Pergamon

PII: S0031-9422(97)01091-1

Phytochemistry, Vol. 48, No. 2, pp. 333–338, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

DIMERIC AND TRIMERIC ELLAGITANNINS FROM *CORYLUS HETEROPHYLLA*

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(*Received* 11 *September* 1997)

Key Word Index—*Corylus heterophylla*; Betulaceae; leaves; tannin; heterophylliin F; heterophylliin G; ellagitannin oligomer.

Abstract—Besides previously reported tannins and related polyphenols, additional two new hydrolysable tannins, heterophylliins F and G, were isolated from the leaf extract of *Corylus heterophylla*, and characterized by spectral and chemical evidence as dimeric and trimeric ellagitannins, respectively, in which monomeric units are linked to each other through a valoneoyl group.

INTRODUCTION

Some betulaceous plants have been reported to contain large quantities of hydrolysable tannins, including pedunculagin and *C*-glucosidic tannins, such as casuarinin and stachyurin [1–3]. One such species, *Corylus heterophylla* is widely grown in Korea and the northern part of China, and its nuts are used as an appetite stimulator, digestive and food in China [4]. We previously isolated 13 polyphenols, including the new ellagitannins, heterophylliins A–E, from the leaf extract of *C. heterophylla* [5]. Among them, heterophylliins B and C were the first dimers possessing the *C*-glucosidic tannin, casuarinin, as a constructing monomeric unit. Considering the recent discovery of many oligomeric hydrolysable tannins with diverse structures in plant kingdom [6], we have reinvestigated the leaf extract for further new oligomers and have isolated oligomers, named heterophylliins G and F. This paper describes the structural elucidation of these new compounds.

RESULTS AND DISCUSSION

The concentrated solution of the aqueous acetone homogenate of the dried leaves of *C. heterophylla* was subjected to repeated column chromatography on Toyopearl HW-40 and Sephadex LH-20 to afford heterophylliins F (**1**) and G (**8**) as amorphous powders. Upon methylation, followed by methanolysis with NaOMe, both of these compounds yielded common products, i.e., methyl tri-*O*-methylgallate, trimethyl octa-*O*-methylvaloneate (**2**) and dimethyl hexamethoxydiphenate (**3**). The electrospray ionization (ESI) mass spectrum of **1** gave a $[M+NH_4]^+$ at *m/z* 1588, suggesting that **1** is a dimeric ellagitannin with M_r 1570. The ^1H NMR spectrum of **1** indicated the presence of three galloyl, a hexahydroxydiphenoyl (HHDP) and a valoneoyl group as revealed by three 2H-singlets and five 1H-singlets in the aromatic region. The atropisomerism of the chiral HHDP and valoneoyl groups was determined to be (*S*)-series, respectively, by a large positive Cotton effect at 230 nm ($[\theta]$ $+3.1\times10^5$) in the CD spectrum [7]. Two sets of seven-spin system in the aliphatic region are indicative of the presence of two glucose cores with *C1*-conformation. On the basis of the ^1H–^1H COSY data (Table 1), one (glucose-II) of the glucose cores was assumed to be fully acylated, whereas the hydroxyl groups at C-2 and C-3 of the other glucose core (glucose-I) are unacylated, as evidenced by appearance of the H-2 and H-3 signals at higher field [δ 3.66 (*dd*, J = 8.5, 10.0 Hz) and 3.80 (*t*, J = 10.0 Hz)] than the corresponding signals of glucose-II. In fact, the chemical shifts of the glucose-I protons coincided with those of strictinin (**4**) [8] (Table 1). Two biphenyl moieties in the molecule were located at O-4/O-6 of each glucose core, based on the large differences (δ 5.12 and 3.66; δ 5.26 and 3.81) between chemical shifts of each proton signal due to the C-6 methylene group [9]. The acyl groups at anomeric positions have β-orientation, as evidenced by large coupling constant (J = 8.5 Hz) of the H-1 and H-1′ signals. Based on these data, the monomeric constituents of heterophylliin G was deduced to be strictinin (**4**) and tellimagrandin II (**5**), which are linked to each other

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Table 1. ^{1}H NMR data of glucose moieties of heterophylliins F (**1**) and G (**8**) (500 MHz, Me_2CO-d_6+D_2O, *J* in Hz)

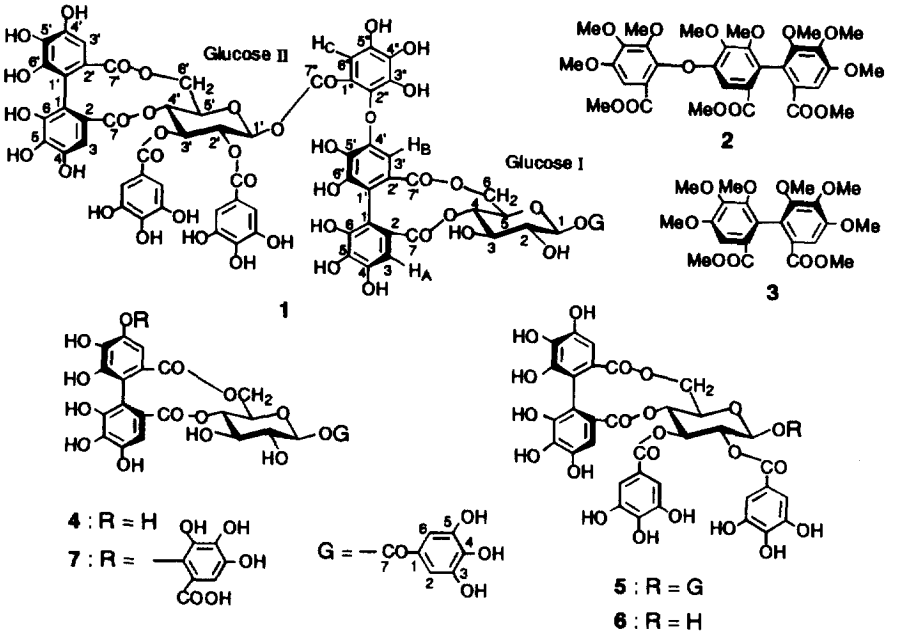
Protons	**1**	**4**	**5**	**8**		**9**		**10**
				α-anomer	β-anomer	α-anomer	β-anomer	
Glucose I								
H-1	5.65 *d* (8.5)	5.76 *d* (7.0)		5.36 *d* (3.5)	4.99 *d* (8.0)	5.40 *d* (3.5)	5.07 *d* (8.0)	
H-2	3.66 *dd* (8.5, 10.0)	3.66 *dd* (7.0, 9.0)		5.01 *dd* (3.5, 9.5)	4.80 *dd* (8.0, 9.5)	5.06 *dd* (3.5, 9.5)	4.83 *dd* (8.0, 9.5)	
H-3	3.80 *t* (10.0)	3.84 *t* (9.0)		5.41 *t* (9.5)	5.16 *t* (9.5)	5.42 *t* (9.5)	5.19 *t* (9.5)	
H-4	4.82 *t* (10.0)	4.91 *t* (9.0)		4.95 *t* (9.5)	4.95 *t* (9.5)	4.95 *t* (9.5)	4.95 *t* (9.5)	
H-5	4.05 *dd* (6.0, 10.0)	4.11 *dd* (6.0, 9.0)		4.53 *ddd* (1.0, 6.5, 9.5)	4.14 *br dd* (6.5, 9.5)	4.52 *ddd* (1.0, 6.5, 9.5)	4.14 *br dd* (6.5, 9.5)	
H-6	5.12 *dd* (6.0, 13.0)	5.22 *dd* (6.0, 14.0)		5.13 *dd* (6.5, 13.0)	5.14 *dd* (6.5, 13.0)	5.21 *dd* (6.5, 13.0)	5.22 *d* (6.5, 13.0)	
	3.66 *d* (13.0)	3.79 *d* (14.0)		3.65 *dd* (1.0, 13.0)	3.71 *br d* (13.0)	3.64 *dd* (1.0, 13.0)	3.71 *br d* (13.0)	
Glucose II								
H-1′	6.05 *d* (8.5)		6.20 *d* (8.0)		6.07 *d* (8.0)			6.08 *d* (8.0)
H-2′	5.54 *dd* (8.5, 10.0)		5.58 *dd* (8.0, 9.5)		5.25 *d* (8.0, 9.5)			5.23 *dd* (8.0, 9.5)
H-3′	5.76 *t* (10.0)		5.83 *t* (9.5)		5.75 *t* (9.5)			5.77 *t* (9.5)
H-4′	5.15 *t* (10.0)		5.20 *t* (9.5)		5.18 *t* (9.5)			5.16 *t* (9.5)
H-5′	4.43 *dd* (6.5, 10.0)		4.45 *dd* (6.0, 9.5)		4.43 *dd* (6.5, 9.5)			4.45 *dd* (6.5, 9.5)
H-6′	5.26 *dd* (6.5, 13.0)		5.36 *dd* (6.0, 13.0)		5.26 *dd* (6.5, 13.0)			5.27 *dd* (6.5, 13.0)
	3.81 *d* (13.0)		3.87 *d* (13.0)		3.80 *d* (13.0)			3.80 *d* (13.0)
Glucose III								
H-1″					6.09 *d* (8.5)			6.16 *d* (8.5)
H-2″					5.07 *br t* (9.0)			5.14 *br t* (9.0)
H-3″					5.41 *t* (10.0)			5.37 *t* (10.0)
H-4″					5.06 *t* (10.0)			5.08 *t* (10.0)
H-5″					4.43 *dd* (6.5, 10.0)			4.44 *dd* (6.5, 10.0)
H-6″					5.25 *dd* (6.5, 13.5)			5.26 *dd* (6.5, 13.0)
					3.74 *d* (13.5)			3.78 *d* (13.0)

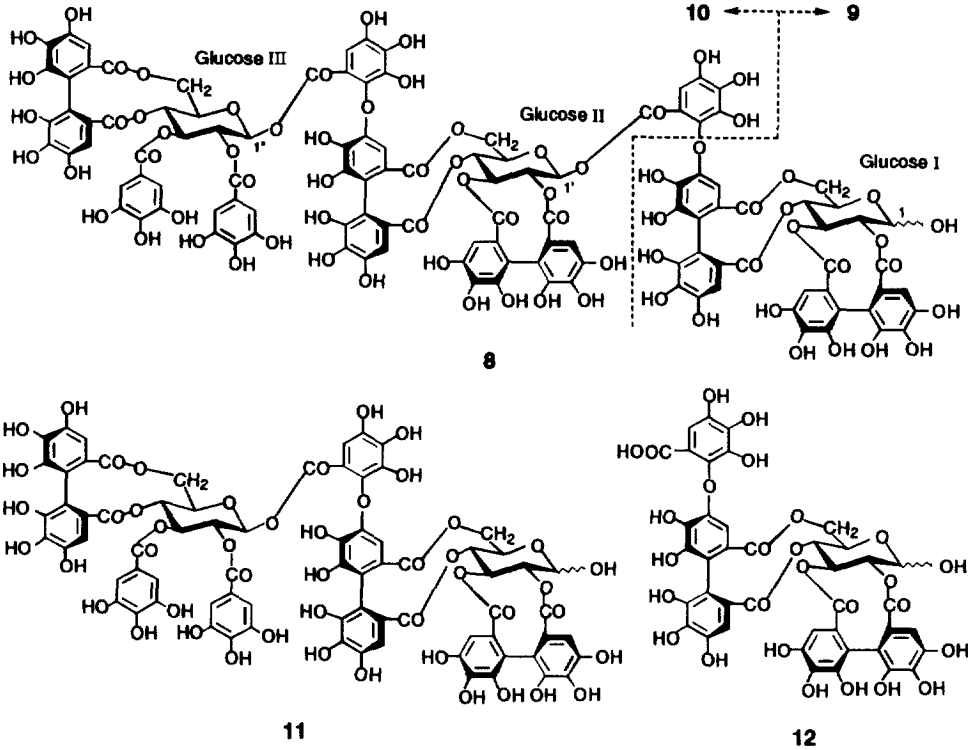
Table 2. ^{13}C-NMR data of glucose moieties of heterohylliins F (**1**) and G (**8**) (126 MHz, Me_2CO-d_6 + D_2O)

				8		**9**		
	1	**4**	**5**	α-anomer	β-anomer	α-anomer	β-anomer	**10**
Glucose I								
C-1	95.7	95.5		91.5	95.1	91.4	95.1	
C-2	74.1	74.7		75.7	78.1	75.4	78.1	
C-3	75.3	75.6		75.6	77.4	75.6	77.4	
C-4	72.2	72.8		69.9	68.9	69.9	69.5	
C-5	73.0	73.2		67.1	72.2	67.1	72.1	
C-6	63.6	63.7		63.5	63.5	63.4	63.5	
Glucose II								
C-1	93.1		93.8	91.8				92.3
C-2	71.7		71.8	75.4				74.9
C-3	73.1		73.3	77.1				77.2
C-4	70.6		70.8	69.5				69.3
C-5	72.9		73.1	73.2				73.5
C-6	63.1		63.1	62.9				63.2
Glucose III								
C-1				93.1				93.4
C-2				71.7				71.9
C-3				73.3				73.5
C-4				70.5				70.7
C-5				72.9				73.1
C-6				63.5				63.2

through formation of a valoneoyl group. The glucose carbon resonances in the ^{13}C NMR of **1** were also in agreement with the combined signals of **4** and **5** [10] (Table 2). The binding mode of the monomers was clarified with the aid of a HMBC (J_{CH} = 6 Hz) spectrum of **1** as follows. Two aromatic proton signals at δ 6.46 and 6.70 were assigned to the HHDP protons based on their correlations with ester carbonyl carbons at δ 167.7 and 168.4, which showed cross-peaks with the H-4′ and H-6′ signals, respectively. The remaining three 1H-aromatic singlets (δ 6.12, 6.61 and 7.09) were thus attributed to the valoneoyl protons. Of these signals, the singlet (δ 6.12) at the highest field among the aromatic protons was assigned to H_B, based on the two-bond correlation with the ethereal phenyl carbon at δ 146.5 [9]. The H_B signal showed a connectivity with H-6 of the glucose-**I** by three-bond couplings with the common ester carbonyl carbon at δ 168.3, thus providing definite evidence for orientation of the valoneoyl group, as shown in the formula. Similar connectivities of three galloyl signals with glucose H-2′, H-3′ and H-1, respectively, were observed, and the H_A and H_C of the valoneoyl group were correlated with glucose H-4 and H-1′ through carbonyl carbons, to lead the structure **1**. Chemical evidence supporting the structure **1** for heterophylliin F was obtained by partial hydrolysis of **1** in hot aqueous solution, yielding tellimagrandin I (**6**) and a partial hydrolysate (**7**), *m/z* 820 $[M+NH_4]^+$. The ^{1}H NMR spectrum of **7** exhibited signals ascribable to a galloyl and valoneoyl group; the glucose proton signals were similar to those of **4**. These data were consistent with structure **7**, which originated from the glucose-**I** residue of **1**.

The trimeric nature of heterophylliin G (**8**) was suggested by a retention time longer than that of **1** on normal-phase HPLC [10] and a $[M+NH_4]^+$ at *m/z* 2672 in the ESI mass spectrum. Although the ^{1}H NMR spectrum of **8** is complicated owing to partial duplications of signals arising from an equilibrium mixture of α- and β-anomers, the aromatic proton signals could be accounted for by the presence of three HHDP, two valoneoyl and two galloyl groups (see Experimental). The number of each acyl constituent was confirmed by the corresponding carbons including 14 ester carbonyl carbons in the ^{13}C NMR spectrum. Based on the ^{1}H–^{1}H COSY spectrum, the sugar parts of **8** were indicated to be composed of three *C1*-glucopyranose residues whose hydroxyl groups are all acylated, except for that at the anomeric position (Table 1). Chemical shifts of the glucose carbon resonances of **8** were comparable to those of pedunculagin (**9**) [11] and rugosin F (**10**) [12] (Table 2), which both co-occur in the plant. The CD spectrum of **8** showed an intensive positive Cotton effect at 229 nm ($[\theta]$ 6.2×10^5), which is also almost superimposable on an additive Cotton effect of **9** and **10**, establishing the chiralities of biphenyl moieties in **8** to be all *S*-configurations. These spectral features clearly suggested that heterophylliin G is a trimer biogenetically





produced by intermolecular C–O oxidative coupling forming a valoneoyl group between **9** and **10**. The orientation of two valoneoyl groups in **8**, which could not be deduced from the above spectral data, was unambiguously established by partial hydrolysis of **8**. The major partial hydrolysates were identified as degalloylrugosin F (**11**) [5] and praecoxin A (**12**) [13], as well as **6**. The trimeric structure **8** was thus assigned to heterophylliin G.

It should be noted that heterophylliin G is the first

trimer found in the Betulaceae, although ca 20 trimers have been hitherto isolated from various plant families [6].

EXPERIMENTAL

General

^1H (500 MHz) and ^{13}C (126 MHz) NMR were measured in Me_2CO-d_6+D_2O and chemical shifts are given in δ on a TMS scale. ESIMS were recorded after injecting a sample soln in 50% aq. MeOH containing 0.1% ammonium acetate. Chromatographic conditions were the same as those described in a previous paper [4].

Plant material

Leaves of *C. heterophylla* Fisch. were collected in Harbin, in July, 1996 and identified by Prof. Liu Ming Yuan, Harbin Educational College. A voucher specimen is deposited at the Herbal Garden of Heilongjiang Commercial College.

Extraction and isolation

The concd soln of the aq. Me_2CO homogenate of the *C. heterophylla* static leaves (2.5 kg) was fractionated by CC over Dia-ion HP-20 (Mitsubishi Kasei Co. Ltd, Japan) with H_2O and aq. MeOH (10 → 20 → 30 → 40% → 50%) and MeOH in a stepwise gradient mode. A part (20 g) of the 40% MeOH eluate (74 g), which was shown to be rich in oligomers by normal phase HPLC, and further submitted to a combination of CC over Toyopearl HW-40 (course and/or fine grades; Toso, Japan) and Sephadex LH-20 with the same solvent system to afford heterophylliin G (**1**) (120 mg) and F(**8**) (48 mg).

Heterophylliin F (**1**)

Off white amorphous. $[\alpha]_D$ +75.3° (MeOH, *c* 1.0). ESIMS *m/z*: 1588 ($[M+NH_4]^+$). UV λ_{max} (MeOH) nm (log ε): 210 (5.34), 285 (4.98). CD (MeOH) [θ] (nm): $+3.1\times10^5$ (230), -11.4×10^4 (261), $+11.1\times10^4$ (283). ^1H NMR: δ 7.15, 6.99, 6.94 [each 2H, *s* galloyl (G)], 7.09 1H, *s* valoneoyl (Val) H-6″], 6.70 (1H, *s*, HHDP H-3′), 6.46 (1H, *s*, HHDP H-3), 6.61 (1H, *s*, Val H-3), 6.12 (1H, *s* Val H-3′). ^{13}C NMR: δ 104.6, 107.8, 108.0 (2C), 109.8 (HHDP C-3, 3′, Val C-3, 3′, 6″), 110.1 (2C), 110.1 (2C), 110.2 (2C) (G, C-2, 6), 112.4 (Val C-1′), 115.6 (Val C-1), 115.9 (HHDP C-1′), 115.9 (HHDP C-1), 117.8 (Val C-1′), 119.8, 120.0, 120.3 (G C-1), 125.5, 125.8, 126.1, 126.2 (HHDP C-2, 2′, Val C-2, 2′), 136.2 (Val C-5), 136.5 (HHDP C-5′), 136.6 (HHDP C-5), 136.9 (Val C-5′), 137.9 (Val C-2″), 139.2, 139.5, 139.5 (G C-4), 140.4 (Val C-3″), 141.3 (Val C-4″), 143.2 (Val C-5″), 144.3, 144.3, 144.5, 144.9 (HHDP C-6, 6′, Val C-6, 6″), 145.1 (HHDP C-4′), 145.2 (2C, HHDP C-4, Val C-4), 146.5 (Val C-4′), 146.0 (2C), 145.9 (2C), 145.7 (2C) (G C-3,5), 168.4 (HHDP C-7′), 168.3 (Val C-7′), 168.2 (Val C-7), 167.7 (HHDP C-7), 166.6, 166.1, 165.7 (G C-7), 162.5 (Val C-7″).

Heterophylliin G (**8**)

Pale brown amorphous. α_D +91.7° (MeOH, *C* 1.0). ESIMS *m/z*: 2672 ($[M+NH_4]^+$). UV λ_{max} (MeOH) nm (log ε): 210 (5.58), 285 (5.24). CD (MeOH) [θ] (nm): $+6.2\times10^5$ (229), -2.6×10^5 (261), $+1.8\times10^5$ (282). ^1H NMR: δ 6.99, 6.95 (each *s*, 2H in total, G), 7.1, 7.16 (each *s*, 1H in total), 6.59, 6.58 (each *s*, 1H in total), 6.36 (1H, *s*), 6.21, 6.20 (each *s*, 1H in total), 7.11, 7.08 (each *s*, 1H in total), 6.63 (1H, *s*), 6.60, 6.59 (each *s*, 1H in total), 6.51 (1H, *s*), 6.47, 6.45 (each *s*, 1H in total), 6.39, 6.38 (each *s*, 1H in total), 6.19 (1H, *s*), 6.18 (1H, *s*) (HHDP and Val). ^{13}C NMR: δ 104.8 (2C), 107.3 (2C), 107.6 (1C), 107.5 (2C), 108.4 (2C), 109.7, (2C), 109.9 (1C) (HHDP C-3, 3′, Val C-3, 3′, 6″), 110.1 (4C), (G C-2, 6), 112.6 (1C), 113.3 (1C), 114.2 (1C), 114.3 (1C), 114.7 (1C), 114.8 (1C), 115.4 (1C), 115.6 (1C), 115.8 (1C), 115.9 (1C), 117.1 (1C), 117.7 (1C) (HHDP C-1, 1′, Val C-1, 1′, 1″), 119.9 (1C), 120.0 (1C) (G C-1), 125.2 (1C), 125.3, 125.4 (1C in total), 125.5 (1C), 125.6 (1C), 125.7 (1C), 126.0 (1C), 126.1 (1C), 126.3 (1C), 126.4 (1C), 126.6 (1C) (HHDP C-2, 2′, Val C-2, 2′), 135.9, 136.0 (1C in total), 136.2 (1C), 136.3 (2C), 136.4 (2C), 136.5 (2C), 136.8 (1C), 137.0 (1C), 137.5 (1C), 137.8 (1C) (HHDP C-5, 5′, Val C-5, 5′, 2″) 139.2 (1C), 139.5 (1C) (G C-4), 140.4 (2C), 141.2 (1C), 141.3 (1C) (Val C-3″, 4″), 134.2 (1C), 134.4 (1C), 144.2 (2C), 144.3 (3C), 144.6 (1C), 144.7 (1C), 145.0 (2C), 145.2 (1C), 145.7 (4C), 145.9 (4C), 146.6 (1C), 146.7 (1C) (HHDP C-4, 4′, 6, 6′ Val C-4, 4′, 6 6′, 5″), 145.1 (2C), 145.2 (2C) (G-3, 5), 169.4, 169.3 (1C in total), 169.3 (1C), 169.2 (1C), 169.1, 168.9 (1C in total), 168.1 (1C), 168.0 (1C), 167.98 (1C), 167.9 (1C), 167.8 (1C), 167.7 (1C), 166.5 (1C), 166.0 (1C), 162.9 (1C), 162.3 (1C) (ester carbonyl).

Methylation of heterophylliins F (**1**) *and G* (**8**) *followed by methanolysis*

A mixt. of **1** (or **8**) (5 mg), Me_2SO_4 (5 μl) and K_2CO_3 (20 mg) in Me_2CO (5 ml) was stirred overnight at room temp. and then heated under reflux for 3 h. After removal of inorganic material by centrifugation, the supernatant was evapd *in vacuo*. The residue was subjected to prep. TLC (silica gel: $CHCl_3$–Me_2CO, 10:1) to afford methyl tri-*O*-methylgallate, **2** and **3** which were identified with authentic samples by direct comparison of TLC, HPLC [normal phase, *n*-hexane–EtOAc (2:1)] and EIMS.

Partial hydrolysis of **1**

A soln of **1** (10 mg) in H_2O (1 ml) was heated at 100° for 15 min. The concd soln was submitted to CC over MCI-gel CHP-20P with aq. MeOH to give

tellimagrandin I (**6**) (2 mg) and a partial hydrolysate (**7**) (3 mg).

Partial hydrolysate (**7**)

ESIMS *m/z*: 820 ($[M+NH_4]^+$). ^1H NMR [Me_2CO-d_6]: δ 7.14 (2H *s*, G), 7.05, 6.70, 6.22 (each 1H *s*, Val), 5.46 (*d*, J = 8.5 Hz, H-1), 3.65 (*dd*, J = 8.5, 10 Hz, H-2), 3.77 (*t*, J = 10 Hz, H-3), 4.81 (*t*, J = 10 Hz, H-4), 4.03 (*dd*, J = 6, 10 Hz, H-5), 5.09 (*dd*, J = 6, 13 Hz, H-6), 3.66 (*d*, J = Hz, H-6).

Partial hydrolysis of **8**

Heterophylliin G (**8**) (20 mg) was treated with hot H_2O (2 ml) in a way similar to that described above, to give **6** (3 mg), degalloylrugosin F (**11**) (5 mg) and praecoxin A (**12**) (1 mg), which were identified by direct comparison with authentic samples by HPLC and ^1H NMR.

Acknowledgements—We are grateful to the SC-NMR Laboratory of Okayama University for measuring NMR. One of us (Z.-X.J.) thanks the National Education Commission of China for a scholarship.

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