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LIQUIDAMBIN, AN ELLAGITANNIN FROM *LIQUIDAMBAR FORMOSANA*

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Key Word Index—*Liquidambar formosana*; Hamamelidaceae; tannin; hydrolysable tannin; ellagitannin; liquidambin; equilibration; biogenesis; casuarinin; pedunculagin.

Abstract—A new ellagitannin, named liquidambin, which could be biogenetically closely correlated with casuarinin and pedunculagin, has been isolated from the leaves of *Liquidambar formosana*. Its structure was determined as 5-*O*-galloyl-2,3,4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-D-glucose. The structural equilibration due to hydration of the aldehyde group of the glucose core in this tannin was shown from its ^{1}H and ^{13}C NMR spectra.

INTRODUCTION

Previously, we reported remarkable changes in the tannin composition in the leaves of *Liquidambar formosana* Hance during the spring [1]. This change is in accord with the proposed biogenetic pathway [2] from galloylglucoses and tellimagrandin II to *C*-glucosidic casuarinin (1) via pedunculagin (2). We now describe the isolation and structure elucidation of an additional new tannin in the leaves, named liquidambin, which could be biogenetically closely correlated to both 1 and 2.

RESULTS AND DISCUSSION

Liquidambin (3) was isolated from the *n*-BuOH soluble portion of the extract of *L. formosana* leaves by means of centrifugal partition chromatography (CPC) [3] in combination with CC over MCI gel CHP-20P.

The tannin, $C_{41}H_{28}O_{26} \cdot 7H_2O$, was obtained as an off-white amorphous powder. Although liquidambin shows a complicated ^{1}H NMR spectrum (in Me_2CO-d_6) due to the formation of an equilibrium mixture, the signals of a galloyl group [δ7.12 (2H, *s*)] and two hexahydroxydiphenoyl (HHDP) groups [δ6.75, 6.74, 6.72 and 6.59 (1H each, *s*)] of the major component in the equilibrium mixture of 3, and those of the minor component [δ7.15 (2H, *s*) (galloyl); 6.75, 6.65, 6.63 and 6.61 (1H each, *s*) (HHDP)] were observed.

Reduction of 3 by $NaBH_4$ afforded a product, $C_{41}H_{30}O_{26} \cdot 2H_2O$ (4). Its ^{1}H NMR spectrum (400 MHz, in Me_2CO-d_6) indicates the presence of a galloyl group [δ7.12 (2H, *s*)], two HHDP groups [δ6.74, 6.70, 6.58 and 6.57 (1H each, *s*)] and a glucitol core [δ5.61 (*dd*, J = 1, 8.5 Hz, H-4), 5.60 (*dd*, J = 2.5, 8.5 Hz, H-5), 5.51 (*dd*, J

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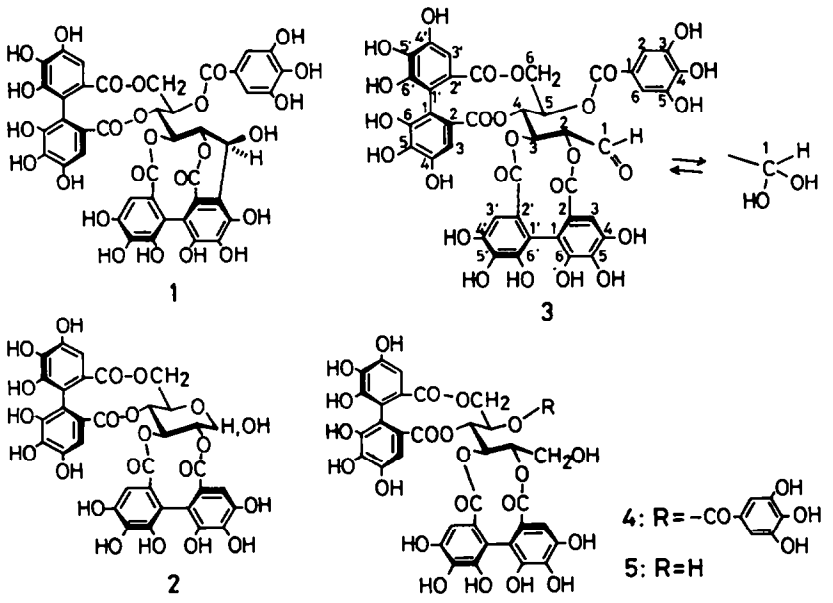

= 1, 9.5 Hz, H-3), 5.09 (*ddd*, J = 2, 5, 9.5 Hz, H-2), 4.88 (*dd*, J = 2.5, 13 Hz, H-6_a), 4.32 (*t*, J = 6 Hz, proton of the hydroxyl group at C-1), 4.10 (*d*, J = 13 Hz, H-6_b), 4.05 (*ddd*, J = 5, 6, 12 Hz, H-1_a) and 3.97 (*ddd*, J = 2, 6, 12 Hz, H-1_b)].

Although liquidambin is not identical with casuarictin [= 1-*O*-galloyl-2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucose] [4] or potentillin [= 1-*O*-galloyl-2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-α-D-glucose] [5], partial hydrolysis of liquidambin (**3**) with tannase gave pedunculagin, 2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-D-glucose (**2**) [4]. Comparison of the chemical shifts of the glucitol protons of **4** with those of 2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-D-glucitol (**5**), which was produced from **2**, shows a downfield shift of H-5 in **4** from that in **5** (δ4.27 → 5.60). Therefore, the position of the galloyl group in **3** was assigned to be at O-5 of the glucose core. This assignment was further verified by treatment of **3** with *p*-toluenesulphonic acid, which afforded casuarinin (**1**).

Thus, liquidambin has structure **3**, in which two (*S*)-hexahydroxydiphenoyl groups are located at O-2–O-3 and O-4–O-6 of the glucose core, and the galloyl group is located at O-5. Although the presence of an aldehyde group at C-1 of the glucose core of **3** was shown by a signal at δ9.77 in the ^{1}H NMR spectrum and shown by a signal at δ194.61 in the ^{13}C NMR spectrum of **3**, the aldehyde group is to a large extent hydrated as shown by the signal at δ87.99 in the ^{13}C NMR spectrum. The ratio of the major and minor components was estimated to be 9:7, based on the peak areas of the aromatic protons in the ^{1}H NMR spectrum.

The facile chemical transformation of liquidambin into casuarinin suggests that liquidambin could be a precursor of casuarinin biosynthesis in *L. formosana*. Although the result of enzymatic transformation of liquidambin into pedunculagin suggests that biosynthesis of pedunculagin may occur by this route, pedunculagin will not be produced from liquidambin in most species, as liquidambin has not been found in the other plant species in spite of the wide distribution of pedunculagin [2, 6].

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were measured at 400 MHz and 100 MHz, respectively with TMS as int. std. TLC was carried out on Avicel SF cellulose plates (0.3 mm) using 7% HOAc as developer.

Isolation. Fresh leaves (3.5 kg) were collected in May, 1986, from a tree of *L. formosana* Hance grown at Okayama University, and were homogenized in Me_2CO–H_2O (7:3, 14 l). After filtration, the filtrate was concd *in vacuo* and extracted with Et_2O, EtOAc and *n*-BuOH, successively. An aliquot (3 g) of the *n*-BuOH extract (36 g) was subjected to CPC on a Sanki L-90 centrifugal partition chromatograph [3] using *n*-BuOH–*n*-PrOH–H_2O (4:1:5) and reversed-phase development; 10 g portions of the eluate were collected. Combined fractions 16–30 were evapd and subjected to CPC using the same solvent system but normal-phase development: 5 g fractions were collected. Combined fractions 30–46 were evapd and dissolved in H_2O. The aq. soln was acidified to pH 2 with 10% HCl and subjected to CC over MCI gel CHP-20P (75–150 μm) with H_2O and then MeOH as eluants. The MeOH eluate was further purified by CC over MCI gel CHP-20P with 20% MeOH as eluant to afford liquidambin (20 mg).

Liquidambin (**3**). Pale yellow amorphous powder, R_f on TLC, 0.41. $[\alpha]_D$ +69° (Me_2CO; *c* 0.5). (Found: C, 46.43; H, 3.67. $C_{41}H_{28}O_{26}\cdot 7H_2O$ requires: C, 46.34; H, 3.98.) UV λ_{max}^{MeOH} nm (log ε): 219 (4.88), 270 sh (4.55). IR ν_{max}^{KBr} cm^{-1}: 3400, 1740–1710, 1610, 1510, 1450, 1360–1310, 1230–1180, 1030. ^{1}H NMR (Me_2CO-d_6): δ7.12 (2H, *s*, galloyl), 6.75, 6.74, 6.72, 6.59 (1H each, *s*, 2 × HHDP), 5.62 [*dd*, J = 1.5, 9 Hz, glucose (glu) H-4], 5.57 (*dd*, J = 3.5, 9 Hz, glu H-5), 5.47 (*dd*, J = 1.5, 9 Hz, glu H-3), 5.05 (*dd*, J = 2, 9 Hz, glu H-2), 4.87 (*dd*, J = 3.5, 13 Hz, glu H-6_a), 4.12 (*d*, J = 13 Hz, glu H-6_b) [major (hydrated) form]; δ9.77 (1H, *s*, glu H-1), 7.15 (2H, *s*, galloyl), 6.75, 6.65, 6.62, 6.61 (1H each, *s*, 2 × HHDP) 5.69 (*dd*, J = 1, 9 Hz, glu H-4), 5.67 (*dd*, J = 3, 9 Hz, glu H-5), 5.54 (*dd*, J = 1, 9.5 Hz, H-3), 5.52 (*d*, J = 9.5 Hz, glu H-2), 5.03 (*dd*, J = 3, 13 Hz, glu H-6_a), 4.09 (*d*, J = 13 Hz, glu H-6_b) [minor (aldehydo) form]. ^{13}C NMR (Me_2CO-d_6): δ64.79, 64.83 (glu C-6), 70.08, 70.16, 70.37, 70.75, 72.56, 74.98, 75.80, 77.92, (glu C-2–C-5), 87.99 (glu C-1), 107.40, 107.60, 107.64, 107.76, 107.83, 107.91, 108.28, 108.41 (HHDP C-3,3'), 110.35 (galloyl C-2,6), 113.68, 113.97, 114.02, 114.07, 115.20, 115.35, 116.07, 116.40 (HHDP C-1,1'), 120.72, 120.89 (galloyl C-1), 124.88, 125.24, 125.97, 126.71, 126.79, 127.12, 127.31, 127.40 (HHDP C-2,2'), 136.06, 136.09, 136.14, 136.22, 136.30, 136.52, 136.92, 137.20 (HHDP C-5,5'), 139.36, 139.46 (galloyl C-4), 144.00, 144.31, 144.37, 144.50, 144.64, 144.70, 144.86, 144.97, 145.24, 145.31, 145.37, 145.41, 145.51 (HHDP C-4,4',6,6'), 146.02, 146.08 (galloyl C-3,5), 165.81, 165.88, 167.92, 168.01, 168.19, 168.38, 168.64, 168.72, 168.92, 168.98 (ester carbonyl), 194.61 (glu C-1).

Reduction of liquidambin. To a MeOH soln (1 ml) of liquidambin (12 mg) was added $NaBH_4$ (10 mg). The soln was left to stand for 10 min at room temp. and then it was acidified with HOAc (0.5 ml) and evapd. The residue was dissolved in H_2O (1 ml) and acidified to pH 2 with 10% HCl. The aq. soln was passed through a SEP-PAK C_{18} cartridge (Waters Associates), and the cartridge was eluted with H_2O and then with MeOH. The MeOH eluate was evapd to give 5-*O*-galloyl-2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-D-glucitol (**4**) (11 mg). R_f on TLC, 0.46, $[\alpha]_D$ +98° (MeOH; *c* 0.5). (Found: C, 50.28; H, 3.88. $C_{41}H_{30}O_{26}\cdot 2H_2O$ requires C, 50.52; H, 3.52.) ^{1}H NMR: see text.

Partial hydrolysis of liquidambin. An aq. soln (1 ml) of liquidambin (10 mg) was treated with tannase at 37° for 22 hr. The resulting soln was acidified to pH 2 with 10% HCl and then subjected to SEP-PAK C_{18} cartridge treatment eluting with H_2O and then MeOH. The MeOH eluate was evapd and then subjected to prep. TLC to afford pedunculagin (**2**) (3 mg) [4].

Reduction of pedunculagin (**2**). A MeOH soln (1 ml) of pedunculagin (10 mg) was treated with $NaBH_4$ (10 mg) for 5 min. The soln was then acidified with HOAc (0.5 ml) and evapd. The residue was dissolved in H_2O (1 ml) and acidified to pH 2 with 10% HCl. The soln was subjected to CC on MCI gel CHP-20P using H_2O and then MeOH as eluants. The MeOH eluate was evapd to give 9 mg of 2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-D-glucitol (**5**). R_f on TLC, 0.62, $[\alpha]_D$ +186° (MeOH; *c* 0.5). (Found: C, 47.13; H, 3.85. $C_{34}H_{26}O_{22}\cdot 4H_2O$ requires C, 47.56; H, 3.99.) ^{1}H NMR (Me_2CO-d_6 + D_2O): δ6.65 (*s*), 6.64 (2H, *s*), 6.52 (*s*) (2 × HHDP), 5.61 (*dd*, J = 1.5, 9.5 Hz, glucitol H-3), 5.22 (*dd*, J = 1.5, 8.5 Hz, H-4), 5.05 (*ddd*, J = 2.5, 5, 9.5 Hz, H-2), 4.70 (*dd*, J = 3, 12 Hz, H-6_a), 4.27 (*dd*, J = 2.5, 8.5 Hz, H-5), 3.97 (*dd*, J = 2.5, 13 Hz, H-1_a), 3.94 (*d*, J = 12 Hz, H-6_b), 3.94 (*dd*, J = 5, 13 Hz, H-1_b).

Transformation of liquidambin (**3**) *into casuarinin* (**1**). A mixture of liquidambin (10 mg), *p*-toluenesulphonic acid (5 mg) and dioxan (1 ml) was heated at 100° in a sealed tube for 1 hr and then evapd. The residue was dissolved in H_2O (1 ml) and subjected to a SEP-PAK C_{18} cartridge treatment using H_2O and then MeOH as eluants. The MeOH eluate was further purified by prep. TLC to give casuarinin (**1**) (4 mg) [4].

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