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THREE PHENYLPROPANOID GLYCOSIDES FROM *MUSSATIA*

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Key Word Index—*Mussatia*; Bignoniaceae; phenylpropanoid glycosides; mussatioside I; mussatioside II; mussatioside III; cinnamic acid; dimethylcaffeic acid; *p*-methylcoumaric acid; isolation.

Abstract—Three new phenylpropanoid glycosides, mussatioside I, mussatioside II and mussatioside III were isolated from the methanolic extract of the bark of a new *Mussatia* species. On the basis of the chemical and spectral evidence their structures were determined as [β-(4′-hydroxyphenyl)ethyl-*O*-β-D-glucopyranosyl(6 → 1)]-*O*-β-D-xylopyranosyl(1 → 3)-α-L-(4-*O*-*t*-cinnamoyl)rhamnopyranoside, [β-(4′-hydroxyphenyl)-ethyl-*O*-β-D-glucopyranosyl(6 → 1)]-*O*-β-D-xylopyranosyl(1 → 3)-α-L-(4-*O*-dimethylcaffeoyl)rhamnopyranoside and [β-(4′-hydroxyphenyl)ethyl-*O*-β-D-glucopyranosyl(6 → 1)]-*O*-β-D-xylopyranosyl(1 → 3)-α-L-(4-*O*-*p*-methylcoumaroyl)rhamnopyranoside, respectively. *M. hyacinthina* was also found to contain mussatioside I.

INTRODUCTION

Many species of the Bignoniaceae have been reported to have medicinal properties, and they are widely used in South American folk medicine. In our search for natural products with biological activity, we have investigated a new *Mussatia* species and *M. hyacinthina*, which occur in South America. In Peru and Bolvia, these plants are mixed with coca or chewed alone for sweetening, euphoric or medicinal effects [1–3]. The present paper describes the isolation and characterization of three new phenylpropanoid glycosides (mussatioside I, mussatioside II and mussatioside III) which differ from other phenylpropanoid glycosides isolated in recent years [4–6] in the nature of the sugars and their linkages and of the aglycone.

RESULTS AND DISCUSSION

Mussatioside I (**1**), which is the major phenylpropanoid glycoside of the bark of *Mussatia* sp. nov., was isolated as an amorphous powder, $C_{34}H_{44}O_{16}$. The presence of glucose, rhamnose and xylose moieties in **1** was suggested by acid hydrolysis with 4 N hydrochloric acid, confirmed by PC and their ratio established as 1:1:1 by GC. Acetylation of **1** with acetic anhydride–pyridine gave an octa-acetate (**2**). Alkaline hydrolysis of **1** with methanolic sodium hydroxide followed by treatment with methanol gave descinnamoyl mussatioside I (**3**) and methyl cinnamate. Methylation of **1** by Hakomori's method [7] gave descinnamoyl nona-*O*-methyl mussatioside I (**4**). Milder hydrolysis gave a partially hydrolysed product (**5**) and D-xylose, which was the only sugar detected and thus the terminal unit (see Scheme 1). The mass spectra of **1**, **2** and **4** showed the characteristic fragment peaks due to the cinnamoyl group (*m/z* 131) in **1** and **2**, a terminal xylose in **1**, **2** and **4** (*m/z* 133, 259 and 175), and *p*-hydroxyphenylethyl (*m/z* 121, 163 and 135, respectively) as shown in Scheme 2. These findings indicated the presence of all expected moieties derived from **1**.

The attachment positions of these moieties were determined by the ^{1}H NMR, 2D ^{1}H NMR and ^{13}C NMR of **1** and its derivatives. The assignment of proton and carbon signals in the sugars was based on decoupling experiments and the 2D-homonuclear (COSY) and ^{13}C–^{1}H NMR correlations of **1**.

In the ^{1}H NMR spectrum of **1**, the anomeric proton and the methyl group of the rhamnose moiety were easily recognized: the last at δ1.16 as a doublet (J = 6.2 Hz). This spectrum also exhibits the well-resolved triplet at δ5.23 (1H, *t*, J = 9.7 Hz), which is assignable to the ester-bearing methine proton and disappears in the ^{1}H NMR spectrum of descinnamoyl mussatioside I (**3**). The assignments of H-3R and H-4R in the rhamnose moiety were deduced by irradiation experiments: irradiation of H-5 at δ3.98 collapsed the doublet of the signal due to the rhamnose methyl group at δ1.16 into a singlet, and the triplet at δ5.23 also collapsed into a doublet, for which reason the latter signal was assigned to H-4. Irradiation of H-3R at δ4.1 changed the triplet at δ4.8 and the signal at δ4.08. Furthermore, comparison of the ^{13}C NMR spectral data of **1** (Table 1) with those of **3** revealed that the signals due to C-3, C-4 and C-5 of the rhamnose moiety were shifted by −3.46, +1.39 and −1.72, respectively. The esterification shifts [8] confirm that the cinnamoyl group is linked via the hydroxyl group at C-4 of the rhamnose moiety.

Comparison of the ^{13}C NMR spectral data of **1** (Table 1) with those of **5** (**1** partially hydrolysed) showed that the signals due to C-2, C-3 and C-4 of the rhamnose moiety were shifted by −0.16, +8.39 and −1.32 ppm, respectively, while the signals relative to the glucose moiety kept practically constant. The glycosidation shifts [9] indicated that the xylose moiety is attached to the C-3 hydroxyl group of rhamnose.

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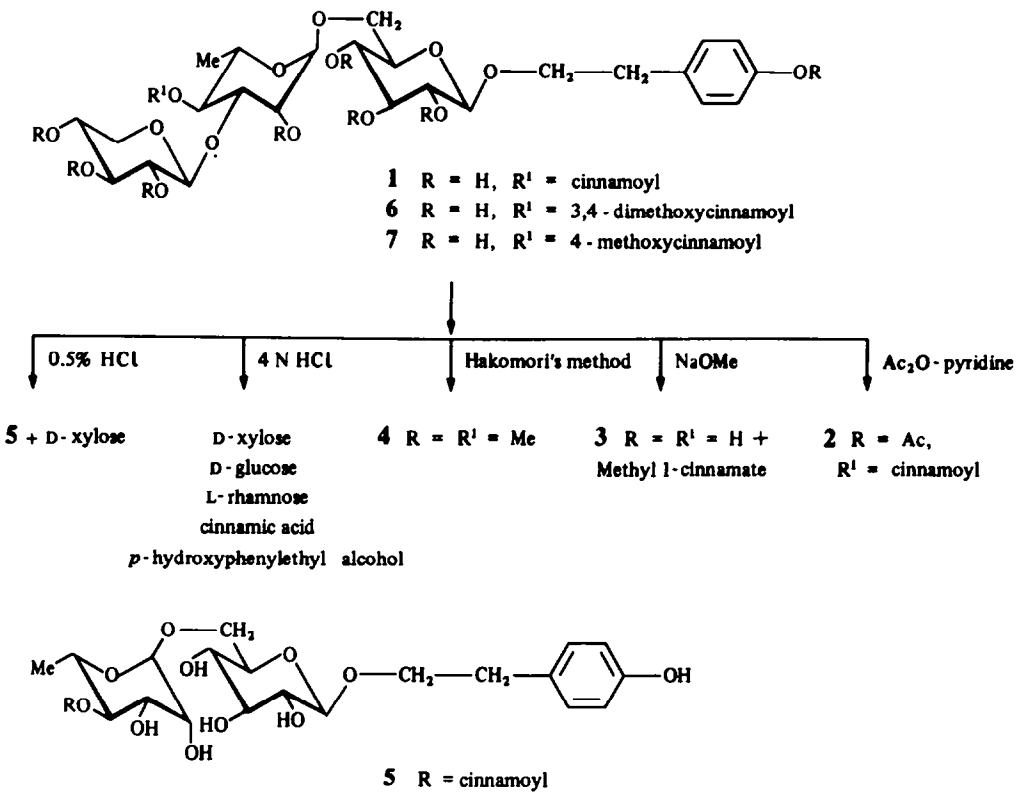
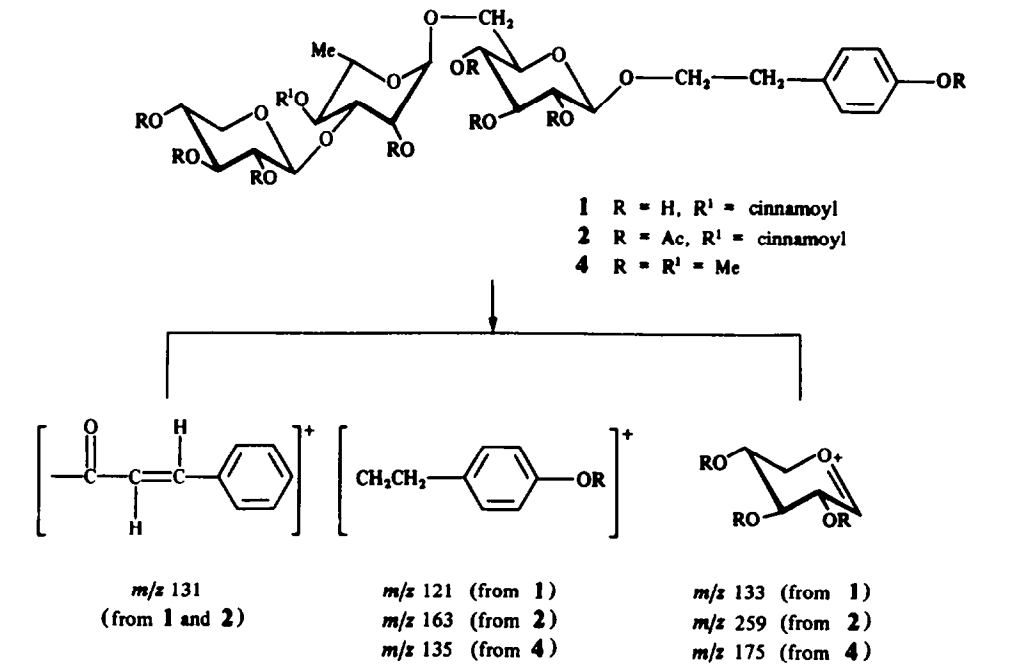


Scheme 1. Structures of phenylpropanoid glycosides 1–3 and products of analysis of mussatioside I (1).



Scheme 2. Mass spectral fragmentation of 1, 2 and 4.

Table 1. ^{13}C NMR peaks of the sugar moieties of 1, 3, 5, 6 and 7 (TMS as internal standard in CD_3OD)

	1	3	5	6	7
Glucose moiety					
1	104.46	104.46	104.56	104.49	104.45
2	75.11	75.06	75.19	75.13	75.09
3	78.04	78.05	78.11	78.08	78.02
4	71.60	71.70	71.59	71.64	71.60
5	76.63	76.79	76.69	76.66	76.61
6	68.33	68.28	68.07	68.35	68.34
Rhamnose moiety					
1	102.04	102.07	102.18	102.07	102.03
2	72.22	71.82	72.38	72.27	72.22
3	78.92	82.36	70.51	78.95	78.86
4	74.28	72.89	75.82	74.17	74.12
5	67.84	69.57	67.76	67.90	67.86
Me-5	17.88	18.01	17.93	17.90	17.88
Xylose moiety					
1	106.38	106.48	—	106.34	106.28
2	74.52	75.23	—	74.57	74.51
3	77.24	77.55	—	77.22	77.17
4	70.99	71.04	—	71.03	70.99
5	66.69	66.86	—	66.71	66.67

The ^{13}C NMR signal of C-6 in the glucose moiety of **1** and its derivatives **3** and **5** (δ68.33, δ68.28 and δ68.07, respectively) was shifted downfield relative to that of C-6 in methyl-β-D-glucopyranoside (δ61.19) [10], indicating that the rhamnose moiety is combined with the hydroxyl group at C-6 of glucose [9]. These linkages between sugars were corroborated by the methanolysis of the permethylated compound **4** with 20% methanolic hydrochloric acid to give methyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside **4a**, methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside **4b**, methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside **4c** and *p*-methoxyphenylethyl alcohol **4d**. The chemical shift of the C-8′ signal at δ72.11 relative to that of *p*-hydroxyphenylethyl alcohol as well as the glucose C-1 signal at δ104.46 suggested the linkage of the glucose moiety to the C-8′ position of *p*-hydroxyphenylethyl alcohol [11].

The ^{1}H NMR spectrum of the peracetylated compound **2** revealed the presence of eight distinct acyl signals (seven aliphatics, δ1.84–2.09, and one aromatic, δ2.31). In the ^{13}C NMR spectrum, the three anomeric carbons of the xylose, glucose and rhamnose moieties are shifted upfield (−4.98, −3.80 and −5.04 ppm, respectively) relative to the ^{13}C NMR spectrum of **1** due to the acetylation of their C-2 hydroxyl groups (β effect of neighbouring acyl groups), and the oxygen functions at C-2 in the xylose, glucose and rhamnose moieties appear as free hydroxyls [12].

The glycoside linkages at C-1 of the glucose and xylose moieties were shown to be β by the chemical shifts (δ4.31 in glucose and δ4.38 in xylose) and the large coupling constant (J = 7.6 and 7.1 Hz, respectively) of the anomeric hydrogen signals in the ^{1}H NMR spectra and by the ^{13}C–^{1}H coupling constants of J = 154.9 and 161.6 Hz, respectively, for the anomeric center. The configuration at the anomeric position of the rhamnosyl group was shown to be α by the small coupling constant in the ^{1}H NMR spectrum (δ4.84, J = 1.5 Hz) and the ^{13}C–^{1}H coupling constant of 168.0 Hz.

Mussatioside II **6** was isolated as an amorphous white powder, $C_{36}H_{47}O_{18}$, which gave glucose, xylose and rhamnose on acid hydrolysis. The ^{1}H NMR and ^{13}C NMR spectral data of **6** were very similar to those of **1**, except for the disappearance of aromatic proton signals corresponding to the cinnamoyl group. The signals at δ3.86 and δ3.87 in the ^{1}H NMR spectrum (each 3H, *s*), and those at δ56.60 and δ56.49 in the ^{13}C NMR spectrum, showed the presence of two aromatic methoxyl groups. Furthermore, the appearance of an ABX system at δ6.94–7.15 in the ^{1}H NMR spectrum (J_{AB} = 8.4 and J_{AX} = 1.5 Hz) indicated the presence of a dimethylcaffeoyl group instead of the cinnamoyl group. This was corroborated by alkaline hydrolysis of **6** with methanolic sodium hydroxide followed by treatment with methanol to give methyl dimethylcaffeate **6′** and a des-dimethylcaffeoyl mussatioside II which was identical to **3** (Scheme 1). The linkages between the sugars, the aglycone and the configurations of the sugar moieties were identical to those in **1**.

Mussatioside III **7** was isolated as an amorphous white powder ($C_{35}H_{45}O_{17}$) which also gave glucose, xylose and rhamnose on acid hydrolysis. In the ^{1}H NMR spectrum, two doublets centred at δ6.93 and δ7.52 (each 2H, J = 8.8 Hz), were assigned to the AB system of the aromatic protons of a methylcoumaroyl group, which also showed two *trans* olefinic proton resonances at δ6.35 and δ7.63 (each 1H, *d*, J = 16.0 Hz) and a signal at δ3.81 due to a methoxy group. The other ^{1}H NMR and ^{13}C NMR spectral data were identical to those of **1**. This structure was verified by alkaline hydrosysis of **7** to give methyl *p*-methylcoumarate **7′** and compound **3** (Scheme 1).

Thus mussatiosides I–III have the structures **1**, **6** and **7** respectively. In a similar manner, bark of *M. hyacinthina* yielded **1** and TLC evidence showed a similar phenylpropanoid glycoside spectrum to that of *Mussatia* sp. nov.

EXPERIMENTAL

NMR spectra were measured at 250 MHz for ^{1}H NMR and 62.83 MHz for ^{13}C NMR (Bruker WM-250) with $CDCl_3$ and CD_3OD as solvents. Chemical shifts are given on δ (ppm) scale with TMS as internal standard (*s*, singlet; *d*, doublet; *t*, triplet; *br*, broad). HRMS (FAB) were performed by the Midwest Center for

Table 2. ^{13}C NMR peaks of *t*-cinnamoyl, dimethylcaffeoyl and *p*-methylcoumaroyl moieties of 1, 6 and 7, respectively (TMS as internal standard in CD_3OD)

	t-Cinnamoyl moiety of 1	Dimethylcaffeoyl moiety of 6	*p*-Methylcoumaroyl moiety of 7
C-1″	135.77	128.92	128.39
C-2″	130.96	111.81	116.12
C-3″	129.39	150.85	130.97
C-4″	130.02	150.10	163.25
C-5″	130.79	112.80	130.97
C-6″	130.96	124.22	116.12
C-7″	146.83	146.99	146.74
C-8″	118.89	116.52	115.47
C-9″	168.42	168.81	168.85
OMe	—	56.60, 56.49	55.92

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GC was run on a Hewlett Packard 5710A apparatus with a flame ionization detector, RLCC was performed on a Tokyo Rikakikai Co. apparatus. HPLC separations on a Whatman Partisil ODS column were performed using a chromatograph, equipped with a UV detector operated at 254 nm and a R401 differential refractometer. Neutral alumina (Woelm N, Act. I) was used for CC. Precoated RP-18$F_{254}S$ plates (Merck) were used for TLC. Spots were detected by UV fluorescence and spraying with Liebermann's reagent, followed by heating at 100° for 5–10 min. The conditions for GC were as follows: column GP 3% SP-2330, 2 mm i.d./2 m; column temp., 230°; carrier gas, N_2 (30 ml/min). Paper chromatograms were done on Whatman no. 1 paper.

Plant materials. The major extraction and isolation was carried out on bark of *Mussatia* sp. nov. supplied by E. Wade Davis, Botanical Museum of Harvard University. This was collected on the upper Apurimac River (Peru), a voucher deposited (EWD no. 1322) at Harvard University and identified by A. Gentry as a new species of *Mussatia*, related to *M. hyacinthina* (Standl.) Sandw. A specific taxonomic classification is awaiting additional botanical collections. A similar extraction and isolation to that reported in detail below was carried out on *M. hyacinthina*, 'blanco' form, purchased by Davis in the Rurrenabaque market. A voucher of *M. hyacinthina*, Davis no. 1206, was deposited at Harvard University and also determined by Gentry. The phenylpropanoid glycoside spectrum of both barks was found to be similar.

Extraction and isolation. The bark (290 g) of *Mussatia* sp. nov. was defatted with hexane and the residue extracted with MeOH and lyophilized. This extract (45 g) was dissolved in a minimal amount of MeOH, the soln thus obtained was dropped into Me_2CO and centrifuged and the filtrate was concentrated and partitioned between H_2O and water-saturated *n*-BuOH. The *n*-BuOH extract was concd (24.8 g), loaded on a column of neutral Al_2O_3 (260 g) and rapidly eluted with BuOH–H_2O–MeOH (7:3:1). The eluate was concentrated (17.05 g) and a 1 g sample was fractionated by rotation locular counter-current chromatography (RLCC) using a $CHCl_3$–MeOH–H_2O (7:13:8) solvent system (the upper layer as the mobile phase, the lower layer as the stationary phase and a flow of 0.8 ml/min) to give a mixture of three phenylpropanoid glycosides (285 mg) which were separated by reversed phase HPLC with MeOH–H_2O (1:1) as solvent and a flow of 3.5 ml/min: mussatioside II (**6**, R_t 45 min; 15 mg), mussatioside I (**1**, R_t 57 min; 110 mg) and mussatioside III (**7**, R_t 69 min; 20 mg).

Mussatioside I (**1**). Amorphous white powder, $[\alpha]_D$ −14.54° (MeOH; *c* 0.27); ^{1}H NMR (CD_3OD): see Table 3. ^{13}C NMR (CD_3OD): aglycone moiety: δ 130.79 (C-1'), 116.31 (C-2'), 131.58 (C-3'), 156.81 (C-4'), 131.58 (C-5'), 116.31 (C-6'), 36.49 (C-7'), 72.11 (C-8'); glucose, rhamnose and xylose moieties: see Table 1; cinnamoyl moiety: see Table 2; FABMS-HR *m/z* (rel. int): 715.279056 $[C_{34}H_{44}O_{16}(M)+Li]^+$ (75).

Mussatioside II (**6**). Amorphous white powder, $[\alpha]_D$ −10° (MeOH; *c* 0.30); ^{1}H NMR (CD_3OD): aglycone moiety: δ 2.85 (2H, *t*-like, *J* = 7.1 Hz, H-7'), 3.75 (1H, *m*, H-8'), 3.98 (1H, *m*, H-8'), 6.69 (2H, *d*, *J* = 8.4 Hz, H-3' and H-5'), 7.06 (2H, *d*, *J* = 8.4 Hz, H-2' and H-6'); glucose moiety: δ 4.32 (1H, *d*, *J* = 7.6 Hz, H-1), 3.20 (1H, *t*, *J* = 7.6, 8.5 Hz, H-2); rhamnose moiety: δ 4.82 (1H, *br s*, H-1), 5.21 (1H, *t*, *J* = 9.8 Hz, H-4), 1.17 (3H, *d*, *J* = 6.1 Hz, Me-5); xylose moiety: δ 4.37 (1H, *d*, *J* = 6.9 Hz, H-1), 3.10 (1H, *t*, *J* = 6.9, 8.5 Hz, H-2); and dimethylcaffeoyl moiety: δ 6.41 (1H, *d*, *J* = 15.9 Hz, H-8"), 7.64 (1H, *d*, *J* = 15.9 Hz, H-7"), 7.19 (1H, *d*, *J* = 1.5 Hz, H-2"), 6.95 (1H, *d*, *J* = 8.3 Hz, H-5"), 7.15 (1H, *dd*, *J* = 1.5, 8.3 Hz, H-6"), 3.86 and 3.87 (6H, each *s*, 2 × OMe); ^{13}C NMR (CD_3OD): aglycone moiety: 130.81 (C-1'), 116.33 (C-2'), 130.96 (C-3'), 156.86 (C-4'), 130.96 (C-5'), 116.33 (C-6'), 36.51 (C-7'), 72.10 (C-8'); glucose, rhamnose and xylose moieties: see Table 1; *p*-methylcoumaroyl moiety: see Table 2. FABMS *m/z* (rel. int.): 769 $[C_{36}H_{48}O_{18}(M)+H]^+$ (8.5).

Table 3. ^{1}H NMR peaks of mussatioside I (1) (TMS as internal standard in CD_3OD)

	H	δ
t-Cinnamoyl moiety	2"	7.56 (*m*)
	3"	
	4"	7.39 (*m*)
	5"	
	6"	7.56 (*m*)
	7"	7.69 (*d*, 16.0)
	8"	6.51 (*d*, 16.0)
Aglycone moiety	2'	7.07 (*d*, 8.5)
	3'	6.69 (*d*, 8.5)
	5'	6.69 (*d*, 8.5)
	6'	7.07 (*d*, 8.5)
	7'	2.87 (*t*, 7.1)
	8'	3.75 3.98 (*m*)
Glucose moiety	1	4.31 (*d*, 7.6)
	2	3.20 (*t*, 7.6–8.5)
	3	3.43 (*t*, 9.0)
	4	3.38 (*t*, 9.0)
	5	3.27 (*m*)
	6	3.70 4.02 (*m*)
Rhamose moiety	1	4.82 (*d*, 1.0)
	2	4.08 (*dd*, 1.0, 3.0)
	3	4.11 (*dd*, 9.7, 3.0)
	4	5.23 (*t*, 9.7)
	5	3.90 (*m*)
	6	1.16 (*d*, 6.2)
Xylose moiety	1	4.38 (*d*, 7.1)
	2	3.10 (*dd*, 7.1, 9.0)
	3	3,38 (*t*, 9.0)
	4	3.47 (*m*)
	5	3.15 3.85 (*m*)

Splitting patterns and *J* values (Hz) are given in parentheses.

Mussatioside III (**7**). Amorphous white powder, $[\alpha]_D$ −30° (MeOH; *c* 0.20); ^{1}H NMR (CD_3OD): aglycone moiety: δ 2.85 (2H, *t*-like, *J* = 7.1 Hz, H-7'), 6.68 (2H, *d*, *J* = 8.5 Hz, H-3' and H-5'), 7.06 (2H, *d*, *J* = 8.5 Hz, H-2' and H-6'), 3.75 (1H, *m*, H-8'), 3.90 (1H, *m*, H-8'), glucose moiety: δ 4.30 (1H, *d*, *J* = 7.6 Hz, H-1), 3.20 (1H, *t*, *J* = 7.6 and 8.5 Hz, H-2); rhamnose moiety: δ 4.80 (1H, *d*, *J* = 1.1 Hz, H-1), 4.10 (1H, *dd*, *J* = 9.7 and 3.0 Hz, H-3), 5.18 (1H, *t*, *J* = 9.7 Hz, H-4), 3.90 (1H, *m*, H-5), 1.15 (1H, *d*, *J* = 6.2 Hz, Me-5); xylose moiety: δ 4.35 (1H, *d*, *J* = 7.0 Hz, H-1), 3.10 (1H, *t*, *J* = 7.0 Hz, H-2); *p*-methylcoumaroyl moiety: 6.35 (1H, *d*, *J* = 15.9 Hz, H-8"), 7.63 (1H, *d*, *J* = 15.9 Hz, H-7"), 6.93 (2H, *d*, *J* = 8.8 Hz, H-3" and H-5"), 7.52 (2H, *d*, *J* = 8.8 Hz, H-2" and H-6"), 3.81 (3H, *s*, OMe); ^{13}C NMR (CD_3OD): aglycone moiety: δ 130.79 (C-1'), 116.31 (C-2' and C-6'), 131.18 (C-3' and C-5'), 156.81 (C-4'), 36.49 (C-7'), 72.10 (C-8'); glucose, rhamnose and xylose moieties: see Table 1; *p*-methylcoumaroyl moiety: see Table 2. FABMS *m/z* (rel. int.): 739 $[C_{35}H_{46}O_{17}(M)+H]^+$ (42.5).

Acid hydrolysis of compound **1**. A soln of **1** (16 mg) in 4 N HCl (2 ml) was refluxed for 1 hr. H_2O was added and the mixture extracted with $CHCl_3$. The aq. layer was neutralized with Ag_2CO_3, the ppt filtered off and the filtrate evaporated *in vacuo* giving a residue in which the sugars were identified by PC with *n*-BuOH–benzene–pyridine–H_2O (5:1:3:1.5). The residue was also reduced with $NaBH_4$ and then acetylated, the hexitolacetates produced being quantitated by GC.

Acetylation of **1**. Compound **1** (33 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) to give octaacetyl mussatioside I (**2**, 48 mg) as an amorphous powder. ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, *d*, J = 6.3 Hz, rhamnose Me-5), 1.88–2.12 (5 × OAc), 2.26 (1 × OAc), 4.47 (1H, *d*, J = 7.8 Hz, glucose H-1), 4.67 (1H, *d*, J = 6.8 Hz, xylose H-1), 4.78 (1H, *br s*, J = 1.4 Hz, rhamnose H-1), 2.87 (2H, *t*-like, J = 9.0 Hz, H-7'). ^{13}C NMR ($CDCl_3$): δ 170.21, 169.97, 169.71, 169.37, 169.26, 169.16, 165.71, 149.23, 145.85, 136.11, 134.09, 130.57, 129.81, 128.94, 128.17, 121.29, 117.31, 101.40, 100.66, 97.86, 74.57, 73.06, 72.83, 72.37, 71.42, 71.18, 70.60, 70.17, 69.05, 68.88, 66.70, 66.46, 61.94, 35.17, 21.93, 20.85, 20.71, 20.48, 20.42, 20.23, 17.26.

Alkaline hydrolysis of compound **1**. To a soln of **1** (50 mg) in absolute MeOH (2 ml) was added 5% NaOMe–MeOH (0.2 ml) and the reaction mixture was refluxed for 1.5 hr. The mixture was passed through an Amberlite IR-120 (H^+) column and the eluate was concd under red. pres. The residue was dissolved in H_2O and extracted with Et_2O. Concentration of the aq. layer gave descinnamoyl mussatioside I (**3**, 12 mg) as an amorphous powder, and concn of the Et_2O layer gave methyl-*t*-cinnamate (**3'**). ^{1}H NMR (CD_3OD) of **3**: δ 1.26 (3H, *d*, J = 6.1 Hz, rhamnose Me-5), 4.29 (1H, *d*, J = 7.0 Hz, glucose H-1), 4.45 (1H, *d*, J = 7.0 Hz, xylose H-1), 4.78 (1H, *br s*, rhamnose H-1). ^{13}C NMR (CD_3OD) of **3**: aglycone moiety: δ 130.83 (C-1'), 116.28 (C-2' and C-6'), 130.97 (C-3' and C-5'), 156.76 (C-4'), 36.39 (C-7'), 72.20 (C-8'); glucose, rhamnose and xylose moieties: see Table 1.

Per-O-methylation of compound **1** *by Hakomori's method*. **1** (164 mg) in DMSO (5 ml) was treated with dimsyl carbanion soln (5 ml) prepared from NaH (1 g) and DMSO (8 ml), and the solution was stirred at room temp. for 30 min under N_2. MeI (5 ml) was then added and stirring was continued for a further 2.5 hr in the dark. The reaction mixture was poured into ice H_2O (70 ml) and extracted with Et_2O (3 × 80 ml). The combined Et_2O extract was washed with H_2O, 3% $Na_2S_2O_3$ soln and then H_2O, dried, and concd under red. pres. The residue was purified by prep. TLC ($CHCl_3$–Me_2CO, 6:1; R_f = 0.55) to give descinnamoyl nona-*O*-methyl mussatioside I (**4**, 100 mg) as a colourless syrup. ^{1}H NMR ($CDCl_3$): δ 1.29 (3H, *d*, J = 6.7 Hz, rhamnose Me-5), 3.44–3.65 (3H, each *s*, 8 × OMe), 3.78 (3H, *s*, 1 × OMe), 4.24 (1H, *d*, J = 7.7 Hz, glucose H-1), 4.46 (1H, *d*, J = 7.3 Hz, xylose H-1), 4.78 (1H, *br s*, rhamnose H-1), 6.82 (2H, *d*, J = 7.5 Hz, H-2' and H-6'), 7.15 (2H, *d*, J = 7.5 Hz, H-3' and H-5'). ^{13}C NMR ($CDCl_3$): δ 158.14, 130.53, 129.75, 113.77, 104.51, 103.20, 98.31, 86.39, 85.23, 83.73, 83.60, 82.28, 80.52, 79.87, 79.51, 78.96, 74.31, 70.64, 67.87, 66.88, 62.98, 60.61, 60.44, 60.37, 60.29, 60.23, 60.16, 59.35, 58.42, 55.07, 35.19, 17.69.

Methanolysis of compound **4** *with* 20% *methanolic* HCl. A soln of **4** (80 mg) in 20% methanolic HCl (2 ml) was refluxed for 2 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated to dryness *in vacuo*, and the residue was subjected to prep. TLC (benzene–acetone 2:1) to give two fractions. Fraction 1 was separated by reversed phase HPLC with MeOH–H_2O (48:52) into methyl-2,3,4-tri-*O*-Me-α,β-D-glucopyranoside (**4c**) and methyl 2,3,4-tri-*O*-Me-α,β-D-xylopyranoside (**4a**), which were identified by comparison of ^{1}H NMR and ^{13}C NMR ($CDCl_3$) spectral data with authentic samples. Fraction 2 was also separated by reversed phase HPLC, (MeOH–H_2O 58:42), into *p*-methoxyphenylethyl alcohol (**4d**). ^{1}H NMR ($CDCl_3$): δ 7.15 (2H, *d*, J = 8.6 Hz, H-3' and H-5'), 6.86 (2H, *d*, J = 8.6 Hz, H-2' and H-6'), 2.81 (2H, *t*, J = 6.5 Hz, H-7'), 3.82 (2H, *t*, J = 6.5 Hz, H-8'), 3.79 (3H, *s*, OMe) and methyl 2,4-di-*O*-Me-α-L-rhamnopyranoside (**4b**), ^{1}H NMR ($CDCl_3$): δ 4.72 (1H, *br s*, H-1), 3.46 (1H, *dd*, J = 1.3 and 3.7 Hz, H-2), 3.80 (1H, *dd*, J = 9.4 and 3.7 Hz, H-3), 2.97 (1H, *t*, J = 9.4 Hz, H-4), 3.5 (1H, *m*, J = 9.4 and 6.2 Hz, H-5), 1.32 (3H, *d*, J = 6.2 Hz, Me-5), 3.36, 3.49, 3.58 (3 × OMe). ^{13}C NMR ($CDCl_3$): δ 97.30 (C-1), 80.63 (C-2), 71.32 (C-3), 83.86 (C-4), 67.06 (C-5), 17.81 (C-6), 54.74 (MeO-1), 58.86 (MeO-2), 60.79 (MeO-4) agreed with those reported in lit. [13].

Partial hydrolysis of **1**. **1** (60 mg) in 0.5% HCl (2.5 ml) was refluxed at 90° for 1 hr, then H_2O added to the soln and the mixture extracted with *n*-BuOH. The aq. layer was neutralized with Ag_2CO_3 and the ppt filtered off. The filtrate was evaporated *in vacuo* and the residue was identified as xylose by PC and GC. The *n*-BuOH extract was concd under red. pres. and separated by prep. TLC (*n*-BuOH–H_2O–MeOH, 7:3:1) to give **5** (15 mg), ^{1}H NMR (CD_3OD): δ 4.31 (1H, *d*, J = 7.7 Hz, glucose H-1), 4.81 (1H, *br s*, rhamnose H-1), 1.16 (3H, *d*, J = 6.3 Hz, rhamnose Me-5), 5.06 (1H, *t*, J = 9.4 Hz, rhamnose H-4). ^{13}C NMR (CD_3OD): aglycone moiety: δ 130.10 (C-1'), 116.28 (C-2' and C-6'), 131.61 (C-3' and C-5'), 156.94 (C-4'), 36.58 (C-7'), 72.15 (C-8'); cinnamoyl moiety: 135.84 (C-1''), 130.99 (C-2'' and C-6''), 129.37 (C-3'' and C-5''), 130.10 (C-4''), 118.98 (C-7''), 146.72 (C-8''), 168.52 (C-9''); glucose and rhamnose moieties: see Table 1.

Alkaline hydrolysis of compound **6**. To a soln of **6** (30 mg) in absolute MeOH (4 ml) was added 5% NaOMe–MeOH (0.4 ml) and the reaction mixture refluxed for 1.5 hr. The reaction mixture was treated as for **1**. The residue obtained was dissolved in H_2O and extracted with Et_2O. Concentration of the aq. layer gave desdimethyl caffeoyl mussatioside II (8 mg), whose ^{13}C NMR and ^{1}H NMR spectral data were identical to those of **3**. The Et_2O extract was dried and concd to give methyl dimethylcaffeate (**6'**).

Alkaline hydrolysis of compound **7**. A mixture of **7** (45 mg) in absolute MeOH (2 ml) and 5% NaOMe–MeOH (0.2 ml) was refluxed for 1.5 hr. After the usual workup, the residue was dissolved in H_2O and extracted with Et_2O. The aq. layer, after concn, gave des-*p*-methylcoumaroyl mussatioside III (14 mg), whose ^{13}C NMR and ^{1}H NMR spectral data were identical to those of **3**. The Et_2O extract was dried and concd to give methyl *p*-methylcoumarate (**7'**).

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