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HYDROLYSABLE TANNINS AND RELATED COMPOUNDS FROM *CASTANEA MOLLISSIMA**

HUIMING FENG, GEN-ICHIRO NONAKA† and ITSUO NISHIOKA†‡

Department of Forest Products, Chemistry and Engineering, Nanjing Forestry University, Nanjing, China,† Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

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Key Word Index—*Castanea mollissima*, Fagaceae, tannins, 3,4,5-trihydroxybenzylalcohol, 3,4,5-trihydroxybenzaldehyde, gallic acid, *m*-dehydrodigallic acid, *p*-dehydrodigallic acid

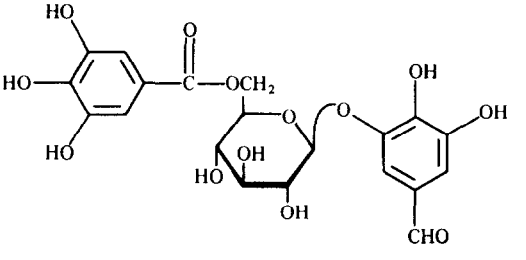
Abstract—A chemical examination of the leaves of *Castanea mollissima* has led to the characterization of four new polyphenolic compounds, castamollissin, isochesnatin, isochestanin and castanin In addition, the occurrence of 20 known hydrolysable tannins and related compounds was demonstrated

INTRODUCTION

In contrast to widely distributed gallic acid esters, the derivatives of its probable metabolite, 3,4,5-trihydroxybenzylalcohol, have not commonly been encountered in nature Previous work [1–7] demonstrated that the Fagaceous plants, *Castanea crenata* Sieb *et* Zucc and *Castanopsis cuspidata* var *sieboldii* Nakai, accumulate such benzylalcohol derivatives in large amounts Considering that gallic acid is usually metabolised in higher plants to hexahydroxydiphenic acid [8, 9] and dehydrodigallic acid [10], and further to flavogallonic acid [11], sanguisorbic acid [12], valoneaic acid [13], tergallic acid [14], gallagic acid [15], etc. through oxidative carbon–carbon and/or carbon–oxygen coupling(s), the metabolism of gallic acid in these plants is rather unusual To extend our work, we have now undertaken the chemical analysis of the polyphenolic compounds occurring in the allied species, *Castanea mollissima* Bl., which is a large deciduous tree planted in China and Korea for ornamental and food purposes

RESULTS AND DISCUSSION

The leaves of *C mollissima*, were extracted with 60% aqueous acetone Subsequent combined chromatography of the extract over several types of reverse-phase gels afforded 23 compounds (**1–23**) The isolated compounds were classified into four groups based on their structural features. The first class of compounds (**1–8**) possessed 3,4,5-trihydroxybenzylalcohol and/or dehydrodigallic acid ester moiety in their molecule, and among these, **4–8** were identified as dehydrodigallic acid [10], chesnatin [2], chestanin [7], cretanin [4] and a mixture of 6′- and 6″-*O*-galloyl chestanin [1], respectively, by comparisons of their spectral data with those of authentic samples previously obtained from *C crenata* The second class (**9–14**) consisted of ellagitannins with a *C*-glycosidic linkage, among which **9–11**, **13** and **14** were identified as casuarinin [16], casuariin [16], stachyurin [16], vescalagin [17, 18] and grandinin [unpublished results], respectively Casuarinin (**9**) was the major polyphenol in this plant material, whereas in *C crenata* galloyl hamameloses and 3,4,5-trihydroxybenzylalcohol glycosides are the major metabolites [1, 19]. Compound **12** (named castanin) was a desgalloyl stachyurin as revealed by ^{1}H and ^{13}C NMR examinations and has not yet been isolated from a natural source Confirmation of its structure was achieved by partial hydrolysis of stachyurin (**11**) with tannase, which afforded gallic acid and **12**. The third group (**15–22**) included ellagitannins based on a glucopyranose core with $^4C_1$ conformation. Comparison of their ^{1}H NMR data with those of previously isolated samples established their identities as 2,3-hexahydroxy (HHDP)-1-*O*-(sanguin H-5) (**15**) [20], 2,3-HHDP-4-(**16**) [unpublished results], 4,6-HHDP-1-*O*-(strictinin) (**17**) [16], 4,6-HHDP-2-*O*-(**18**) [unpublished results], 4,6-HHDP-3-*O*-(gemin D) (**19**) [21] and 4,6-HHDP-2,3-di-*O*-galloylglucoses (**22**) [9], 2,3·4,6-HHDP-glucose (pedunculagin) (**20**) [22, 23] and 2,3-HHDP-4,6-valoneaylglucose (**21**) [24]. The remaining compound was found to be identical with the simple gallic acid ester, β-D-glucogallin (**23**).

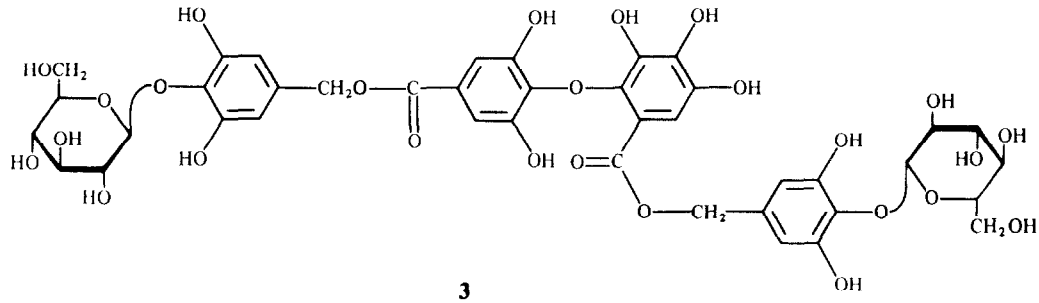


1

Compound **1** appeared to be new and was designated as castamollissin The ^{1}H NMR spectrum showed a two-proton singlet at δ7.19 typical of a gallic acid ester and

*Part 65 in the series 'Tannins and Related Compounds' For Part 64 see ref [1]

‡Author to whom correspondence should be addressed



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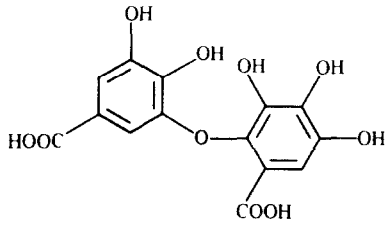

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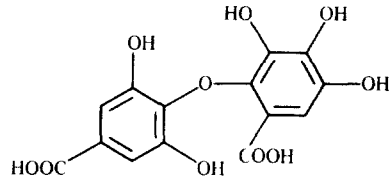
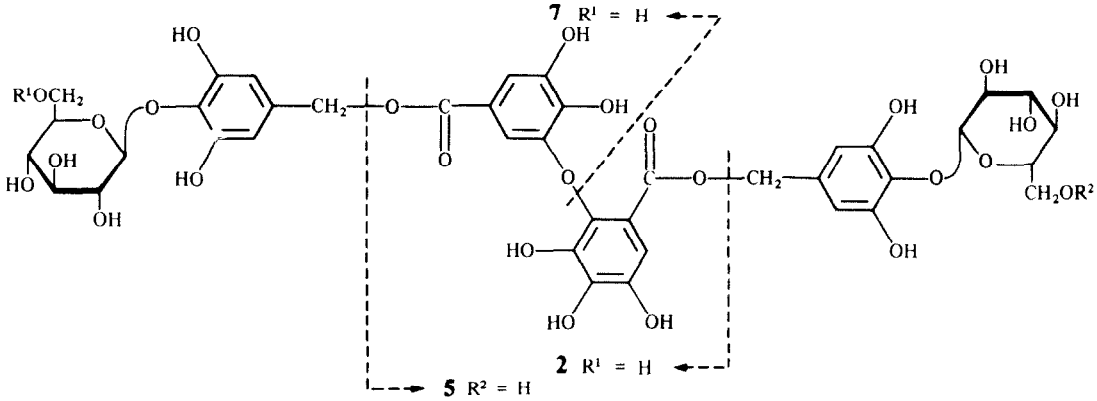
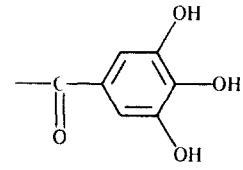


3a



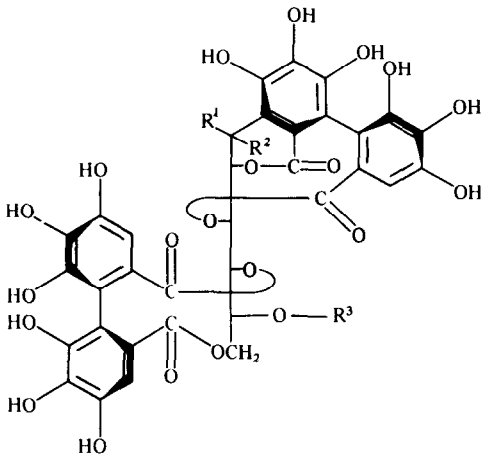
6 R^1, R^2 = H Gal



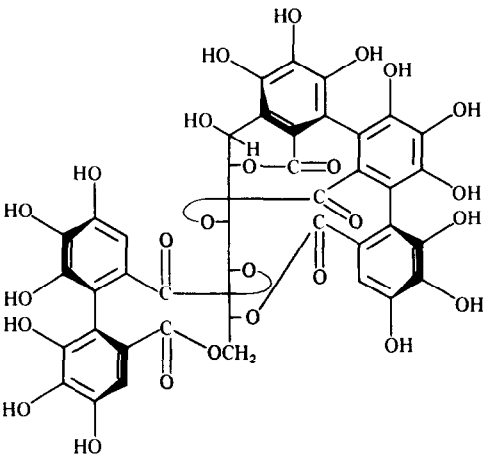
8 R^1, R^2 = H, Gal

two *meta*-coupled aromatic signals at δ 7 18 (J = 2 Hz) and 7 31 (J = 2 Hz) The most outstanding feature was the observation of an aldehyde proton signal at δ 9 58 The presence of a β-glucoside moiety was confirmed by the ^{13}C NMR chemical shifts of six aliphatic carbon signals, which were almost identical with those observed in 6-*O*-galloyl-β-D-glucosides [25] The location of the galloyl group at the glucose C-6 position was also confirmed by the lowfield shifts (δ 4 63, J = 2, 12 Hz, δ 4 30, J = 6, 12 Hz) of the corresponding methylene proton signals in the ^{1}H NMR spectrum The ^{13}C NMR data were consistent with a 3,4,5-trihydroxybenzaldehyde structure, showing an aromatic aldehyde signal at δ 192 9 and signals for an aromatic ring with a 3,4,5-trihydroxy-substitution system. The configuration of the glucose anomeric center was determined to be β from the ^{1}H NMR coupling constant (J = 7 Hz) of the anomeric signal Based on these observations, castamollissin was concluded to be 3,4,5-trihydroxybenzaldehyde 3-*O*-(6′-*O*-galloyl)-β-D-glucopyranoside (**1**)

Compound **2** (isochesnatin) gave ^{1}H NMR spectrum exhibiting two *meta*-coupled aromatic signals (δ 6 98, J = 2 Hz and δ 7 25, J = 2 Hz) and a one-proton singlet (δ 7 01) characteristic of a dehydrodigallic acid ester The appearance of a two-proton aromatic singlet at δ 6 49, together with an aliphatic two-proton singlet at δ 5 17



	R^1	R^2	R^3
9	H	OH	Gal
10	H	OH	H
11	OH	H	Gal
12	OH	H	H



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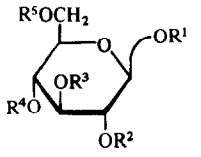
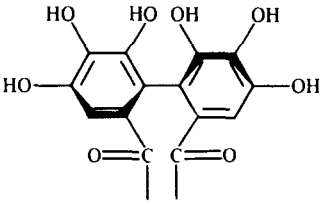
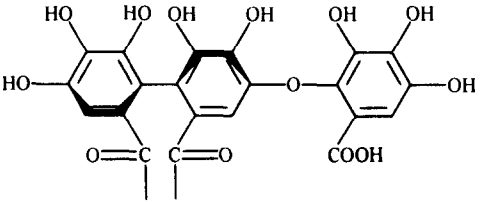


	R^1	R^2	R^3	R^4	R^5
15	Gal	HHDP		H	H
16	H	HHDP		Gal	H
17	Gal	H	H	HHDP	
18	H	Gal	H	HHDP	
19	H	H	Gal	HHDP	
20	H	HHDP		HHDP	
21	H	HHDP		Val	
22	H	Gal	Gal	HHDP	

HHDP



Val



assignable to a hydroxymethyl group attached to an ester function, indicated the presence of a 3,4,5-trihydroxybenzylalcohol moiety in the molecule The ^{13}C NMR spectrum showed sugar signals, the chemical shifts being almost identical with those found in β-D-glucosides such as chesnatin (**5**) and chestanin (**6**). Although these spectral data, as well as the negative FABMS [*m/z* 637 (M $-$H)$^-$], seemed to be consistent with those of chesnatin (**5**), their ^{1}H and ^{13}C NMR spectra were not superimposable Accordingly, **2** has been concluded to be isomeric with chesnatin in the orientation of the dehydrodigalloyl ester group.

Compound **3** (isochestanin) gave ^{1}H NMR data almost identical to chestanin (**6**), except that aromatic signals appeared as a two-proton singlet at δ7.15 instead of two *meta*-coupled doublets, indicative of a symmetrical substitution of one of the aromatic rings in the dehydrodigalloyl moiety On acid or enzymatic hydrolysis **3** yielded glucose, 3,4,5-trihydroxybenzylalcohol and a new phenolcarboxylic acid (**3a**). The ^{1}H NMR spectrum of **3a** showed, after D_2O exchange, only two singlets at δ6 96 and 7.13 each corresponding to one and two protons. The ^{13}C NMR spectrum of **3a** exhibited 14 carbon signals including two carboxylic acid resonances at δ168 0 and 170 7. These observations indicated that the new acid (**3a**) is a gallic acid dimer, which is isomeric with dehydrodigallic acid, and therefore we propose the trivial name *p*-dehydrodigallic acid for this compound. The negative FABMS with the prominent peak at *m/z* 937 was the same as that of **6**, consistent with the proposed structure (**3**).

The present study has shown that the polyphenolic

constituents of *Castanea mollissima* are different from those of *C. crenata*, especially in the absence of galloylhamameloses. 3,4,5-Trihydroxybenzaldehyde may be an intermediate in the biosynthesis of the corresponding benzylalcohols occurring in this plant, and therefore its isolation is of significance from the viewpoint of the metabolism of gallic acid.

EXPERIMENTAL

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper [1].

Plant material. *Castanea mollissima* was grown on the campus of Nanjing Forestry University, China and the leaves collected in September 1986.

Extraction and isolation. The air-dried powdered leaves (2.8 kg) were extracted × 3 at room temp. with 60% aq. Me_2CO. After concn., the resulting ppt. was removed by filtration. The filtrate was applied to a column of Sephadex LH-20. Elution with H_2O containing increasing proportions of MeOH yielded five fractions: I (50 g), II (8 g), III (16 g), IV (300 g) and V (35 g). Subsequent separation of fraction IV on Sephadex LH-20 CC with EtOH yielded six further fractions. Fraction IV-1 was repeatedly chromatographed on reverse-phase gels such as MCI-gel CHP-20P, Fuji gel ODS-G3 and Bondapak C_{18} Porasil B with H_2O containing increasing amounts of MeOH, furnished castamollissin (**1**) (17 mg), isochesnatin (**2**) (24 mg), *m*-dehydrodigallic acid (**4**) (2.2 g), chesnatin (**5**) (4.8 g), chestanin (**6**) (4.7 g) and cretanin (**7**) (12 mg). Repeated CC of fraction IV-2 on MCI-gel CHP-20P with H_2O–MeOH (7:3) and on Sephadex LH-20 with 60% aq. MeOH yielded isochestanin (**3**) (310 mg), a mixture of 6′- and 6″-galloyl chestanins (**8**) (257 mg), sanguin H-5 (**15**) (15 mg), 2,3-HHDP-4-*O*-galloylglucose (**16**) (12 mg), strictinin (**17**) (79 mg), 4,6-HHDP-2-*O*-galloylglucose (**18**) (21 mg), gemin D (**19**) (14 mg) and β-D-glucogallin (**23**) (10 mg). On similar CC, fraction IV-3 afforded grandinin (**17**) (79 mg), 4,6-HHDP-2,3-di-*O*-galloylglucose (**22**) (482 mg) and pedunculagin (**20**) (212 mg), while fractions IV-4 and IV-5 gave castanin (**12**) (20 mg), casuarinin (**9**) (ca. 10 g), casuarin (**10**) (48 mg), stachyurin (**11**) (48 mg), vescalagin (**13**) (125 mg) and 2,3-HHDP-4,6-valoneaylglucose (**21**) (178 mg).

Castamollissin (**1**). Colourless needles (H_2O), mp 209–211°, $[\alpha]_D^{20}$ −58.8° [Me_2CO–H_2O (1:1), *c* 1.06]. ^1H NMR (Me_2CO-d_6 + D_2O) δ 4.30 (1H, *dd*, *J* = 6, 12 Hz, glc H-6), 4.63 (1H, *dd*, *J* = 2, 12 Hz, glc H-6), 5.06 (1H, *d*, *J* = 7 Hz, glc H-1), 7.18 (1H, *d*, *J* = 2 Hz, aromatic H), 7.19 (2H, s, galloyl H), 7.31 (1H, *d*, *J* = 2 Hz, aromatic H), 9.58 (1H, s, CHO). ^{13}C NMR (Me_2CO-d_6 + D_2O) δ 64.6 (glc C-6), 70.9 (glc C-4), 74.0 (glc C-2), 75.1 (glc C-5), 76.5 (glc C-3), 102.7 (glc C-1), 109.9 (galloyl C-2, C-6), 111.0, 112.2 (C-2, C-2′), 121.0 (galloyl C-1), 128.8 (C-1), 139.0 (galloyl C-4), 143.0 (C-4), 146.0 (2C, galloyl C-3, C-5), 146.8, 147.0 (C-3, C-5), 167.4 (–COO–), 192.9 (–CO–). (Found: C, 47.33; H, 4.56. $C_{20}H_{20}O_{13}$·2H_2O requires: C, 47.62; H, 4.80%).

Isochenatin (**2**). An off-white amorphous powder, $[\alpha]_D^{20}$ −20.9° [Me_2CO–H_2O (1:1), *c* 0.65]. Negative FABMS *m/z* 637 $[M-H]^-$. ^1H NMR (Me_2CO-d_6 + D_2O) δ 4.60 (1H, *d*, *J* = 7 Hz, glc H-1), 5.17 (2H, s, $-CH_2O-$), 6.49 (2H, s, benzylalcohol H-2, H-6), 6.98, 7.25 (each 1H, *d*, *J* = 2 Hz, dehydrodigalloyl H), 7.01 (1H, s, dehydrodigalloyl H). ^{13}C NMR (DMSO-d_6 + D_2O) δ 60.4 (–CH_2O–), 65.0 (glc C-6), 69.3 (glc C-4), 73.3 (glc C-2), 75.6 (glc C-5), 77.1 (glc C-3), 163.9, 168.0 (–COO–). (Found: C, 48.79; H, 4.19. $C_{27}H_{26}O_{19}$·3.2H_2O requires: C, 48.73; H, 4.39%).

Isochetanin (**3**). An off-white amorphous powder, $[\alpha]_D^{20}$ −84.8° [Me_2CO–H_2O (1:1), *c* 1.0]. Negative FABMS *m/z* 937 $[M-H]^-$. ^1H NMR (Me_2CO-d_6 + D_2O) δ 4.59 (1H, *d*, *J* = 7 Hz, glc H-1), 5.11, 5.17 (each 2H, s, $-CH_2O-$), 6.50, 6.54 (each 2H, s, benzylalcohol H-2, H-6), 6.93 (1H, s, *p*-dehydrodigalloyl H), 7.15 (2H, s, *p*-dehydrodigalloyl H). ^{13}C NMR (Me_2CO-d_6 + D_2O) δ 61.6 (2C, glc C-6), 66.7, 67.0 (–CH_2O–), 70.3 (2C, glc C-4), 74.4 (2C, glc C-2), 77.0 (2C, glc C-5), 78.2 (2C, glc C-3), 107.5 (2C, glc C-1), 107.9 (4C, benzylalcohol C-2, C-6), 108.2, 110.4 (2C), 114.4, 126.2, 133.9, 150.3 (*p*-dehydrodigalloyl C), 135.0, 135.3 (benzylalcohol C-1), 142.2, 142.3 (benzylalcohol C-4), 150.9 (2C, benzylalcohol C-3, C-5), 166.5, 168.1 (–COO–). (Found: C, 50.95; H, 4.86. $C_{48}H_{42}O_{29}$ requires: C, 51.17; H, 4.48%).

Acid hydrolysis of **3**. A soln. of **3** (100 mg) in 2 N HCl (5 ml) was heated at 70° for 2.5 hr, and the reaction mixture was directly applied to a column of MCI-gel CHP-20P. Elution with H_2O containing increasing amounts of MeOH yielded three fractions. The first fraction eluted with H_2O contained a sugar, which was identified as glucose by co-chromatography [Avicell SF cellulose, *n*-BuOH–pyridine–H_2O (6:4:3)] with an authentic sample. The next fraction was analysed by TLC (Si gel, C_6H_6–HCO_2Et–HCO_2H (3:6:1)] and 3,4,5-trihydroxybenzylalcohol was identified. Crystallization of the last fraction from H_2O yielded *p*-dehydrodigallic acid (**3a**) (15 mg) as colourless needles, mp 250–254° (dec.). ^1H NMR (Me_2CO-d_6 + D_2O) δ 6.96 (1H, s, aromatic H), 7.13 (2H, s, aromatic H). ^{13}C NMR (Me_2CO-d_6 + D_2O) δ 108.4, 110.6 (2C), 114.7, 127.4, 134.5, 138.5, 140.0, 142.3, 150.5 (2C) (aromatic C), 168.0, 170.0 (–COO–).

Enzymatic hydrolysis of **3**. A soln. of **3** (100 mg) in H_2O (5 ml) was incubated with crude hesperidinase at 37° for 80 min. After concn. of the reaction mixture, the residue was treated with MeOH. The MeOH-soluble portion was subjected to CC on Sephadex LH-20. Elution with EtOH afforded glucose, *p*-dehydrodigallic acid (**3a**) (4.2 mg) and 3,4,5-trihydroxybenzylalcohol (11 mg).

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