Tetrahedron 73 (2017) 500–507

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Enzymatic oxidation of ellagitannin and a new ellagitannin metabolite from *Camellia japonica* leaves

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ARTICLE INFO

Article history:
Received 12 October 2016
Received in revised form
8 December 2016
Accepted 13 December 2016
Available online 18 December 2016

Keywords:
Camellia japonica
Ellagitannin
Oxidation
Catechin
2*H*-2-Oxo-pyran-6-carboxylic acid
Camelliatannin

ABSTRACT

Polyphenols in the leaves of *Camellia japonica* L. at different stages of growth were analyzed by HPLC. Pedunculagin [2,3; 4,6-bis-(*S*)-hexahydroxydiphenoyl-D-glucose] was the major polyphenol in the youngest leaves. The levels of this compound and (+)-catechin decreased as the leaves matured. The level of (−)-epicatechin did not change with leaf maturity. Enzymatic oxidation of pedunculagin was examined to investigate the mechanism of the decrease. Pedunculagin was not directly oxidized by treatment with oxidative enzymes. However, when (+)-catechin was added to the reaction mixture, the pyrogallol rings of the 4,6-hexahydroxydiphenoyl group was oxidatively cleaved to give 2*H*-2-oxo-pyran-6-carboxylic acid. The in vitro oxidation products were not detected in fresh leaves, but two pedunculagin oxidation products conjugated with flavan-3-ol were isolated. A new metabolite, camelliatannin I, was isolated and characterized by spectroscopic and density functional theory calculation of NMR chemical shifts.

1. Introduction

Tannins are widely distributed in the plant kingdom, and form part of the plant defense system because of their ability to precipitate proteins that cause unpleasant astringent and bitter tastes. These compounds inhibit the digestive enzymes of herbivores, and decrease nutrient absorption.[1] Unlike highly toxic alkaloids and terpenoids, which are targeted to specific biogenic molecules or biological functions, the toxicity of tannins is nonspecific. Therefore, large quantities of tannins are synthesized and accumulate in tannin-containing plants for effective protection against herbivores and microorganisms.[2] However, the relationship between the structures of tannins and their biological functions is unclear. Feeny showed that seasonal changes in the tannins in oak leaves affect feeding behavior of larvae of the winter moth (*Operophtera brumata* L.).[3] In persimmon, proanthocyanidins precipitate when the seeds are ready for germination, and this makes the fruit more enticing to animals to eat and disperse the seeds.[4] Seasonal changes in the tannins in other plant species have also been reported and discussed from various viewpoints, such as chemical ecology and tannin biosynthesis.[5] However, chemical mechanism of the seasonal change of tannins is not fully understood. In our chemical studies on tannin metabolism in plants, we found changes in the tannin composition in the young leaves of *Camellia japonica* L., which is a well-known ornamental flowering tree originating from Japan, Korea, and China. Young leaves of *C. japonica* collected in April contained the ellagitannin pedunculagin (**1**) and (+)-catechin (**2**), (−)-epicatechin (**3**), and procyanidins as major polyphenols. However, the levels of **1** and **2** in leaves collected in August were negligible. Leaves on a single twig collected in May also showed decreased levels of **1** and **2** with increasing leaf maturity (Figs. 1 and 2). In this paper, we studied enzymatic degradation of **1** assuming that the levels of **1** in the leaves decreased because of oxidative degradation. In addition, the oxidation metabolites of ellagitannins in the leaves were also investigated.

2. Results and discussion

2.1. Enzymatic oxidation of pedunculagin

Previous studies indicated that *C. japonica* leaves contain dimeric ellagitannins and flavan-3-ol conjugated ellagitannins,[6] which are biogenetically related to **1**. Therefore, conversion of **1** to these metabolites during leaf growth is possible. However, preliminary experiments involving fractionation of extracts of young and mature leaves by Sephadex LH-20 column chromatography,

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http://dx.doi.org/10.1016/j.tet.2016.12.027
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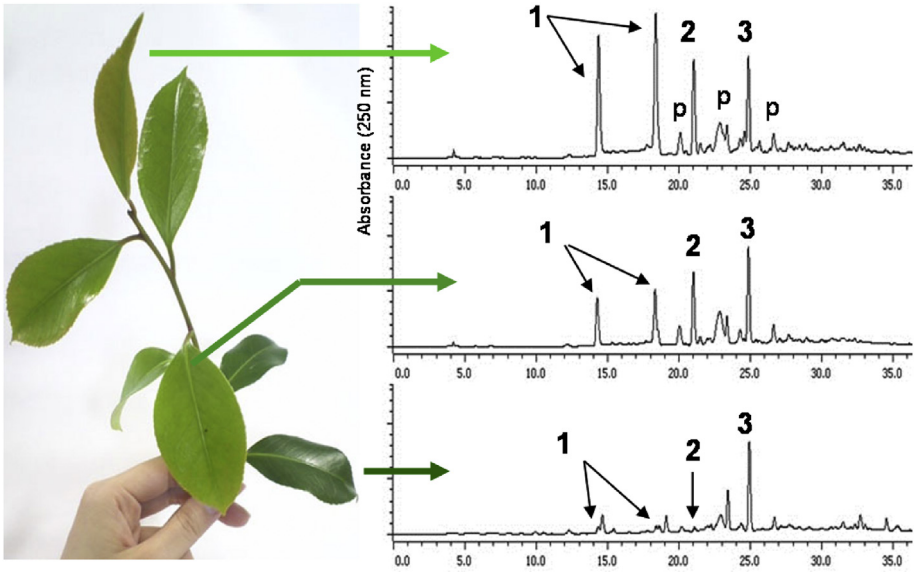

Fig. 1. HPLC profiles of 60% ethanol extracts of the leaves of *C. japonica*. Compound labels are: **1**, α- and β-anomers of pedunculagin; **2**, catechin; **3**, epicatechin; and p, procyanidins.

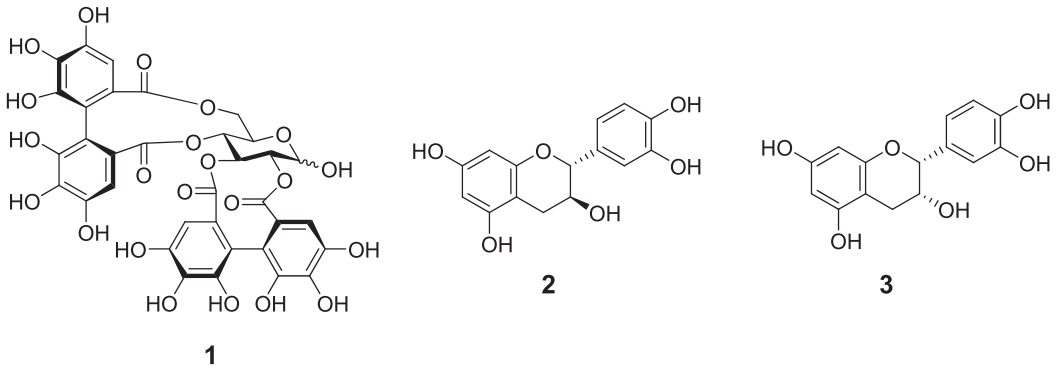

Fig. 2. Structures of major polyphenols found in *C. japonica* leaves.

and subsequent HPLC and TLC analyses of the phenolic fractions, did not show an apparent increase of specific ellagitannin metabolites, including the ellagitannin dimers. Therefore, we assumed that the decrease in **1** was caused by enzymatic oxidation of the pyrogallol rings, because electron rich polyphenols are easily oxidized both chemically and enzymatically as observed in tea leaves during black tea production.[7] We used a homogenate of Japanese pear fruit as a source of polyphenol oxidase by taking account of strong activity of polyphenol oxidase, and the oxidation products of tea catechins are almost the same as that produced during black tea production. Furthermore, Japanese pear fruit homogenate shows practically no background peaks in HPLC analysis and is easily applied to large-scale experiments.[7b,c] In this study, pedunculagin (**1**) was first treated with the Japanese pear fruit homogenate, but this did not oxidize **1** and its levels did not decrease (Fig. 3). Next, (+)-catechin (**2**) was added to the reaction mixture (0.5 molar equivalents), since **2** is also present in *C. japonica* leaves and its level decreases along with that of **1**. Addition of **2** dramatically changed the reaction, and **1** immediately disappeared.

By contrast, when **2** was treated solely with the Japanese pear fruit homogenate, the level of **2** decreased rapidly to give a complex mixture of oxidation products, including dehydrodicatechin-type oligomers.[7c,8] The rate of decrease of **2** was much faster than that in the reaction in the presence of **1**. This could be explained by a

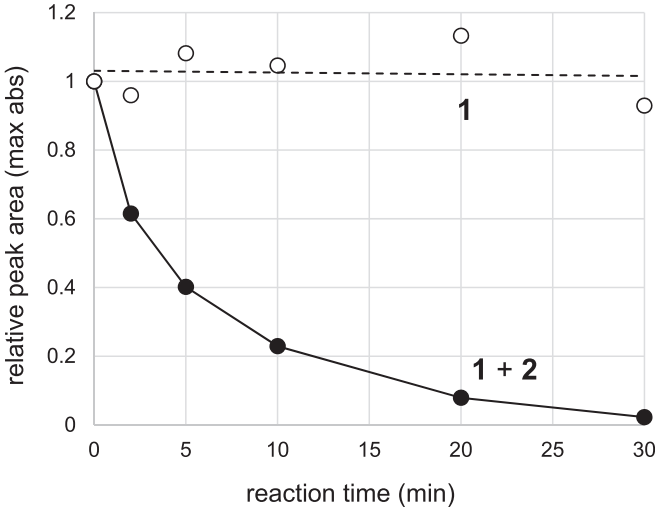

Fig. 3. Decrease in the level of **1** on treatment with polyphenol oxidase Open circle: **1** + polyphenol oxidase, solid circle: **1** + **2** + polyphenol oxidase.