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SEASONAL CHANGES IN THE TANNINS OF *LIQUIDAMBAR FORMOSANA* REFLECTING THEIR BIOGENESIS

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Key Word Index—*Liquidambar formosana*; Hamamelidaceae; tannin; hydrolysable tannin; galloylglucose; gallotannin; ellagitannin; biogenesis; seasonal change of structures.

Abstract—Fractionation of the tannins in the leaf of *Liquidambar formosana* collected at different seasons showed that tellimagrandin II is the main component in early spring, while casuarinin and pedunculagin are the main tannins in autumn. Hydrolysable tannins structurally related to them, tellimagrandin I, casuariin, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose and casuarictin were isolated from the leaf collected in November, and 1,2,6-tri-*O*-galloyl-β-D-glucose from the leaf in May. From HPLC analyses of leaf extracts collected from April to November, it was clear that the composition changes most rapidly in the spring. The seasonal changes in the structures and amounts of these hydrolysable tannins indicate a particular biogenetic pathway for these substances.

INTRODUCTION

The biogenetic pathway of the hydrolysable tannins has been discussed in terms of their structures [1, 2]. We have proposed a possible pathway [3], since some species of Casuarinaceae, Stachyuraceae and Myrtaceae were found to contain several ellagitannins with inter-related structures. Among these tannins, the *C*-glucosidic ellagitannins [e.g. casuariin (**1**) and casuarinin (**2**)] may be produced from the tannins having both galloyl and hexahydroxydiphenoyl (HHDP) groups [e.g. tellimagrandin I (**3**) and II (**4**)] *via* the key compound, pedunculagin (**5**) [3]. One of them, tellimagrandin II (**4**) could be produced from penta-*O*-galloyl-β-D-glucose (**6**) through the oxidative coupling of two galloyl groups at O-4 and O-6 of glucose in **6** [2].

Our present investigation of hydrolysable tannins in the leaf of *Liquidambar formosana* Hance (Hamamelidaceae), a Chinese medicinal plant [4], showed the presence of several tannins which are identical to those found in the species of the three families mentioned above. The isolation of these tannins from the leaves collected in May and November, and the estimation of the contents of these tannins in the leaves collected in each month from April to November with HPLC, revealed remarkable structural changes related to their biogenesis.

RESULTS AND DISCUSSION

The concentrate from the homogenate of fresh leaf of *L. formosana* collected in November, was extracted with Et_2O, EtOAc and *n*-BuOH, successively. Column chromatography on Sephadex LH-20 and Toyopearl HW-40 of the EtOAc extract gave casuarinin (**2**) and pedunculagin (**5**) as the main components, along with tellimagrandin I (**3**), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (**7**) and casuarictin (**8**). Chromatography of the *n*-BuOH extract afforded **2**, **5** and casuariin (**1**).

Leaves collected in May afforded 1,2,6-tri-*O*-galloyl-β-D-glucose (**9**) and tellimagrandin II (**4**). The analyses of the crude leaf extracts also showed that these tannins are the main components.

The analyses of the seasonal change with HPLC (Table 1) indicated that the concentration of tellimagrandin II (**4**), which is abundant in the leaves collected in early spring, rapidly decreases within a month, and the amount present in summer is almost negligible. Conversely, the amounts of casuarinin (**2**) and pedunculagin (**5**) increase with time, and they become the main tannins in summer and autumn. These results are in keeping with the biogenetic pathway outlined in Scheme 1.

In this pathway, the *C*-glucosidic ellagitannin, casuarinin (**2**) which is abundant in the leaves of summer and autumn, is the product of C–C bond formation accompanied by the opening of the glucopyranose ring in pedunculagin (**5**) or its analogues, and is adequately located near the end of the pathway (Scheme 1). Pedunculagin (**5**) can be regarded as the product of oxidative coupling between two galloyl groups in tellimagrandin I (**3**), although it may also be produced by degalloylation of casuarictin (**8**). The present investigation also shows that penta-*O*-galloyl-β-D-glucose (**6**), which is found only in the early spring leaf, is produced at an early stage in biogenesis, and is a precursor of tellimagrandin II (**4**).

EXPERIMENTAL

Isolation of tannins of Liquidambar formosana. Leaves were collected from a single tree grown in the Herbal Garden of Toyama Medical and Pharmaceutical University, Toyama, Japan, and homogenized immediately after collection.

Fresh leaves (1 kg) collected in November, 1983, were homogenized in Me_2CO–H_2O (7:3). After filtration, the filtrate was

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Table 1. Seasonal variation in tannins (fresh leaf content %)

	Date of collection							
	April 23	May 22	June 22	July 23	August 23	September 30	October 23	November 25
Casuarinin (2)	0.02	0.29	0.42	0.80	0.78	0.62	0.61	0.43
Pedunculagin (5)	0.16	0.36	0.29	0.26	0.31	0.23	0.22	0.19
Casuarictin (8)	0.22	0.13	0.03	0.03	0.01	0.02	0.02	0.01
Tellimagrandin II (4)	0.37	0.03	0.03	0.02	0.01	—*	—	—
1,2,6-Tri-*O*-galloylglucose (9)	0.08	0.01	0.01	0.01	trace	—	—	—
Penta-*O*-galloylglucose (6)	0.03	—	—	—	—	—	—	—

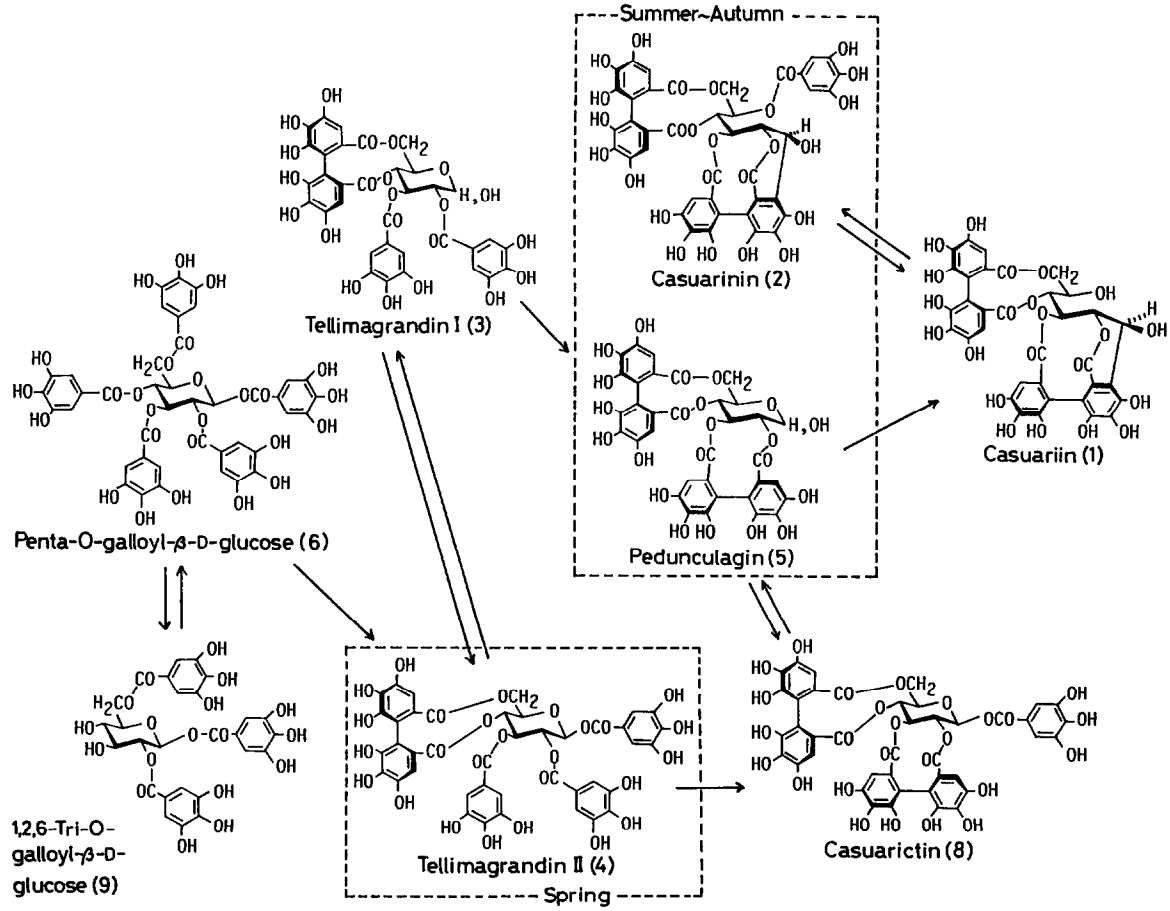
* Less than *ca* 0.003%.

concentrated *in vacuo* below 40°. The resulting aq. soln was extracted with Et_2O, EtOAc and *n*-BuOH, successively, and each soln was evaporated. A portion (5 g) of the EtOAc extract (13 g) was chromatographed over Sephadex LH-20 eluted with EtOH–H_2O (7:3). Combined fractions containing tannins were further purified by CC on Toyopearl HW-40 (fine grade) eluted with EtOH–H_2O (1:1), and afforded tellimagrandin I (3, 11 mg) [3], 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (7, 11 mg) [5], pedunculagin (5, 69 mg) [3] and casuarictin (8, 10 mg) [3]. A portion (6.6 g) of the *n*-BuOH extract (27 g) was submitted to CC on Sephadex LH-20, and gave casuariin (1, 26 mg) [3], pedunculagin (5, 179 mg) and casuarinin (2, 439 mg).

Fresh leaves (1 kg) collected in May, 1984 were similarly treated, and CC of the EtOAc extract (8 g) on Sephadex LH-20 afforded 1,2,6-tri-*O*-galloyl-β-D-glucose (9, 176 mg) [6] and tellimagrandin II (4, 239 mg) [3].

Quantitative analyses of tannins in leaves collected every month. Leaves were collected from a single tree grown at Okayama University, Okayama, Japan, and homogenized immediately after collection.

Fresh leaves (*ca* 12 g) collected in each month from April (when new leaves appear) to November (just before the leaves fall), 1985, were homogenized in (30 ml × 2) Me_2CO–H_2O (7:3) and centrifuged (3000 rpm, 10 min). The combined supernatant was evaporated at under 40°. A portion (6 mg) of the residue was dissolved in MeOH (1 ml) and the soln was analysed by HPLC as follows. Column: YMC A312 (ODS, 150 × 6 mm, Yamamura-Kagaku, Kyoto, Japan). Solvents: (A) 50 mM H_3PO_4 aq.–50 mM



Scheme 1. Biogenesis of hydrolysable tannins in *Liquidambar formosana*.

KH_2PO_4 aq.–MeOH (9:9:2); (B) 50 mM H_3PO_4 aq.–50 mM KH_2PO_4 aq.–MeOH (3:3:4). Elution profile: 0–10 min, solvent A; 10–30 min, 0–100% solvent B in solvent A (linear gradient); 30–50 min, solvent B. Flow rate: 1.2 ml/min. Temperature: 40° (in an oven). Detection and peak identification: 230–400 nm (MCPD-350PC diode-array detector, Union-Giken, Hirakata, Osaka, Japan) and 280 nm (Shimadzu SPD-6A, Shimadzu, Kyoto, Japan). R_ts of tannins: pedunculagin, 4.3 and 8.8 min (anomeric mixture); casuarinin, 19.3 min; casuarictin, 24.4 min; 1,2,6-tri-*O*-galloyl-β-D-glucose, 25.7 min; tellimagrandin II, 26.6 min; penta-*O*-galloyl-β-D-glucose, 31.7 min. A Shimadzu CR3-A integrator (Shimadzu) was used for quantitation.

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