein band of ca. M_r 62,000 was detected (cf. Fig. 1).

2.3. Inhibitor studies

The oxidation of pentagalloylglucose to tellimagrandin II displayed no apparent cofactor requirement; in particular, addition of the most common proton acceptors, NAD^+ or $NADP^+$, had virtually no effect. It appeared thus very likely that the phenol coupling reaction was catalyzed by some sort of phenol oxidase or peroxidase. To achieve more information on this question, a series of inhibitor experiments was conducted whose results are summarized in Table 2. Hydrogen peroxide had a strong inhibitory effect, and performing the reaction in a nitrogen atmosphere under strictly anaerobic conditions completely eliminated the enzyme activity. It was thus concluded that the enzyme was no peroxidase but belonged to the vast class of oxygen dependent (poly)phenol oxidases. This latter group is usually divided into (i) *o*-diphenol: O_2 oxidoreductases (EC 1.10.3.1) which are known under several trivial names, like *o*-diphenol oxidase, catecholase, etc., and that also comprise the 'tyrosinases' (EC 1.14.18.1), and (ii) the closely related *p*-diphenol: O_2 oxidoreductases (EC 1.10.3.2). The latter are known as 'laccases' from fungi, but have also been reported to occur in plants (for references, see Walker and McCallion, 1980).

Discrimination between these two enzyme groups can be achieved by investigating the more or less specific effects of a range of inhibitors. Among the tested inorganic compounds (cf. Table 2), Cl^- and hydroxylamine have been reported to inhibit laccase stronger than catecholase (Ferrar and Walker, 1996); the *T. grandiflora* enzyme displayed moderate to strong inhibition with these agents. Ascorbate, which was inactive in our study, has little effect on both enzyme classes (Ferrar and Walker, 1996). Laccase has a higher affinity for the respiratory poisons azide and cyanide than tyrosinase (Leech and Daigle, 1998), and these compounds exerted moderate to strong effects on the enzyme described here. Cinnamic acids are good inhibitors of catecholase, but not of laccase (Walker and McCallion, 1980; Ferrar and Walker, 1996; Pedreño and Ros Barceló, 1999); such compounds were clearly inactive in our experiments. Metal chelators appear to have ambivalent effects: the pharmaceutical desferal (for explanation of acronyms and trivial names, see footnote to Table 2) has been reported to inhibit laccase, but not catecholase (De Pinto and Ros Barceló, 1996), while the opposite effect has been observed for mimosine and tropolone (Kahn and Andrawis, 1985; Ferrar and Walker, 1996; Pedreño and Ros Barceló, 1999). We found strong inhibition with desferal for the pentagalloylglucose oxidizing enzyme, while mimosine and tropolone had no effect. Detergents have also been used to differentiate between the two classes of phenol oxidases. Walker and McCallion (1980) reported that the cationic detergent

Table 2
Inhibitions studies with pentagalloylglucose: O_2 oxidoreductase[a]

Agent class	Compound	Concentration	Inhibition (%)
Inorganics	Hydrogen peroxide	0.01–0.1%	50–100
	Nitrogen, replacing oxygen	100%	100
	Na chloride	100–300 mM	55–70
	Hydroxylamine	0.1–0.8 mM	42–48
	Ascorbate	0.1–1 mM	0
	Na azide	0.1–0.8 mM	56–100
	Na cyanide	0.1–0.8 mM	38–40
Cinnamic acids	Cinnamate	0.25–10 mM	0
	p-Coumarate	0.25–10 mM	0
	Ferulate	0.25–10 mM	0
Chelators	Desferal[b]	5–55 mM	36–75
	Mimosine	0.1–1 mM	0
	Tropolone	0.1–1 mM	0
Detergents	CTAB	1 mM	82
Sulfur compounds	β-Mercaptoethanol	0.1–0.8 mM	25–95
	Glutathione, reduced	0.1–0.8 mM	73–80
	Na hydrogene sulfite	0.1–0.8 mM	68–70

[a] Determinations were performed under standard assay conditions.
[b] Acronyms and trivial names: CTAB, cetyltrimethylammonium bromide; desferal, desferrioxamine mesylate; mimosine, β-(*N*-3-hydroxy-pyridone-4)-α-aminopropionic acid; tropolone, 2-hydroxy-2,4,6-cycloheptatriene-1-one.

CTAB inhibited laccase but did not affect catecholase; we found strong inhibition in the presence of this agent. Significant inhibition was observed in our tests with sulfur compounds (β-mercaptoethanol, reduced glutathione, Na hydrogene sulfite) that have been found to inhibit both catecholase and laccase (Ferrar and Walker, 1996). Finally, *p*-hydroquinone was efficiently oxidized to *p*-benzoquinone with the *T. grandiflora* enzyme. This reaction is characteristic of laccases, while catecholases display no, or only minimal, affinity towards *p*-quinols (Ferrar and Walker, 1996).

Summarizing the above results, it is evident that the tellimagrandin II forming enzyme from *T. grandiflora* is a member of the laccase family of phenol oxidases (EC 1.10.3.2) that is known by its low substrate specificity. It should thus receive the systematic name 'pentagalloylglucose: oxygen oxidoreductase'. The reaction catalyzed by this new enzyme is depicted in Fig. 3.

3. Experimental

3.1. Plant material

Plants of the weed *T. grandiflora* (Pursh) Lindley (fringe cups, Saxifragacea) were grown in the open air or in the University greenhouses. Young leaves (2–4 months old) were washed with dist. H_2O and either immediately used as enzyme source, or frozen in liquid N_2 and stored at −20 °C in evacuated plastic bags where they could be kept for more than 6 months without apparent loss of enzyme activity.

3.2. Chemicals and general experimental procedures

[*U*-^{14}C]Pentagalloylglucose was prepared by photoassimilation of $^{14}CO_2$ with leaves of the gallotannin producing plant *Rhus typhina* (staghorn sumac) and isolated in >99% purity by repeated chromatography on Sephadex LH-20 (Nishizawa et al., 1980) and preparative reversed-phase HPLC (Rausch and Gross, 1996); unlabelled pentagalloylglucose was isolated from dried leaves of *R. typhina* by the same techniques. Methods employed for native and SDS-PAGE and for protein determinations have been reported recently (Niemetz and Gross, 1998). Standard enzyme activity assays, containing 12.5 μg (42 Bq) pentagalloylglucose and enzyme (4 pkat) in 50 μl acetate buffer (50 mM, pH 5.0), were incubated at 30 °C for 30 min, stopped by heating (100 °C, 5 min), and analyzed by isocratic RP-18 HPLC under the following conditions: Reprosil NE, 5 μm, 250×4 mm i.d.; solvent 0.01% aq. TFA/EtOH/EtOAc (10:2:1; by vol.); flow rate 0.5 ml min^{-1}. Eluted substances were monitored at 280 nm; radioactivity was determined by fractionation of eluates and subsequent liquid-scintillation counting. Acid-denatured enzyme was used as a control.

3.3. Enzyme purification

All operations were performed at 0–4 °C. Fresh or frozen leaves (80 g) of *T. grandiflora* were homogenized in liquid N_2, extracted with 250 ml Tris–HCl (1.5 M, pH 8.0)/Na borate (0.2 M, pH 7.5) (1:1, by vol.) and centrifuged (30,000 *g*, 30 min). The supernatant was depleted of phenolics by stirring with Amberlite XAD (8 g,

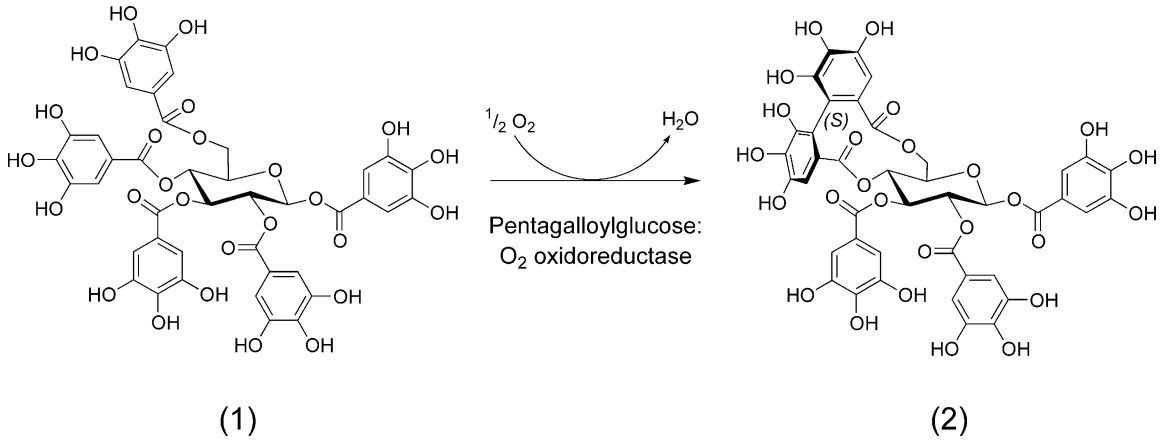

Fig. 3. Reaction scheme of the oxidation of pentagalloylglucose (**1**) to the ellagitannin, tellimagrandin II (**2**), by the laccase (EC 1.10.3.2)-type phenol oxidase, pentagalloylglucose: O_2 oxidoreductase, from leaves of *Tellima grandiflora* (fringe cups, Saxifragaceae).

borate form) for 20 min, filtered through glass wool and fractionated with solid $(NH_4)_2SO_4$. The pellet precipitating at 30–80% saturation was resuspended in acetate buffer (50 mM, pH 5.0), desalted on Pharmacia Biotech PD-10 columns, concentrated by ultrafiltration (Filtron Macrosep; exclusion limit 10,000 Da) and chromatographed on a Sephacryl S-300 (Pharmacia) column (40×2.4 cm i.d.) in acetate buffer. The active frs were combined, supplemented with 1.25 M ammonium sulfate and subjected to HIC on butyl-sepharose 'fast flow' (Pharmacia; column 17×16 mm i.d.) equilibrated with 50 mM acetate buffer (pH 5.0) plus 1.25 M ammonium sulfate. The column was successively washed with decreasing concs. of ammonium sulfate (1.25 M, 0.625 M, 0 M) in acetate buffer (pH 5.0), followed by washing with redist. H_2O. Enzyme activity was eluted in steps 2–4, the bulk being found in the salt-free buffer eluate. This fraction was chromatographed on a 10×12 mm i.d. column of hydroxyapatite (Bio-Gel HTP; Bio-Rad) equilibrated in 50 mM acetate buffer, pH 5.0. Washing with this buffer eliminated the bulk of inactive protein, while the phenol oxidase was eluted with 300 mM Na phosphate buffer, pH 5.0. The enzyme was subjected to a final chromatofocussing step on a 200×5 mm i.d. Mono P column (Pharmacia). As buffers were employed: A = 0.025 M bis-Tris (2[bis(2-hydroxyethyl)imino]-2-(hydroxymethyl)-1,3-propanediol; Serva, Heidelberg), adjusted to pH 7.1 with iminodiacetic acid (Sigma); B = Polybuffer 74 (Pharmacia), diluted 1:10 with redist. H_2O and adjusted to pH 4.0 with iminodiacetic acid. After equilibration with buffer A, elution of applied protein samples (100 µl) was achieved by a linear gradient (0–45 min) of 0–100% B in A (flow rate 0.5 ml min^{-1}). Proteins and pH values of column eluates were monitored by spectrophotometry (UV 280 nm) and with a flow-through pH-electrode.

3.4. Reaction product isolation

The reaction product, tellimagrandin II, was synthesized in a scaled-up enzyme assay mixture (400 ml vol., pH 5, containing 100 mg pentagalloylglucose and 850 mg protein) that was incubated for 30 min at 30 °C. After stopping the reaction by heat-denaturing the enzyme, the mixture was extracted with EtOAc. The contents of the dried organic phase were subjected to semi-preparative RP-18 HPLC on Kromasil (5 µ, 250×20 mm i.d.; gradient: solvent A = 0.01% aq. TFA, B = acetonitrile; 0–1 min 5% B, 1–2 min 5–18% B, then isocratic at 18% B; flow rate 22 ml min^{-1}). Relevant fractions were immediately depleted of organic solvent in vacuo, lyophilized and rechromatographed twice by isocratic RP-18 HPLC on Kromasil (5 µm, 250×20 mm i.d.; solvent 0.01% aq. TFA/EtOH/EtOAc = 10:2:1; by vol.; flow rate 12 ml min^{-1}). The reaction product-containing fractions were neutralized, depleted of solvent and lyophylized, affording 2 mg material of >90% purity as determined by analytical HPLC.

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References

Davin, L.B., Wang, H.-B., Crowell, A.L., Bedgar, D.L., Martin, D.M., Sarkanen, S., Lewis, N.G., 1997. Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. Science 275, 363-366.

De Pinto, M.C., Ros Barceló, A., 1996. Inhibition of both peroxidase and laccase by desferal (desferrioxamine mesylate). Phytochemistry 42, 283–286.

Erdtman, H., 1935. Phenoldehydrierungen VI. Dehydrierende Kupplung einiger Guajakolderivate. Svenska Kemisk Tidskrift 47, 223–230.

Feldman, S., Sahasrabudhe, K., 1999. Ellagitannin chemistry. Syntheses of tellimagrandin II and a dehydrodigalloyl ether-containing dimeric gallotannin analogue of coriariin A. Journal of Organic Chemistry 64, 209–216.

Ferrar, P.H., Walker, J.R.L., 1996. Inhibition of diphenol oxidase: a comparative study. Journal of Food Biochemistry 20, 15–30.

Fröhlich, B., Niemetz, R., Gross, G.G., 2002. Gallotannin biosynthesis: two new galloyltransferases from *Rhus typhina* leaves preferentially acylating hexa- and heptagalloylglucoses. Planta 216, 168–172.

Haslam, E., 1989. Plant Polyphenols. Vegetable Tannins Revisited. Cambridge University Press, Cambridge.

Haslam, E., 1998. Practical Polyphenols. From Structure to Molecular Recognition and Physiological Action. Cambridge University Press, Cambridge.

Hathway, D.E., 1957. The transformation of gallates into ellagate. Biochemical Journal 67, 445–450.

Kahn, V., Andrawis, A., 1985. Inhibition of mushroom tyrosinase by tropolone. Phytochemistry 24, 905–908.

Khanbabaee, K., van Ree, T., 2001. Strategies for the synthesis of ellagitannins. Synthesis, 1585–1610.

Leech, D., Daigle, F., 1998. Optimisation of a reagentless laccase electrode for the detection of the inhibitor azide. The Analyst 123, 1971–1974.

Lewis, N.G., Davin, L.B., 2000. Phenolic coupling *in planta*: dirigent proteins, dirigent sites and notions beyond randomness. Part 1. Polyphénols Actualités 20, 18–26.

Lineweaver, H., Burk, D., 1934. The determination of enzyme dissociation constants. Journal of the American Chemical Society 56, 658–666.

Mayer, W., Hoffmann, E.H., Lösch, N., Wolf, H., Wolter, B., Schilling, G., 1984. Dehydrierungsreaktionen mit Gallussäureestern. Liebigs Annalen der Chemie, 929–938.

Niehaus, J.U., Gross, G.G., 1997. A gallotannin degrading esterase from leaves of pedunculate oak. Phytochemistry 45, 1555–1560.

Niemetz, R., Gross, G.G., 1998. Gallotannin biosynthesis: purification of β-glucogallin: 1,2,3,4,6-pentagalloyl-β-D-glucose galloyltransferase from sumac leaves. Phytochemistry 49, 327–332.

Niemetz, R., Schilling, G., Gross, G.G., 2001. Ellagitannin biosynthesis: oxidation of pentagalloylglucose to tellimagrandin II by an enzyme from *Tellima grandiflora* leaves. Chemical Communications, 35–36.

Nishizawa, M., Yamagishi, T., Nonaka, T., Nishioka, I., 1980. Structure of gallotannins in *Paeoniae radix*. Chemical and Pharmaceutical Bulletin 28, 2850–2852.

Pedreño, M.-A., Ros Barceló, A., 1999. Inhibition by ferulic acid of the oxidase activity of a cell wall-located basic peroxidase involved in lignification in *Z. elegans*. International Symposium for Plant Peroxidases, Columbus, Ohio, USA.

Rausch, H., Gross, G.G., 1996. Preparation of [^{14}C]-labelled 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose and related gallotannins. Zeitschrift für Naturforschung 51c, 473–476.

Schmidt, O.Th., Mayer, W., 1956. Natürliche Gerbstoffe. Angewandte Chemie 68, 103–115.

Tanaka, N., Shimomura, K., Ishimaru, K., 2001. Ellagic acid formation from galloylglucoses by a crude enzyme of *Cornus capitata* adventitious roots. Bioscience, Biotechnology and Biochemistry 65, 1869–1871.

Walker, J.R.L., McCallion, R.F., 1980. The selective inhibition of *ortho*- and *para*-diphenol oxidases. Phytochemistry 19, 373–377.