Tannins and Related Compounds. LXXXIV.[1] Isolation and Characterization of Five New Hydrolyzable Tannins from the Bark of *Mallotus japonicus*

Reiko SAIJO, Gen-ichiro NONAKA and Itsuo NISHIOKA*

Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received February 8, 1989*

A chemical examination of the bark of *Mallotus japonicus* (THUNB.) MUELLER-ARG. (Euphorbiaceae) has led to the isolation of five new hydrolyzable tannins (16—20), together with fourteen known tannins (1—14). On the basis of chemical and spectroscopic evidence, the structures of compounds 16 and 17 were established as 1,2-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-β-D-glucose and 1-*O*-digalloyl-3,6-(*R*)-hexahydroxydiphenoyl-β-D-glucose, respectively, while compounds 18 (mallojaponin) and 19 (mallonin) were shown to be 1-*O*-galloyl-2,4-elaeocarpusinoyl-3,6-(*R*)-valoneayl-β-D-glucose and 1-*O*-galloyl-2,4-elaeocarpusinoyl-β-D-glucose. Compound 20 (mallotusinin) was characterized as a novel ellagitannin which possesses a unique 1,1′-(3,3′,4,4′-tetrahydroxy)dibenzofurandicarboxyl group. On the other hand, examination of the leaves revealed the presence of hydrolyzable tannins (8—10, 12—15) all containing a β-D-glucopyranose core with $^1C_4$-conformation. Furthermore, the orientation of the valoneayl group in mallotinic acid (13) and mallotusinic acid (14), which had remained unclarified, was determined on the basis of ^1H–^{13}C shift correlation spectral analysis and chemical correlations.

Keywords *Mallotus japonicus*; Euphorbiaceae; mallojaponin; mallonin; mallotusinin; mallotinic acid; hydrolyzable tannin; valoneaic acid; elaeocarpusinic acid; 1,1′-(3,3′,4,4′-tetrahydroxy)dibenzofurandicarboxylic acid

In previous papers,[2] We reported on the isolation and characterization of a series of gallotannins possessing *C*-glycoside and phenol glucoside cores from the bark of *Mallotus japonicus* (THUNB.) MUELLER-ARG. (Euphorbiaceae), which has been used in Japan as a folk medicine for gastric and duodenal ulcers. Further chemical examination of the bark of this plant has now resulted in the isolation and characterization of fourteen known tannins (**1**—**14**) and five new hydrolyzable tannins (**16**—**20**), among which **18**—**20** were designated as mallojaponin, mallonin and mallotusinin, respectively. In addition, examination of the leaf extract has revealed the occurrence of hydrolyzable tannins (**8**—**10**, **12**—**14**), together with terchebin (**15**). This paper deals with the isolation and structural characterization of these tannins, and also describes the determination of the orientation of the valoneayl group in mallotinic acid (**13**) and mallotusinic acid (**14**), which remained to be solved.

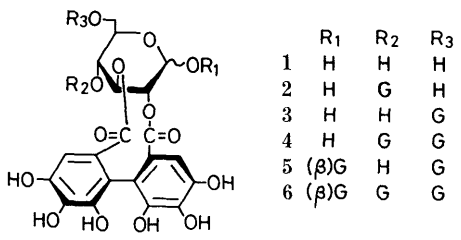
By a combination of adsorption and partition (Sephadex LH-20, MCI-gel CHP-20P, Fuji-gel ODS-G3 and Avicel cellulose) chromatographies, compounds **1**—**14** and **16**—**20** were isolated from the aqueous acetone extract of the bark, while from the leaf extract, compounds **8**—**10** and **12**—**15** were obtained. Among these compounds, **1**—**7** were found to be ellagitannins based on a glucopyranose core with $^4C_1$-conformation, to which a 4,4′,5,5′,6,6′-(*S*)-hexahydroxydiphenoyl[(*S*)-HHDP] ester group is connected. They were identified as 2,3-(*S*)-HHDP-D-glucose (**1**),[3] and its 4-*O*-(**2**) (pterocaryanin B),[4] 6-*O*-(**3**),[3] 4,6-di-*O*-(**4**),[5] 1(β),6-di-*O*-(**5**)[6] and 1(β),4,6-tri-*O*-gallates (**6**) (pterocaryanin C)[4] and 1(β)-*O*-galloyl-2,3,4,6-bis-(*S*)-HHDP-glucose (**7**) [1(β)-*O*-galloylpedunclagin],[7] by means of physical and spectral comparisons with authentic samples. On the other hand, compounds **8**—**15** were found to contain a glucopyranose core with $^1C_4$-conformation, and were identified as corilagin (**8**),[8] punicafolin (**9**),[8] geraniin (**10**),[9] elaeocarpusin (**11**),[9] furosin (**12**),[10] mallotinic acid (**13**),[11] mallotusinic acid (**14**)[11] and terchebin (**15**).[12]

Compound **16**, a tan amorphous powder $[\alpha]_D$ −71.4° (MeOH), $C_{34}H_{26}O_{22}\cdot H_2O$, showed an $(M-H)^-$ ion peak at *m/z* 785 in the negative fast atom bombardment mass spectrum (FAB-MS). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum suggested the presence of two galloyl groups (δ 7.15, 4H, s) and one HHDP ester group (δ 6.74 and 6.95, each 1H, s). Enzymatic hydrolysis of **16** with tannase afforded gallic acid and a hydrolysate which was found to be identical with 3,6-(*R*)-HHDP-D-glucose (**16a**)[8] by direct physical and spectral comparisons.

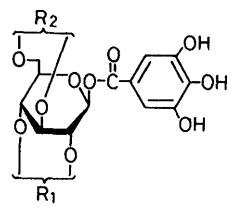
The locations of the galloyl groups were determined spectroscopically as follows. In the ^{1}H-NMR spectrum of **16**, two lowfield signals [δ 5.42 (d, $J=5$ Hz) and 6.64 (d, $J=5$ Hz)] due to glucose methine protons geminal to the galloyl group were observed. One (δ 6.64) of them could apparently be assigned to the anomeric proton from its chemical shift value, while the other signal at δ 5.42 was readily assignable to the C-2 proton by ^1H–^1H shift-correlation spectroscopy (COSY). Therefore, **16** was considered to be 1,2-di-*O*-galloyl-3,6-(*R*)-HHDP-D-glucose. Further support for this structure was obtained by comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) chemical shifts of the glucose signals with those of **8** and **9**. The similar chemical shifts for the anomeric signals in **16** (δ 91.3) and **9** (δ 91.9), and in contrast, the lowfield shift in **8** (δ 94.1) indicated the locations of the galloyl groups at the C-1 position and the adjacent C-2 position in **16**. On the other hand, the chemical shifts for C-4 in **16** (δ 62.1) and **8** (δ 62.0) were almost the same, but differed from that in **9** (δ 64.7), indicating the absence of the galloyl group at the C-4 position.

The configuration at the anomeric center was concluded to be β from the close similarities of the ^{13}C-NMR chemical shifts of anomeric resonances in **16** and **9**. On the basis of these chemical and spectroscopic data, compound **16** was characterized as 1,2-di-*O*-galloyl-3,6-(*R*)-HHDP-β-D-glucose.

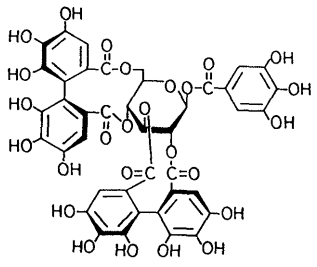
Compound **17**, a tan amorphous powder, $[\alpha]_D$ −112.0° (MeOH), was found to have the same molecular formula $C_{34}H_{26}O_{22}\cdot H_2O$ as that of **16** by elemental analysis and negative FAB-MS [*m/z* 785 $(M-H)^-$]. Upon tannase hydrolysis, **17** yielded **16a** and gallic acid in a molar ratio



	R_1	R_2	R_3
1	H	H	H
2	H	G	H
3	H	H	G
4	H	G	G
5	(β)G	H	G
6	(β)G	G	G



	R_1	R_2
8	H,H	(*R*)HHDP
9	G,G	(*R*)HHDP
10	DHHDP	(*R*)HHDP
11	Ela	(*R*)HHDP
12	DHHDP	H,H
13	H,H	(*R*)Val
14	DHHDP	(*R*)Val
15	DHHDP	G,G



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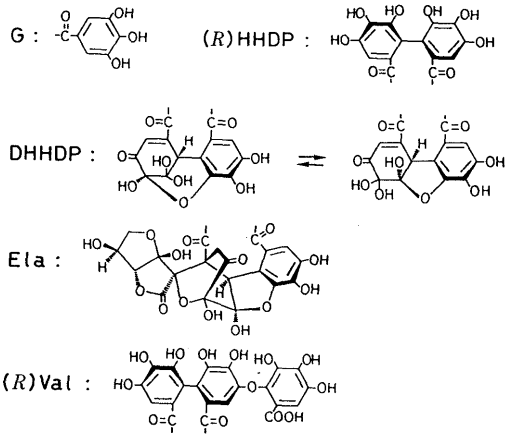


Chart 1

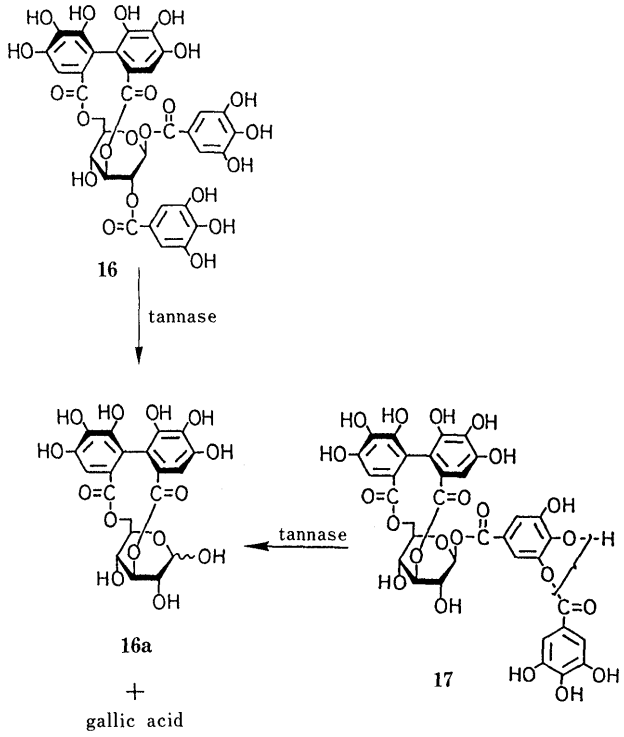


Chart 2

of *ca.* 1:2. In the ^{1}H-NMR spectrum of **17**, the chemical shifts and coupling patterns of the glucose moiety were virtually identical to those of **8**, thus suggesting that at least one galloyl group is located at the anomeric position, and that the extra galloyl group is linked depsidically. These suggestions were further confirmed by conversion of **17** to **8** on methanolysis with methanolic acetate buffer (pH 5.4).[13] The location of the extra galloyl group was determined by inspection of the ^{1}H- and ^{13}C-NMR spectra, which clearly showed signals arising from an equilibrium mixture of *m*- and *p*-digallates[14] (see Experimental). From these findings, compound **17** was assigned as 1-*O*-digalloyl-3,6-(*R*)-HHDP-β-D-glucose.

Mallojaponin (**18**), a tan amorphous powder, $[\alpha]_D$ +15.9° (MeOH), $C_{57}H_{38}O_{37}\cdot 6H_2O$, and mallonin (**19**), a tan amorphous powder, $[\alpha]_D$ +13.3° (MeOH), $C_{33}H_{28}O_{24}\cdot 2H_2O$, were extremely unstable in solution, and gradually formed yellow substances, which showed larger *Rf* values than the original compounds on thin-layer chromatography (TLC).

The ^{1}H- and ^{13}C-NMR spectra of **18** showed sugar resonances, the chemical shifts and coupling patterns being almost identical with those found in **11**. In addition, the observation of characteristic ^{1}H-NMR signals due to an isolated methylene [δ 2.19 (d, J=20 Hz) and 2.97 (dd, J=3, 20 Hz)] and a benzylic methine [δ 5.69 (d, J=3 Hz)], which are mutually coupled through four bonds (W-rule),[15] were suggestive of the presence of an elaeocarpusinoyl ester group. Among four aromatic one-proton singlets, the singlet at δ 7.23 was assigned to the aromatic proton in the elaeocarpusinoyl group, based on a comparison of the chemical shifts. The remaining three singlets at δ 6.82, 6.89 and 7.15, one more than in the case of **11**, suggested that the HHDP ester group in **11** is replaced by a valoneayl group. This is further supported by the fact that methylation of **18** with dimethyl sulfate and potassium carbonate in dry acetone gave the heptadecamethyl ether (**18a**) [field desorption mass spectrum (FD-MS): *m/z* 1516 (M^+)].

Compound **11** has been reported to decompose readily into **10** on heating in aqueous solution.[9] A similar reaction occurred in the case of **18** to liberate L-ascorbic acid (**18b**) and **14**. Furthermore, **18** was prepared by condensation of **14** and **18b** in 0.2 M acetic acid solution. On the basis of these findings, **18** was characterized as 1-*O*-galloyl-2,4-

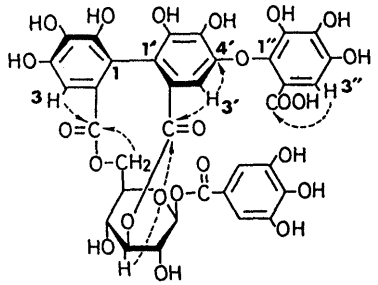
TABLE I. ^{13}C-NMR Spectral Data for Compounds **11**, **13** and **18**—**20**

	13[a]	**18**[b]	**11**[a]	**19**[b]	**20**[b]
Glucose					
C-1	93.7	92.0	92.4	93.5	92.2
C-2	70.0	74.2	74.4	77.4	74.2
C-3	73.0	63.7	63.5	62.5	63.4
C-4	62.6	68.9	68.8	74.0	68.6
C-5	75.9	74.7	74.4	78.6	75.8
C-6	64.2	64.5	64.4	63.9	65.0
Galloyl					
C-1	120.8	119.9	120.3	120.1	119.9
C-2,6	111.1 (2C)	110.7 (2C)	110.5 (2C)	110.2 (2C)	110.4 (2C)
C-3,5	145.7 (2C)	146.1 (2C)	146.1 (2C)	146.1 (2C)	146.0 (2C)
C-4	140.3	140.1[c]	139.7	139.7	140.0
2,4-Acyl group					
C-1		51.8	51.8	51.8	115.0[c]
C-2		49.6	49.8	49.9	116.1[c]
C-3		38.0	38.0	38.1	111.0
C-4		198.5	197.7	198.9	144.7[d]
C-5		96.4	96.3	96.4	133.2
C-6		107.8	108.1	107.9	145.1[d]
C-1′		116.2	116.1	116.5	116.1[c]
C-2′		118.8	118.5	118.6	119.7
C-3′		114.1	114.1	113.9	115.0[c]
C-4′		147.6	147.6	147.5	144.7[d]
C-5′		136.7	136.2	136.3	137.5
C-6′		148.6	148.6	148.5	145.1[d]
C-1″		171.1	170.7	171.5	
C-2″		80.6	80.7	80.7	
C-3″		108.9	109.1	108.9	
C-4″		89.4	89.4	89.8	
C-5″		74.2	73.7	74.0	
C-6″		76.5	76.5	76.5	
3,6-Acyl group					
C-1	116.0	116.2	116.5		115.0
C-2	125.8	123.8	125.0		125.2
C-3	108.0	107.8	109.3		108.2
C-4	145.3	145.1[d]	145.1[c]		145.4[d]
C-5	136.7	136.4	137.4[d]		135.8[e]
C-6	145.4[c]	146.1[d]	145.4[c]		145.4[d]
C-1′	119.0	118.1	116.7		116.8
C-2′	125.1	122.7	123.1		124.6
C-3′	108.8	109.3	110.1		109.9
C-4′	146.7	146.7	144.7[c]		144.9[d]
C-5′	138.8	137.4	137.5[d]		136.4[e]
C-6′	145.0[c]	145.1[d]	144.9[c]		144.9[d]
C-1″	143.0	142.7			
C-2″	114.6	114.1			
C-3″	110.0	110.7			
C-4″	139.8[d]	140.1[c]			
C-5″	138.5	138.7[c]			
C-6″	139.6[d]	137.9[c]			
–COO–	165.3	165.2	164.7	165.2	165.4
	167.3	165.4	165.3	165.8	166.7
	167.4	166.6 (2C)	166.4	168.7	167.4
	168.0	168.1	168.0		169.1
		168.5	170.7		169.4

a) Measured in acetone-d_6. *b*) Measured in acetone-d_6 + D_2O. *c*—*e*) Assignments may be interchanged in each column.

elaeocarpusinoyl-3,6-(*R*)-valoneayl-β-D-glucose.

The orientation of the valoneayl group in **18** was determined by ^{1}H–^{13}C long-range COSY spectral analysis[16] using **13**, which was derivable by hot water treatment of **18**. The correlations between three one-proton aromatic singlets and carboxyl signals through two- and three-bond long-range couplings were clearly seen from the spectrum. Among them, the signal at δ 6.68 was correlated with the carbon resonance at δ 146.7, which is assignable to C-4′ of



13

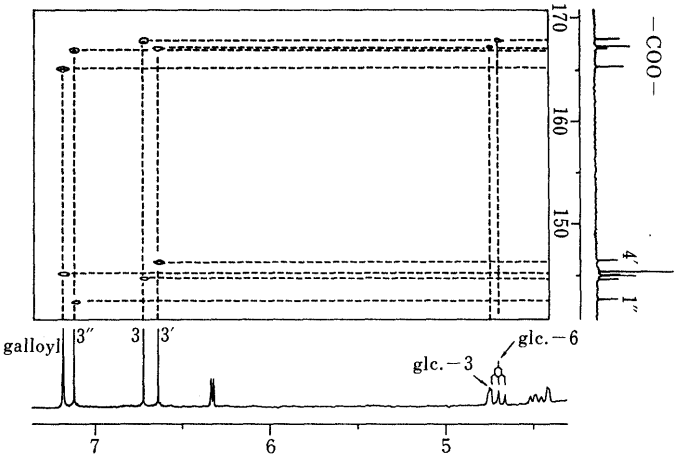


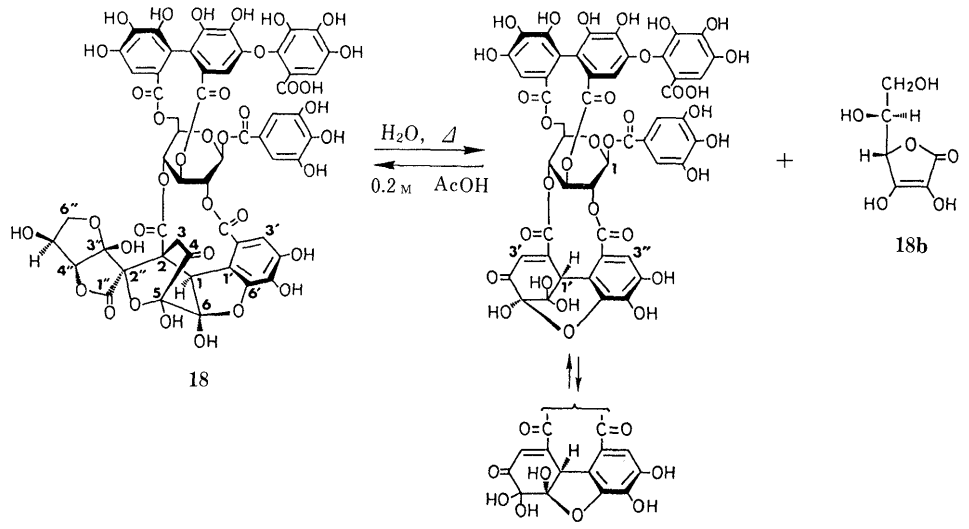
Fig. 1. ^{1}H–^{13}C Long-Range Shift Correlation Spectra of **13** in Acetone-d_6 (J_{CH} = 10 Hz)

the valoneayl group based on the lowfield shift caused by alkylation of the phenolic hydroxyl group. Thus, the signal at δ 6.68 was concluded to correspond to the proton located at the middle aromatic ring (H-3′). Since the signal at δ 7.14 was shown to be correlated with the signal at δ 167.3, which was attributable to the carboxylic acid carbon from its broadness, the signal could be assigned to the proton of the terminal aromatic ring (H-3″). The remaining aromatic signal at δ 6.78 should therefore be assigned to H-3. Furthermore, the carboxylic acid ester signal at δ 167.4 was shown to be coupled with the valoneayl H-3′ and also with the glucose H-3 signal, while the signal at δ 168.0 was correlated with the valoneayl H-3 and glucose H-6 signals. From these findings, the orientation of the valoneayl group in mallotinic acid was concluded to be as shown by the formula **13**. Hence the structures of mallojaponin and mallotusinic acid are represented by the formulae **18** and **14**, respectively.

The ^{1}H-NMR spectrum of **19** showed a two-proton singlet at δ 7.19 and a one-proton singlet at δ7.20 in the aromatic field. The appearance of aliphatic signals [δ 2.22 (d, J = 20 Hz) and 3.02 (dd, J = 3, 20 Hz)], and a benzylic methine [δ 5.72 (d, J = 3 Hz)] was consistent with the presence of an elaeocarpusinoyl ester group in the molecule. In addition, the sugar signal pattern was similar to that found in **8**. These observation suggested **19** to be 1-*O*-galloyl-2,4-elaeocarpusinoyl-β-D-glucose.

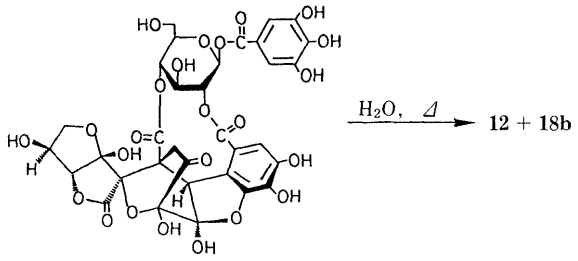
On heating in aqueous solution,[9] **19** afforded **18b** and a hydrolysate, which was found to be identical with **12**. Consequently, mallonin was assigned the structure **19**.

Mallotusinin (**20**), a tan amorphous powder, $[\alpha]_D$ −20.3° (acetone), $C_{41}H_{26}O_{25}\cdot 3H_2O$, gave a ^{1}H-NMR spectrum



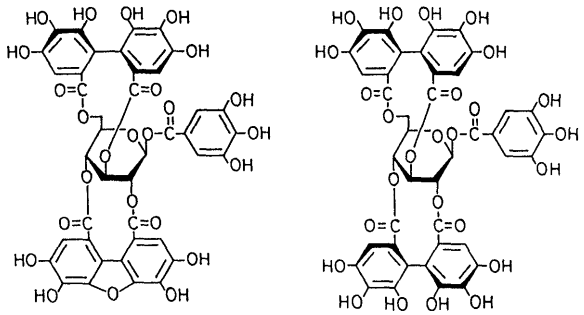
18 14 18b

Chart 3



19

Chart 4



20 21

Chart 5

similar to that of 1-*O*-galloyl-2,4;3,6-bis-(*R*)-HHDP-*β*-D-glucose (**21**),[9] showing signals due to a galloyl group (δ 7.14, 2H, s) and four aromatic protons (δ 6.74, 7.10, 7.14 and 7.37, each 1H, s). The negative FAB-MS of **20** showed an intense $(M-H)^-$ ion peak at *m/z* 917, which was eighteen mass units less than that of **21**. In the ^{1}H-NMR spectrum, the lowfield shifts of all the sugar signals indicated that the sugar hydroxyl groups are exhaustively esterified, while the ^{13}C-NMR spectrum exhibited six sugar signals (δ 63.4, 65.0, 68.6, 74.2, 75.8 and 92.2), which were almost in line with those found in **11** and **18**.

On methylation with dimethyl sulfate and potassium carbonate in dry acetone, **20** afforded the tridecamethyl ether (**20a**). Subsequent alkaline hydrolysis of **20a**, followed by treatment with diazomethane, yielded methyl 3,4,5-trimethoxybenzoate (**20b**), dimethyl 4,4′,5,5′,6,6′-hexamethoxydiphenoate (**20c**) and a methyl carboxylate (**20d**). The molecular formula of **20d** was confirmed to be $C_{22}H_{20}O_9$ by elemental analysis together with the electro-impact mass spectral (EI-MS) data [*m/z* 404 (M^+)]. The ^{1}H-NMR spectrum of **20d** showed signals seemingly due to one aromatic proton (δ 7.40) and three methoxyl groups (δ 3.88, 4.00 and 4.25). The ^{13}C-NMR spectrum also seemingly exhibited six aromatic resonances (δ 111.0, 117.8, 121.0,

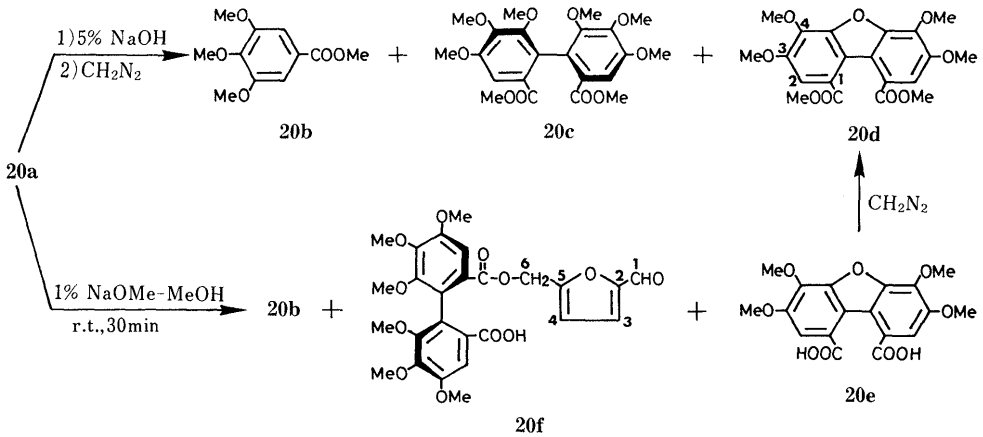


Chart 6

137.1, 149.3 and 150.1), together with a carboxyl (δ 168.1) and methoxyl (δ 56.7, 57.0 and 61.3) signals. Taking the above MS data into account, these ^{1}H- and ^{13}C-NMR observations indicate the presence of a symmetrical substitution system in the molecule of **20d**. Thus, **20d** was concluded to be dimethyl 1,1′-(3,3′,4,4′-tetramethoxy)dibenzofurandicarboxylate.

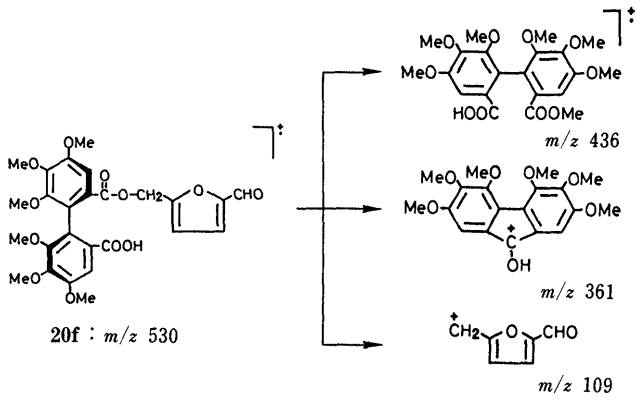


Chart 7

When treated in a weakly alkaline solution (1% methanolic sodium methoxide) at room temperature, **20a** gave **20b** and two carboxylic acids (**20e**, **20f**). In the ^{1}H-NMR spectrum (in dimethyl sulfoxide-d_6), **20e** showed signals seemingly due to one aromatic proton (δ 7.11) and two methoxy groups (δ 3.87 and 3.99). Since the EI-MS of **20e** showed a prominent M^+ ion peak at m/z 346, and **20d** was obtained by diazomethane methylation of **20e**, **20e** was assigned as 1,1′-(3,3′,4,4′-tetramethoxy)dibenzofurandicarboxylic acid. The ^{1}H-NMR spectrum of **20f** exhibited signals corresponding to an aldehyde proton (δ 9.57, 1H, s), two olefinic protons coupled with each other (δ 6.42 and 7.13, each 1H, d, J=4 Hz) and a methylene proton (δ 5.08, 2H, s), together with two aromatic singlets (δ 7.38 and 7.33, each 1H, s) and six methoxyl signals (δ 3.55, 3.57, 3.90 and 3.95, 18H in total, s) assignable to the hexamethoxydiphenoyl ester group. The ^{13}C-NMR spectrum of **20f** showed, besides signals due to the hexame-

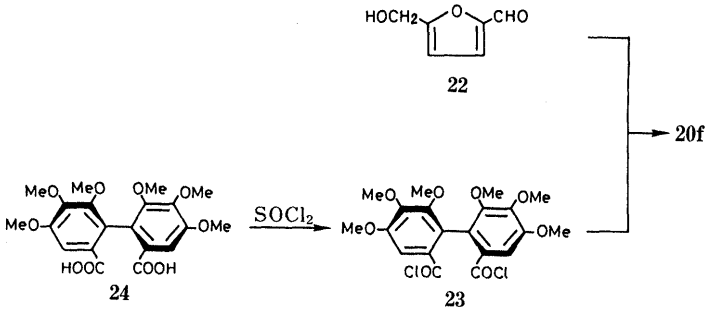


Chart 8

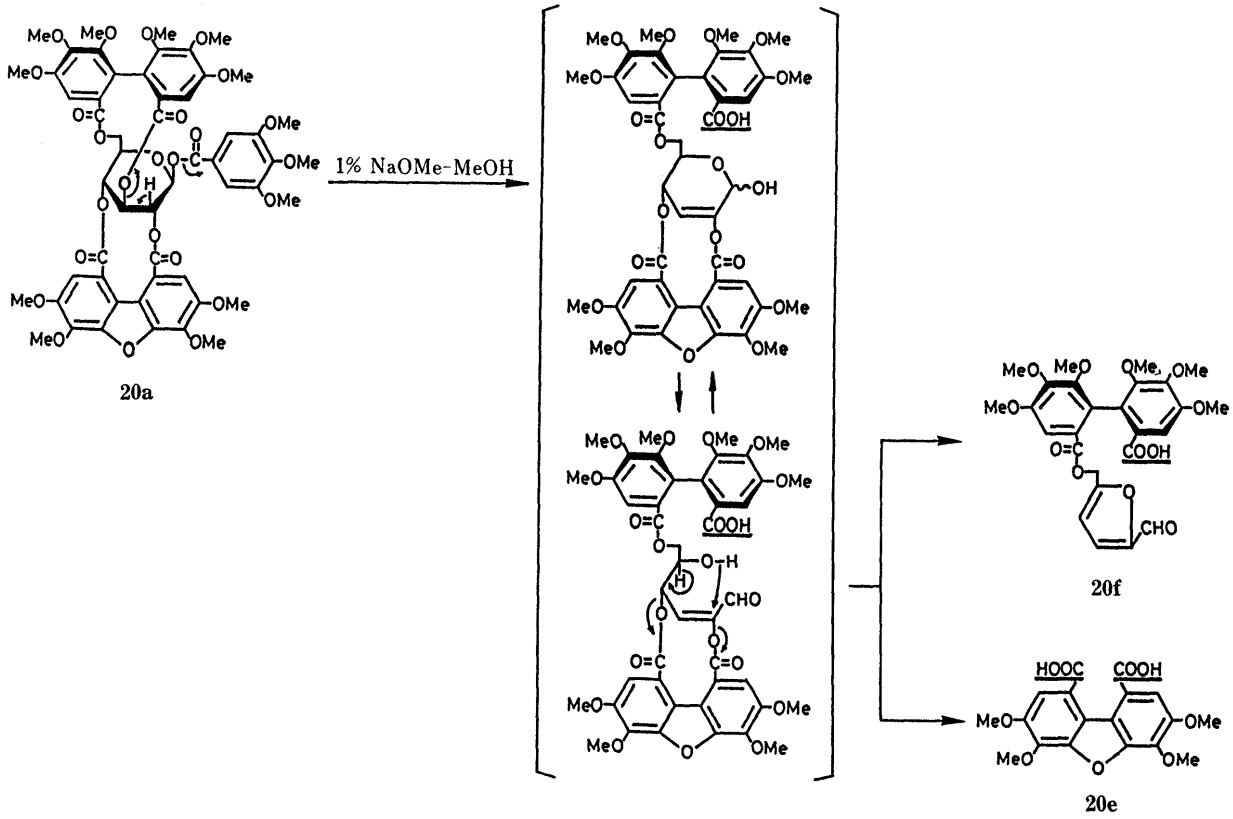


Chart 9

thoxydiphenoyl ester group, six resonances (δ 77.2, 112.5, 121.5, 152.8, 155.3 and 178.0) whose chemical shifts were consistent with the 5-(hydroxymethyl)-2-furaldehyde structure. This was further supported by EI-MS examination, which showed diagnostic fragment peaks (Chart 7).

Confirmation of the structure, including the absolute stereochemistry, was obtained by synthesis of **20f**. Condensation of 5-(hydroxymethyl)-2-furaldehyde (**22**) and an acid chloride (**23**) prepared from (*R*)-hexamethoxydiphenoic acid (**24**) provided, among others, a product identical with **20f**.

Compound **20f** is probably formed from **20a** through elimination of the carboxyl function located at the glucose C-3 position, followed by successive elimination of the 1,1′-(3,3′,4,4′-tetramethoxy)dibenzofurandicarboxyl group from the glucose C-2 and C-4 positions (Chart 9).[17] The proposed pathway is supported by the fact that both **20e** and **20f** were obtained as free carboxylic acid forms, not methyl esters, despite the use of sodium methoxide in methanol. Consequently, the HHDP ester group in **20** was concluded to have *R*-configuration and to be located at the 3,6-positions of the glucose moiety. Mallotusinin was therefore assigned the structure **20**.

Mallojaponin (**18**) and mallonin (**19**) are the second and third examples of hydrolyzable tannins which possess an elaeocarpusinoyl ester group in the molecule, whereas mallotusinin (**20**) represents the first ellagitannin containing the novel 1,1′-(3,3′,4,4′-tetrahydroxy)dibenzofurandicarboxyl group. Taking into account the co-occurrence of geraniin (**10**) in this plant material, the 1,1′-(3,3′,4,4′-tetrahydroxy)dibenzofurandicarboxylic acid is considered to be biosynthetically formed from the dehydrohexahydroxydiphenoic acid through reductive aromatization.

Finally, it should be noted that the hydrolyzable tannins in the leaves of this plant all possess 2,4- and/or 3,6-bridged acyl group(s) attached to the glucose moiety with $^1C_4$-conformation, whereas those of the bark contain 2,3- and/or 4,6- and 2,4-and/or 3,6-positioned acyl group(s). This fact indicates that the enzymic oxidative carbon-to-carbon condensation is non-specific in the bark, which is another characteristic feature of the methabolism of tannins in this plant.

Experimental

Details of the instruments and chromatographic conditions used throughout this work are the same as described in the previous paper.[1]

Isolation of Tannins **a) From the Bark** The fresh bark (43.5 kg) of *M. japonicus*, collected at Fukuoka prefecture, was chopped into small pieces and extracted with 80% aqueous acetone at room temperature. The acetone was removed by evaporation under reduced pressure and the resulting precipitates were filtered off. The filtrate was, after concentration, subjected to Sephadex LH-20 chromatography. Elution with H_2O containing increasing amounts of MeOH and then with H_2O–acetone (1 : 1, v/v) afforded five fractions; I (860 g), II (580 g), III (420 g), IV (140 g) and V (250 g). Fraction I was rechromatographed over MCI-gel CHP 20P with H_2O–MeOH (1 : 0—0 : 1, v/v) and then over Sephadex LH-20 with EtOH to give 2,3-(*S*)-HHDP-D-glucose (**1**) (1.2 g) and pterocaryanin B (**2**) (51 mg). Fraction II was repeatedly chromatographed over Sephadex LH-20 with EtOH and with 80% aqueous MeOH, MCI-gel CHP 20P and Fuji-gel ODS-G3 with H_2O–MeOH (1 : 0—3 : 2, v/v) to yield 6-*O*-galloyl-2,3-(*S*)-HHDP-D-glucose (**3**) (307 mg), corilagin (**8**) (21.3 g), furosin (**12**) (1.5 g), mallotinic acid (**13**) (668 mg) and mallonin (**19**) (177 mg). Fraction III was repeatedly chromatographed over MCI-gel CHP 20P with H_2O–MeOH (1 : 0—2 : 3, v/v), Avicel micro-crystalline cellulose with 2% AcOH and Sephadex LH-20 with EtOH and with 60% aqueous MeOH to afford 4,6-di-*O*-galloyl-2,3-(*S*)-HHDP-D-glucose (**4**) (448 mg), 1,6-di-*O*-galloyl-2,3-(*S*)-HHDP-β-D-glucose (**5**) (190 mg), pterocaryanin C (**6**) (96 mg), punicafolin (**9**) (250 mg), geraniin (**10**) (55.8 g), elaeocarpusin (**11**) (336 mg), mallotusinic acid (**14**) (651 mg), 1,2-di-*O*-galloyl-3,6-(*R*)-HHDP-β-D-glucose (**16**) (67 mg), 1-*O*-digalloyl-3,6-(*R*)-HHDP-β-D-glucose (**17**) (62 mg) and mallojaponin (**18**) (81 mg). On similar chromatographies, fraction V gave 1(β)-*O*-galloylpedunculagin (**7**) (915 mg) and mallotusinin (**20**) (391 mg).

b) From the Leaves The fresh leaves (32.3 kg) were extracted with 80% aqueous acetone at room temperature. After concentration of the extract under reduced pressure, the resulting precipitates were removed by filtration. The aqueous solution was applied to a column of Sephadex LH-20, and elution with H_2O containing increasing amounts of MeOH yielded six fractions; I (50 g), II (80 g), III (260 g), IV (480 g), V (60 g) and VI (50 g). Fraction III was separated by repeated Sephadex LH-20 chromatography with various solvent systems (EtOH, 60% aqueous MeOH, acetone–H_2O, *etc.*) and by MCI-gel CHP 20P chromatography with H_2O–MeOH (1 : 0—0 : 1, v/v) to give corilagin (**8**) (5.2 g) and mallotinic acid (**13**) (952 mg). On similar chromatographies, fraction IV afforded furosin (**12**) (7.1 g), while fraction VI gave punicafolin (**9**) (44 mg), geraniin (**10**) (922 mg), mallotusinic acid (**14**) (327 mg) and terchebin (**15**) (27 mg).

1,2-Di-*O*-galloyl-3,6-(*R*)-HHDP-β-D-glucose (16) A tan amorphous powder, $[\alpha]_D^{26}$ −71.4° (*c* = 0.5, MeOH). *Anal.* Calcd for $C_{34}H_{26}O_{22}\cdot H_2O$: C, 50.75; H, 3.48. Found: C, 50.64; H, 3.69. Negative FAB-MS *m/z*: 785 $(M-H)^-$. ^{1}H-NMR (acetone-d_6 + D_2O): 4.91 (1H, br s, H-3), 5.42 (1H, d, *J* = 5 Hz, H-2), 6.64 (1H, d, *J* = 5 Hz, H-1), 6.74, 6.95 (each 1H, s, HHDP-H), 7.15 (4H, s, galloyl H). ^{13}C-NMR (acetone-d_6 + D_2O): 62.1 (C-4), 64.7 (C-6), 72.3 (C-2), 73.8 (C-3), 76.9 (C-5), 91.3 (C-1), 108.2, 109.6 (HHDP C-3 and C-3′), 110.2, 110.4 (galloyl C-2 and C-6), 115.9, 116.5 (HHDP C-1 and C-1′), 120.1, 120.3 (galloyl C-1), 125.1, 125.4 (HHDP C-2 and C-2′), 136.7, 137.0 (HHDP C-5 and C-5′), 139.6, 139.8 (galloyl C-4), 144.9, 145.3, 146.0 (galloyl C-3 and C-5, HHDP C-4, C-4′, C-6 and C-6′), 165.6, 166.3, 167.4, 168.5 (COO).

Enzymatic Hydrolysis of 16 with Tannase A solution of **16** (16 mg) in H_2O (2 ml) was shaken with tannase at room temperature for 1 h. Evaporation of the solvent afforded a gum, which was treated with EtOH. The EtOH-soluble portion was applied to a Sephadex LH-20 column and eluted with EtOH to give gallic acid (6 mg) and **16a** (9 mg) as a tan amorphous powder, $[\alpha]_D^{22}$ −1.3° (*c* = 0.3, EtOH). ^{1}H-NMR (acetone-d_6): 6.76, 6.77, 6.78, 6.79 (each s, HHDP-H).

1-*O*-Digalloyl-3,6-(*R*)-HHDP-β-D-glucose (17) A tan amorphous powder, $[\alpha]_D^{26}$ −112.0° (*c* = 1.0, MeOH). *Anal.* Calcd for $C_{34}H_{26}O_{22}\cdot H_2O$: C, 50.75; H, 3.48. Found: C, 50.69; H, 3.72. Negative FAB-MS *m/z*: 785 $(M-H)^-$, 633. ^{1}H-NMR (acetone-d_6 + D_2O): 6.42 (1H, d, *J* = 3 Hz, H-1), 6.69, 6.70, 6.76, 6.86 (total 2H, s, HHDP-H), 7.20 (1/2H, s, *p*-depside galloyl H), 7.26 (2H, s, terminal galloyl H), 7.27, 7.42 (each 3/4H, d, *J* = 2 Hz, *m*-depside galloyl H). ^{13}C-NMR (acetone-d_6 + D_2O): 62.2 (C-4), 64.4 (C-6), 68.9 (C-2), 71.3 (C-3), 75.7 (C-5), 94.9 (C-1), 107.9, 110.2 (HHDP C-3 and C-3′), 115.3, 115.9 (HHDP C-1 and C-1′), 125.3, 125.4 (HHDP C-2 and C-2′), 136.5, 137.1 (HHDP C-5 and C-5′), 144.7, 144.9, 145.3, 146.0 (HHDP C-4, C-4′, C-6 and C-6′), 110.6, 120.3, 139.5, 146.0 (terminal galloyl C), 109.9, 127.7, 132.4, 151.2 (*p*-depside galloyl C), 116.7, 117.9, 120.3, 139.9, 144.2, 146.5 (*m*-depside galloyl C), 164.7, 165.3, 165.4, 167.4, 168.7 (COO).

Enzymatic Hydrolysis of 17 with Tannase A solution of **17** (15 mg) in H_2O (2 ml) was incubated with tannase at room temperature for 3 h. The reaction mixture was worked up in the same way as described above to give gallic acid (5 mg) and **16a** (7 mg).

Methanolysis of 17 with Methanolic Acetate Buffer A solution of **17** (28 mg) in a mixture of MeOH (1.5 ml) and 0.1 M acetate buffer (1.5 ml, pH 5.4) was refluxed for 1.5 h. The solution was concentrated to dryness under reduced pressure, and the residue was applied to an MCI-gel CHP 20P column. Elution with H_2O containing increasing amounts of MeOH yielded methyl gallate (9 mg) and **8** (16 mg) as an off-white amorphous powder, $[\alpha]_D^{23}$ −230.2° (*c* = 0.9, MeOH). ^{1}H-NMR (acetone-d_6 + D_2O): 6.38 (1H, d, *J* = 2 Hz, H-1), 6.71, 6.84 (each 1H, s, HHDP-H), 7.13 (2H, s, galloyl H).

Mallojaponin (18) A tan amorphous powder, $[\alpha]_D^{13}$ +15.9° (*c* = 0.4, MeOH). *Anal.* Calcd for $C_{54}H_{38}O_{37}\cdot 6H_2O$: C, 46.75; H, 3.61. Found: C, 47.01; H, 3.61. Negative FAB-MS *m/z*: 1277 $(M-H)^-$. ^{1}H-NMR (acetone-d_6 + D_2O): 2.17 (1H, d, *J* = 20 Hz, H-3), 2.97 (1H, dd, *J* = 3, 20 Hz, H-3), 4.92 (1H, d, *J* = 4 Hz, glucose H-2), 5.69 (1H, d, *J* = 3 Hz, H-1), 6.52 (1H, d, *J* = 4 Hz, glucose H-1), 6.82, 6.89, 7.15 (each 1H, s, valoneayl H), 7.23 (1H, s, H-3′), 7.26 (2H, s, galloyl H). ^{13}C-NMR: Table I.

Methylation of 18 A mixture of **18** (36 mg), dimethyl sulfate (0.2 ml) and anhydrous potassium carbonate (0.4 g) in dry acetone (4 ml) was

refluxed for 1.5 h with stirring. After removal of inorganic salts by filtration, the filtrate was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (5 : 1, v/v) furnished **18a** (16 mg) as a white amorphous powder, $[\alpha]_D^{24}$ +130.0° (c=0.5, $CHCl_3$). *Anal.* Calcd for $C_{71}H_{72}O_{37}\cdot 2H_2O$: C, 54.90; H, 4.90. Found: C, 55.08; H, 4.91. FD-MS m/z: 1516 (M^+). ^{1}H-NMR ($CDCl_3$)ppm: 2.23 (1H, d, J=20 Hz, H-3), 2.79 (1H, dd, J=3, 20 Hz, H-3), 4.88 (1H, s, H-4″), 4.95 (1H, d, J=4 Hz, glucose H-3), 5.37 (1H, d, J=4 Hz, glucose H-2), 5.67 (1H, d, J=3 Hz, H-1), 5.91 (1H, d, J=4 Hz, glucose H-4), 6.57 (1H, d, J=4 Hz, glucose H-1), 6.82, 7.06, 7.25 (each 1H, s, aromatic H), 7.29 (2H, s, galloyl H), 7.31 (1H, s, H-3′).

Treatment of 18 with Hot Water A solution of **18** (40 mg) in H_2O (2 ml) was heated on a boiling water-bath for 3 h. The reaction mixture was directly applied to a column of MCI-gel CHP 20P. Elution with H_2O–MeOH (1 : 0—0 : 1, v/v) furnished **18b** (3 mg) and **14** (9 mg). **18b**: A white amorphous powder, $[\alpha]_D^{22}$ +52.5° (c=0.4, H_2O–MeOH). ^{1}H-NMR (D_2O)[18]: 3.74 (1H, d, J=7 Hz, H-6), 3.75 (1H, d, J=6 Hz, H-6), 4.08 (1H, ddd, J=2, 6, 7 Hz, H-5), 4.96 (1H, d, J=2 Hz, H-4). TLC Rf 0.48 [solvent: benzene–ethyl formate–formic acid (1 : 5 : 1.5)]. **14**: A pale-yellow amorphous powder, $[\alpha]_D^{22}$ −58.4° (c=0.3, acetone). ^{1}H-NMR (acetone-d_6): 4.90 (1/3H, d, J=2 Hz, H-1′), 5.12 (2/3H, s, H-1′), 6.22 (1/3H, d, J=2 Hz, H-3′), 6.40 (1H, br s, H-1), 6.52 (2/3H, s, H-3′), 6.65, 6.66, 6.96, 7.08, 7.13, 7.16, 7.20 (total 4H, s, aromatic H), 7.29 (2H, s, galloyl H). **14** has been reported on derivation to **13**.[11] **13**: An off-white amorphous powder, $[\alpha]_D^{23}$ −75.7° (c=0.9, MeOH). ^{1}H-NMR (acetone-d_6): 3.99 (1H, br s, H-2), 4.08 (1H, dd, J=7, 10 Hz, H-6), 4.42 (1H, br s, H-4), 4.49 (1H, br t, J=10 Hz, H-5), 4.71 (1H, t, J=10 Hz, H-6), 4.75 (1H, br s, H-3), 6.33 (1H, d, J=3 Hz, H-1), 6.64, 6.72, 7.13 (each 1H, s, valoneayl H), 7.19 (2H, s, galloyl H). ^{13}C-NMR: Table I.

Preparation of 18 A mixture of **14** (210 mg) and **18b** (200 mg) in 0.2 M AcOH [H_2O–MeOH (1 : 1)] (3 ml) was kept at 40°C for 8 h. The MeOH was removed by evaporation under reduced pressure and the aqueous solution was applied to an MCI-gel CHP 20P column. Elution with H_2O–MeOH (1 : 0—7 : 3, v/v) yielded a product (153 mg) as a tan amorphous powder, which was found to be identical with **18** by physical and spectral comparisons.

Mallonin (19) A tan amorphous powder, $[\alpha]_D^{26}$ +13.3° (c=0.9, MeOH). *Anal.* Calcd for $C_{33}H_{28}O_{24}\cdot 2H_2O$: C, 46.92; H, 3.79. Found: C, 47.34; H, 4.08. Negative FAB-MS m/z: 807 $(M-H)^-$. ^{1}H-NMR (acetone-d_6 + D_2O): 2.22 (1H, d, J=20 Hz, H-3), 3.02 (1H, dd, J=3, 20 Hz, H-3), 5.22 (1H, d, J=5 Hz, glucose H-2), 5.72 (1H, d, J=3 Hz, H-1), 6.50 (1H, d, J=5 Hz, glucose H-1), 7.19 (2H, s, galloyl H), 7.20 (1H, s, H-3′). ^{13}C-NMR: Table I.

Treatment of 19 with Hot Water A solution of **19** (108 mg) in H_2O (5 ml) was heated on a boiling water-bath for 3 h. The products were separated in the same way as described for **18** to furnish **18b** (10 mg) and **12** (49 mg). **12**: A pale-yellow crystal line powder (H_2O), mp 197—198 °C (dec.), $[\alpha]_D^{23}$ −142.1° (c=1.0, MeOH). ^{1}H-NMR (acetone-d_6 + D_2O): 5.34 (1H, s, H-1′), 6.46 (1H, d, J=2 Hz, H-1), 6.53 (1H, s, H-3′), 7.23 (H, s, galloyl H), 7.28 (1H, s, H-3″).

Mallotusinin (20) A tan amorphous powder, $[\alpha]_D^{23}$ −20.3° (c=0.6, acetone). *Anal.* Calcd for $C_{41}H_{26}O_{25}\cdot 3H_2O$: C, 50.62; H, 3.29. Found: C, 51.02; H, 3.36. Negative FAB-MS m/z: 917 $(M-H)^-$. ^{1}H-NMR (acetone-d_6 + D_2O): 4.36 (1H, dd, J=6,12 Hz, H-6), 4.71 (1H, dd, J=8, 12 Hz, H-6), 4.94 (1H, dd, J=6, 8 Hz, H-5), 5.37 (1H, br d, J=4 Hz, H-4), 5.47 (1H, dt, J=1,4 Hz, H-2), 6.32 (1H, d, J=4 Hz, H-1), 6.69 (1H, dd, J=1,4 Hz, H-3), 6.74, 7.10, 7.14, 7.37 (each 1H, s, aromatic H), 7.14 (2H, s, galloyl H). ^{13}C-NMR: Table I.

Methylation of 20 A mixture of **20** (170 mg), dimethyl sulfate (1.5 ml) and anhydrous potassium carbonate (1.3 g) in dry acetone (25 ml) was refluxed for 1 h with stirring. The reaction mixture was worked up as described for **18** to give **20a** (75 mg) as colorless needles ($CHCl_3$–MeOH), mp 204—205 °C (dec.), $[\alpha]_D^{22}$ −51.4° (c=0.4, $CHCl_3$). *Anal.* Calcd for $C_{54}H_{52}O_{25}\cdot 1/2H_2O$: C, 58.43; H, 4.78. Found: C, 58.33; H, 4.91. FD-MS m/z: 1100 (M^+). ^{1}H-NMR ($CDCl_3$): 5.49 (1H, br s, H-2), 5.54 (1H, br d, J=4 Hz, H-4), 6.49 (1H, s, H-1), 6.80 (1H, br s, H-3), 6.78, 6.80, 6.99, 7.42 (each 1H, s, aromatic H), 7.22 (2H, s, galloyl H).

Alkaline Hydrolysis of 20a A solution of **20a** (45 mg) in 5% methanolic sodium hydroxide (15 ml) was refluxed for 35 min. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form), and the solvent was evaporated off under reduced pressure. The residue was treated with ethereal diazomethane for 1 h, and the solution was concentrated to a syrup, which was chromatographed on silica gel. Elution with hexane–acetone (5 : 1—3 : 1, v/v) yielded **20b** (8 mg) as colorless needles (MeOH), mp 81—82 °C, **20c** (12 mg) as a colorless syrup, $[\alpha]_D^{18}$ +24.3° (c=1.0, $CHCl_3$) and **20d** (14 mg) as colorless needles ($CHCl_3$–MeOH), mp 184—185 °C. *Anal.* Calcd for $C_{22}H_{20}O_9$: C, 59.41; H, 4.95. Found: C, 58.75; H, 5.03. FD-MS m/z: 404 (M^+). ^{1}H-NMR ($CDCl_3$): 3.88, 4.00, 4.25 (each 6H, s, OMe), 7.40 (2H, s, aromatic H). ^{13}C-NMR ($CDCl_3$): 56.7, 57.0, 61.3 (OMe), 111.0 (C-3), 117.8 (C-1), 121.0 (C-2), 137.1 (C-5), 149.3 (C-4), 150.1 (C-6), 168.1 (COO).

Treatment of 20a with Methanolic MeONa **20a** (26 mg) was treated with 1% methanolic MeONa (5 ml) at room temperature for 30 min. The resulting precipitates were collected by filtration, and subjected to silica gel chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) to afford **20e** (9 mg) as a white amorphous powder, EI-MS m/z: 346 (M^+), ^{1}H-NMR (DMSO-d_6): 3.87, 3.99 (each 6H, s, OMe), 7.11 (2H, s, aromatic H). **20e** was treated with diazomethane to give the dimethyl ester, which was identified as **20d** by TLC and spectral comparisons. The above filtrate was neutralized with Amberlite IR-120B (H^+ form), and the solvent was evaporated off under reduced pressure. The residue was chromatographed on silica gel. Elution with $CHCl_3$–MeOH (40 : 1—20 : 1, v/v) yielded **20b** (7 mg) and **20f** (8 mg) as a yellow syrup, $[\alpha]_D^{22}$ +3.2° (c=0.5, $CHCl_3$). *Anal.* Calcd for $C_{26}H_{26}O_{12}\cdot H_2O$: C, 56.93; H, 5.11. Found: C, 56.40; H, 5.04. EI-MS m/z: 530 (M^+, 100%), 436 (71%), 361 (42%), 109 (39%). ^{1}H-NMR ($CDCl_3$): 5.08 (2H, s, H-6), 6.42 (1H, d, J=4 Hz, H-4), 7.13 (1H, d, J=4 Hz, H-3), 7.33, 7.38 (each 1H, s, HMDP[19]-H), 9.57 (1H, s, H-1). ^{13}C-NMR ($CDCl_3$): 55.9, 56.0, 57.9, 60.6, 60.8 (OMe), 77.2 (C-6), 109.3, 109.5 (HMDP C-3 and C-3′), 112.5 (C-4), 121.5 (C-3), 123.7, 123.9 (HMDP C-2 and C-2′), 146.0 (HMDP C-5 and C-5′), 151.1, 151.3, 152.0, 152.3 (HMDP C-4, C-4′, C-6 andC-6′), 152.8 (C-5), 155.3 (C-2), 166.0, 169.7 (COO), 178.0 (C-1).

Preparation of 20f A mixture of **24** (100 mg) and thionyl chloride (2 ml) was refluxed for 3 h with stirring. The reaction mixture was concentrated under reduced pressure, and the oily residue was dissolved in dry pyridine (3 ml). **22** (0.2 ml) was added to the solution, and then the mixture was stirred at room temperature for 3 h. The reaction mixture was acidified with 1 N HCl and extracted with benzene. The organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated to give a residue, which was subjected to silica gel chromatography. Elution with benzene–acetone (1 : 0—1 : 1, v/v) furnished a yellow syrup (75 mg), which was found to be identical with **20f** by physical and spectral comparisons.

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