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A DIMERIC HYDROLYSABLE TANNIN FROM *CAMELLIA OLEIFERA**

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Key Word Index—*Camellia oleifera*; Theaceae; leaves; tannins; ellagitannins; camellioferin A; camelliins A and B.

Abstract—Camellioferin A, a new dimeric ellagitannin, was isolated, together with 13 known tannins, including camelliins A and B, from *Camellia oleifera*. The structure of the new tannin elucidated by spectroscopic methods.

INTRODUCTION

Camellia oleifera is widely grown in southern China and Taiwan for its oil which is used as a hair-dressing and for treating skin diseases [2], and also the leaf and root for treating haemorrhages [2, 3]. Although the occurrence of flavonoids and triterpenoids (sapogenins) in this species has been reported [4, 5], little is known about tannins which might be responsible for its pharmacological activity. On the other hand, recent works have shown that species of the genera *Camellia* and *Schima* (Theaceae) produce tannins with diverse structures, such as macrocyclic hydrolysable tannin dimers, *C*-glucosidic ellagitannins and complex tannins [6–9]. We have now investigated the tannins and other polyphenols of *C. oleifera* and have isolated 14 polyphenolic compounds, including a new dimeric hydrolysable tannin, named camellioferin A.

RESULTS AND DISCUSSION

The dried leaves of *C. oleifera* were homogenized in aqueous acetone and filtered. The concentrated filtrate was extracted with *n*-butanol–methanol (9:1). Repeated column chromatography of the butanol-soluble portion afforded a new tannin, camellioferin A (**14**) and 13 other polyphenols. The latter (**1**–**13**) were identified as chlorogenic acid (**1**), 1,4,6-tri-*O*-galloyl-β-D-glucose (**2**), 2,3,4-tri-*O*-galloyl-D-glucose (**3**), 1,2-di-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-β-D-glucose (**4**) [10], gemin D (**5**) [11], tellimagrandin II (**6**) [12, 13], pedunculagin (**7**) [12], casuarictin (**8**) [12], cornusiin B (**9**) [14], casuariin (**10**) [12], schimawalin B (**11**) [9], camelliins B (**12**) and A (**13**) [6].

Camellioferin A (**14**), an amorphous powder, showed a $[M+Na]^+$ ion peak at *m/z* 1743 in the FAB-mass spectrum. Upon acid hydrolysis, it yielded gallic acid (**15**), ellagic acid (**16**) and valoneic acid dilactone (**17**). Camellioferin A was thus presumed to be a dimeric hydrolysable tannin having a valoneoyl group as the connecting unit between monomers, like **11**–**13**. The ^{1}H NMR spectrum of **14** showed duplicate signals for each proton, which is attributable to equilibration between anomers. The spectrum indicated the presence of a valoneoyl, a hexahydroxydiphenoyl (HHDP) and two galloyl groups by seven pairs of one-proton singlets and two pairs of two-proton singlets in the aromatic region (see Experimental). Although the sugar proton signals were complicated, the ^{1}H–^{1}H COSY and 2D *J*-resolved spectra allowed their full assignments (see Experimental). The large coupling constants ($J = 8$–10 Hz) between *vicinal* protons were characteristic of the glucopyranose ring with the $^4C_1$ conformation. As evidenced by the duplicate signals in the ^{1}H NMR spectrum, one of the anomeric hydroxyl groups was unacylated to cause the equilibration of α- and β-anomers (*ca* 1:1), while the other (δ_H 5.56, $J = 8$ Hz) is acylated with a β-orientation. The chemical shifts of the protons in one (glucose-II) of the glucose cores were similar to those of pedunculagin (**7**) [12], suggesting that HHDP and/or HHDP moiety of the valoneoyl unit were located at O-2/O-3 and O-4/O-6 of glucose-II whose anomeric centre was unacylated. A large difference ($\Delta\delta$ 1.6–1.8 ppm) of the chemical shifts between *geminal* protons at C-6 of glucose-I, like those observed for glucose-II, is characteristic of ellagitannins having an HHDP group (or its analogue) at O-4/O-6 [6–9, 13]. These data, along with the fact that all the hydroxyl groups of glucose-I are acylated, as revealed by the chemical shifts of H-1–H-6, indicated that two galloyl groups and the galloyl part of the valoneoyl group are located at O-1, O-2 and O-3 of glucose-I. A significant upfield shift of the acylated anomeric proton (δ 5.56, H-1), relative to that of

*Part 6 in the series 'Tannins from theaceous plants'. For part 5 see ref. [1].

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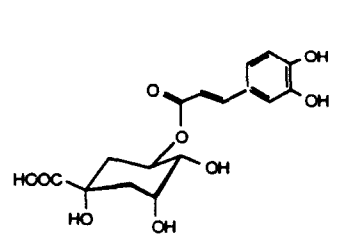

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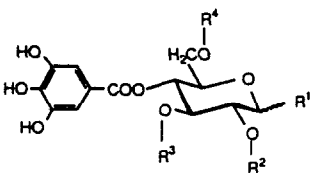
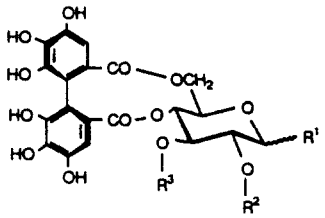
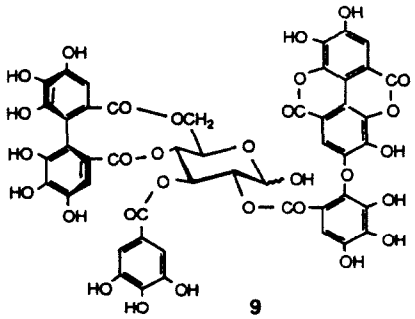


2 : R^1=(β)-OG, R^2=R^3=H, R^4=G
3 : R^1=OH, R^2=R^3=G, R^4=H



	R^1	R^2	R^3
4 : (β)-OG	G	H	
5 : OH	H	G	
6 : (β)-OG	G	G	
7 : OH	(*S*)-HHDP		
8 : (β)-OG	(*S*)-HHDP		



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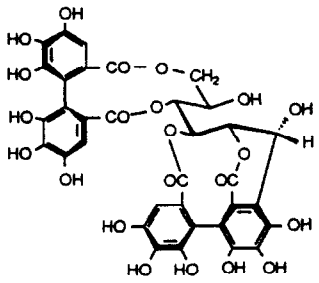

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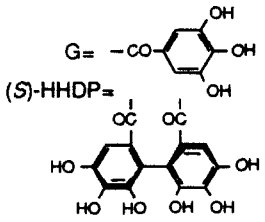
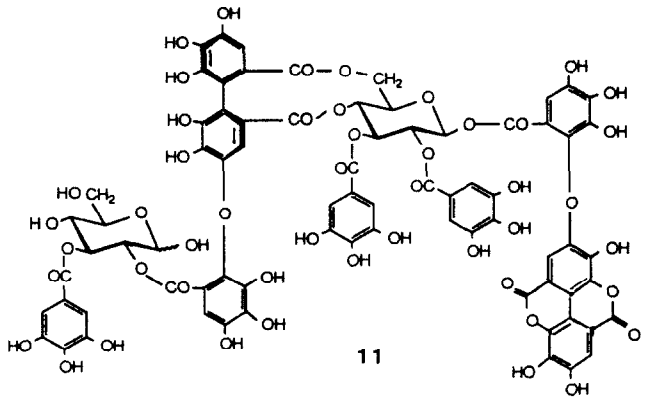


G= -CO-
(*S*)-HHDP=



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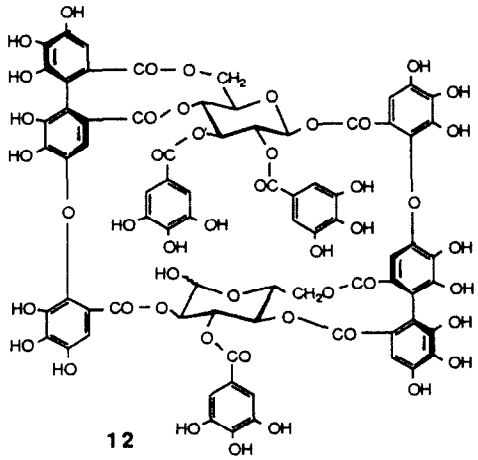


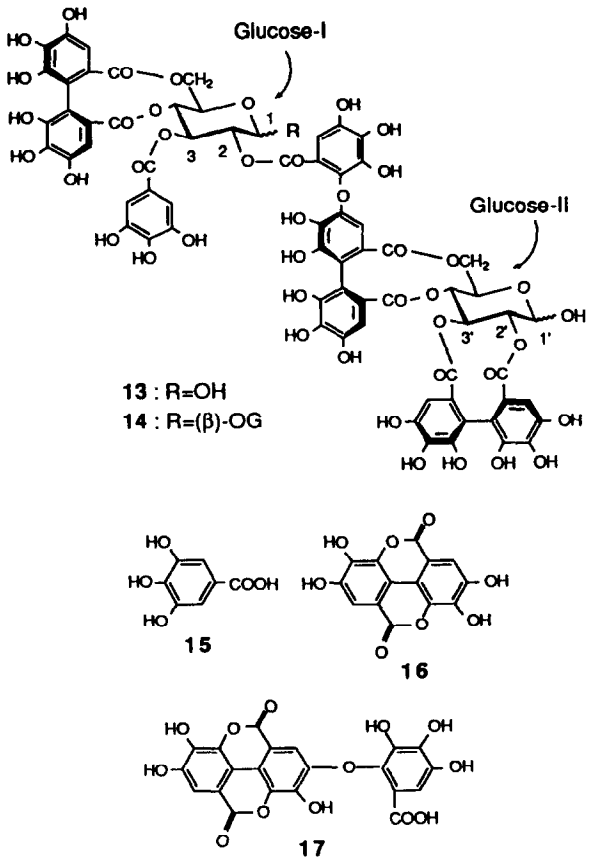
12

6 (δ6.18), is attributable to the anisotropic effect of the valoneoyl group [6, 14, 15], suggesting that the galloyl part of the valoneoyl group was at O-2. The chirality of the biphenyl (HHDP and valoneoyl) moieties was determined to be all *S* by the strong Cotton effects at 225 and 230 nm in the CD spectrum of **14** [16]. Based on these chemical and spectral data along with biogenetic consideration of the co-existence of **14** with **12** and **13**, camellioferin A was presumed to be a monogallate of camelliin A. This structure **14** was finally confirmed by partial hydrolysis with tannase yielding a degalloylated product which was identified as **13**.

EXPERIMENTAL

UV and CD spectra were taken in MeOH, ^{1}H and ^{13}C NMR in $(CD_3)_2CO$–D_2O. FAB-MS were recorded using 3-nitrobenzylalcohol as matrix agent. HPLC was performed on a Superspher SI60 (Merck) column (4 × 125 mm) developed with *n*-hexane–MeOH–HCO_2H (55:33:11:1) containing oxalic acid (450 mg l^{-1}) (flow rate 1.5 ml min^{-1}; detection 280 nm) at room temp., and on a LiChrospher RP-18 column (5 μm; 4 × 250 mm) developed with 10 mM H_3PO_4–10 mM KH_2PO_4–MeCN (9:9:2) (flow rate 1 ml min^{-1}; detection 280 nm) at 40°. CC was carried out on Dia-ion HP-20, MCI-gel CHP-20P (Mitsubishi Kasei) and Toyopearl HW-40 (coarse and fine grades. Tosoh). Prep. TLC was conducted on a Kieselgel 60 PF_{254} with C_6H_6–Me_2CO (4:1).

Plant material. Leaves of *C. oleifera* were collected in October at Lien-hua-chin Experimental Forest, Nan-tou County, Taiwan. A voucher specimen is deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Okayama University.



Isolation of tannins. The 70% aq. Me_2CO homogenate of dried leaves (1.2 kg) was concd and extracted with *n*-BuOH–MeOH (9:1) to give, after evapn, a BuOH extract (45.63 g) and a H_2O extract (42 g). A part (33.12 g) of the BuOH extract was subjected to CC over Diaion HP-20 (7 cm i.d. × 50 cm) with H_2O→aq. MeOH (10%→20%→30%→40%MeOH)→MeOH. The 10% MeOH eluate (3.9 g) gave chlorogenic acid (**1**) (2 mg), gemin D (**5**) (4 mg) and casuariin (**10**) (10 mg). The 20% MeOH eluate (1.5 g) was further chromatographed over Toyopearl HW-40 (coarse) with aq. MeOH to give 2,3,4-tri-*O*-galloyl-D-glucose (**3**) (22 mg) and pedunculagin (**7**) (145 mg). A part (3.05 g) of the 40% MeOH eluate was also chromatographed over Toyopearl HW-40 with 70%EtOH→EtOH–H_2O–Me_2CO (8:1:1→7:2:1→3:1:1) to yield 1,4,6-tri-*O*-galloyl-β-D-glucose (**2**) (10 mg), 1,2-di-*O*-galloyl-β-D-glucose (**4**) (6 mg), cornusiin B (**9**) (18 mg) and a mixt. of dimeric hydrolysable tannins which was sepd by CC on MCI-gel CHP-20P into camelliin A (**13**) (70 mg), camellioferin A (**14**) (43 mg), camelliin B (**12**) (4 mg) and schimawalin B (**11**) (10 mg). The 50% MeOH eluate (3 mg) was rechromatographed over Toyopearl HW-40 with 70% MeOH to give tellimagrandin II (**6**) (42 mg) and schimawalin B (**11**) (15 mg).

CC of another portion (2.07 g) of the 40% MeOH eluate from Diaion HP-20 CC gave further portions of **13** (182 mg) and **14** (9 mg), in addition to casuarictin (**8**) (3 mg).

Camellioferin A. Light brown amorphous powder. $[\alpha]_D$ +67° (MeOH; *c* 1.0). UV λ_{max} nm (log ε): 218 (5.00), 265 (4.62). CD [θ] (nm): $+14.8 \times 10^4$ (225), $+14.4 \times 10^4$ (236), -8.9×10^4 (260), $+5.9 \times 10^4$ (282). ^{1}H NMR: δ 7.02, 7.01 (each *s*, 2H in total, galloyl), 7.00 (2H, *s*, galloyl), 7.03 (1H, *s*), 6.39, 6.38 (each *s*, 1H in total), 6.09, 6.08 (each *s*, 1H in total) (valoneoyl), 6.63 (1H, *s*), 6.59, 6.58 (each *s*, 1H in total), 6.55, 6.50 (each *s*, 1H in total), 6.52 (1H, *s*) (HHDP × 2), 5.56 (*d*, *J* = 8 Hz, Glc H-1), 5.60 (*dd*, *J* = 8, 10 Hz, Glc H-2), 5.82 (*t*, *J* = 10 Hz, Glc H-3), 5.19 (*t*, *J* = 10 Hz, Glc H-4), 4.38 (*m*, Glc H-5), 3.88 (*d*, *J* = 13.5 Hz, Glu H-6), 5.32 (*dd*, *J* = 6.5, 13.5 Hz, Glu H-6); 5.36 (*d*, *J* = 3.5 Hz, Glc H-1′), 5.00 (*dd*, *J* = 3.5, 10 Hz, Glc H-2′), 5.40 (*t*, *J* = 10 Hz, Glc H-3′), 4.94 (*t*, *J* = 10 Hz, Glc H-4′), 4.30 (*m*, Glc H-5′), 3.60 (*d*, *J* = 12 Hz, Glc H-6′), 5.40 (*dd*, *J* = 5.5, 12 Hz, Glc H-6′) (α-anomer), 5.00 (*d*, *J* = 8 Hz, Glc H-1′), 4.80 (*dd*, *J* = 8, 9.5 Hz, Glc H-2′), 5.20 (*t*, *J* = 9.5 Hz, Glc H-3′), 4.94 (*t*, *J* = 9.5 Hz, Glc H-4′), 4.13 (*dd*, *J* = 6.5, 9.5 Hz, Glc H-5′), 3.68 (*d*, *J* = 12 Hz, Glc H-6′), 5.08 (*dd*, *J* = 6.5, 12 Hz, Glc H-6′) (β-anomer).

Acid hydrolysis of camellioferin A. A soln of **14** (1 mg) in 1% H_2SO_4 (1 ml) was heated at 100° for 8 hr. After cooling, the reaction mixt. was extracted with EtOAc and the EtOAc extract analysed by the reversed-phase HPLC, which showed peaks with R_ts identical with those authentic specimens of **15**–**17**.

Partial hydrolysis of camellioferin A. A soln of **14** (10 mg) in H_2O was incubated with tannase [17] (10 drops) at 37° for 36 hr. The reaction mixt. was acidified with dil. HCl and then passed through a Sep-Pak C18 cartridge. After washing with H_2O, the product was eluted with 30% MeOH to give the degalloylated product (5 mg) which was identified as camelliin A (**13**) by comparison of HPLC and ^{1}H NMR data with those of an authentic sample.

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