Tannins and Related Compounds. XCIV. 1) Isolation and Characterization of Seven New Hydrolyzable Tannins from the Leaves of *Macaranga tanarius* (L.) MUELL. et ARG.

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A chemical examination of the tannins in the leaves of *Macaranga tanarius* (L.) MUELL. et ARG. (Euphorbiaceae) has led to the isolation of seven new hydrolyzable tannins (22—28), together with twenty-one known tannins (1—21). On the basis of chemical and spectroscopic evidence, the structures of compounds 22—28 were established as 1,4-di-O-galloyl-D-glucopyranose (22), 3,4-di-O-galloyl-D-glucopyranose (23), galloylpunicafolin (24), galloylgeraniin (25), 1-O-galloyl-3-O-brevifolincarboxyl-β-D-glucopyranose (26), 1,2,4-tri-O-galloyl-3,6-(S)-hexahydroxyphenoyl-β-D-glucopyranose (27) and 1,2,4-tri-O-galloyl-3,6-(R)-hexahydroxyphenoyl-β-D-glucopyranose (macaranganin) (28) and 1,2,4-tri-O-galloyl-3,6-(R)-dehydrohexahydroxyphenoyl-β-D-glucopyranose (tanarinin) (28).

Keywords *Macaranga tanarius*; Euphorbiaceae; hydrolyzable tannin; S-hexahydroxyphenic acid; R-dehydrohexahydroxyphenidic acid; brevifolincarboxylic acid; macaranganin; tanarinin; atropisomerism

*Macaranga tanarius* (L.) MUELL. et ARG. is a common tropical tree distributed from southern Asia to northern Australia. 2) The bark and leaves are known to be rich in tannins and have been used as a folk medicine for diarrhoea and wounds, and also as an antiseptic. 3) As part of our studies on tannins of Euphorbiaceous plants, we have examined the leaves of *M. tanarius*, and isolated seven new hydrolyzable tannins (22—28), along with twenty-one known compound (1—21). This paper deals with the isolation and structure elucidation of these tannins.

The aqueous acetone extract of the leaves, collected in Taiwan, was subjected to a combination of column chromatographies over Sephadex LH-20, MCI-gel CHP 20P, Fuji-gel G-3, Bondapak C18/Porasil B and Avice cellulose, to afford compounds 1—21. Compounds 1—21 were identified as known galloylgallocatechins [1(β)-O- (1), 4) 4-O-(2), 6-O- (3), 6) 2,3-di-O- (4), 7) 1(β),2,6-tri-O- (5), 8) 2,4,6,tri-O- (6), 9] 1(β),2,3,4,6-tetra-O- (7), 10) 1(β),2,4,6-tetra-O- (8) 11) and 1(β),2,3,4,6-penta-O- (9) 12) galloylglucose, galloyl-(−)-shikimic acids [3-O- (10) 13) and 5-O- (11) 14) galloylshikimic acids, galloylquinic acids [4-O- (12) 15) and 3,4-di-O- (13) 16) galloylquinic acids, 3,6-(S)-hexahydroxydiphenoyl (HHDP)-D-glucopyranose (14), 16) corilagin (15), 17) punicafolin (16), 18) furosine (17), 19) terchebin (18), 20) geraniin (19), 21) mallotusinic acid (20) 22) and repandusinic acid A (21), 22) by comparisons of their spectroscopic and physical data with those of authentic samples.

Compounds 22 and 23 showed a dark blue coloration with the ferric chloride reagent and exhibited the same [M−H]− ion peak at m/z 483 in the negative ion fast atom bombardment mass spectra (FAB-MS). 23) Acid hydrolysis of these compounds yielded gallic acid and glucose. In the proton nuclear magnetic resonance (1H-NMR) spectra, the appearance of singlet signals at δ 7.25 and 7.19 (each 2H) in

<chemical structures>
22 and δ 7.03, 7.04 and 7.10 (4H in total) in 23 indicated the presence of two galloyl ester groups in each molecule. The result of 1H-1H shift correlation spectroscopy (COSY) of 22 clearly showed that the two lowfield methine signals at δ 6.36 (1H, d, J=4 Hz) and 5.13 (1H, t, J=10 Hz) were attributable to the glucose anomic and C₄ protons, respectively, thus confirming the location of the two galloyl groups at these positions. The small coupling constant (J=4 Hz) of the anomic proton signal showed the configuration of the anomic center to be α. Accordingly, compound 22 was characterized as 1,4-di-O-galloyl-α-D-glucopyranose (22).

On the other hand, in the 1H-NMR spectrum of 23, complex signal patterns arising from the glucose moiety, as well as the appearance of the anomic signals at δ 5.32 (d, J=4 Hz, α-form) and δ 4.82 (d, J=8 Hz, β-form) in the ratio of 2:1, indicated that 23 is an anomeric mixture. Furthermore, the observation of two pairs of triplet signals in the lowfield region [δ 5.69 and 5.20 (each 2/3, t, J=10 Hz, α-form), δ 5.47 and 5.17 (each 1/3H, t, J=10 Hz, β-form)] suggested the location of the galloyl groups to be at the C₆ and C₄ positions of the glucose core. Further support for this was obtained by methylation of 23, which yielded the α- and β- anomers (23a and 23b). The 1H-NMR spectrum of the α-anomer (23a) clearly indicated that the two lowfield triplets (δ 5.33 and 5.73 (each 1H, J=10 Hz)) are not coupled with the anomeric signal [δ 4.96 (d, J=4 Hz)]. Based on these findings, the structure of 23 was established to be 3,4-di-O-galloyl-D-glucopyranose (23).

Compounds 24 and 25 gave 1H-NMR spectra closely related to those of punicaflavin (16) and geraniin (19), respectively, except for the complexity of the aromatic signals. In the negative FAB-MS, 24 and 25 exhibited [M−H]⁻ ion peaks at m/z 1089 and 1103, which were 152 mass units (corresponding to one galloyl group) more than those of 16 (m/z 937) and 19 (m/z 951), respectively.

Mild methanolysis with methanolic acetate buffer (pH 5.4) yielded punicaflavin (16) and geraniin (19), along with methyl gallate. This result indicated that the additional galloyl group is attached desipically to the phenolic hydroxyl group in each molecule. As for the location of the depside galloyl group in 24, it is evident from the appearance of the 1H-NMR galloyl signals as doublets [δ 7.42, 7.46, 7.52, 7.54 (each d, J=2 Hz)] that the depside galloyl...
group is attached to the proximal galloyl group, but its location could not be definitely determined owing to the lack of sufficient sample. In the case of 25, the $^1$H-NMR spectrum of the phenazine derivative (25a) clearly showed the splitting of the galloyl signals into doublets [δ 7.25, 7.31 (each d, J = 2 Hz)], thus indicating that the depsidically linked galloyl group is located at the anomic galloyl hydroxyls. In addition, taking into account the observation of small galloyl singlet peaks (δ 7.16 in 24, δ 7.06 in 25a), a p-depside galloyl derivative was considered to exist as a minor component in each case. On the basis of these observations, compounds 24 and 25 were concluded to be galloylpuvacifolin and 1-O-di-galloyl-2,4-R-dehydrohexahydroxydiphenoyl (DHHDP)-3,6-(R)-HHDP-β-d-glucopyranose.

Compound 26 showed a prominent [M – H]$^-$ ion peak at m/z 605 in the negative FAB-MS, and exhibited the presence of one galloyl group [δ 7.19 (2H, s)] and a sugar moiety [δ 5.78 (1H, d, J = 8 Hz, anomic H)] in the $^1$H-NMR spectrum. The observation of an aromatic singlet at δ 7.45 and aliphatic ABX-type signals at δ 2.73 (1H, dd, J = 2 and 19 Hz), 3.13 (1H, dd, J = 8 and 19 Hz) and 4.72 (1H, dd, J = 2 and 8 Hz) suggested the occurrence of a brevifolin-carboxylic acid moiety, which was also consistent with the $^{13}$C nuclear magnetic resonance ($^{13}$C-NMR) spectrum [δ 196.0 (carboxyl), 42.3, 38.2 (CH-CH$_2$), 140.6, 150.4 (C=C-O-)]. The component acids and sugar were confirmed unequivocally by acid hydrolysis yielding gallic acid, brevifolincarboxylic acid and glucose.

The location of each acyl group was determined as follows. In the 1H-1H COSY spectrum, the lowfield shifts of the anomer [δ 5.78 (d, J = 8 Hz)] and C$_2$ [δ 5.15 (t, J = 9 Hz)] proton signals apparently indicated these positions to be acylated. Partial hydrolysis with tannase afforded gallic acid and a hydrolysate (26a). In the 1H-NMR spectrum of 26a, the signal arising from the galloyl group disappeared and the anomer proton signal was found to be shifted to upper field [δ 4.64 (d, J = 8 Hz, β-form) and 5.20 (d, J = 4 Hz, α-form)]. This fact indicated the galloyl group to be located at the anomic position. Furthermore, in the 1H-NMR spectrum of 26, a large coupling constant (J = 8 Hz) of the anomic signal indicated the β-configuration. Thus, 26 was represented as 1-O-galloyl-3-O-brevifolin-carboxyl-β-d-glucopyranose (26).

Compound 27 (name macarrangani) showed, in the negative FAB-MS, an [M – H]$^-$ ion peak at m/z 937 identical to that of puvacifolin (16). The $^1$H-NMR spectrum of 27 suggested the presence of three galloyl groups (δ 7.00, 7.14 and 7.26 (each 2H, s)), one HHDP group [δ 7.18 and 7.20 (each 1H, s)] and a β-glucopyranose moiety [δ 6.48 (1H, d, J = 9 Hz, H-1), 5.68–5.46 (2H, m, H-2, 3), 5.19 (1H, d, J = 12 Hz, H-6), 5.07 (1H, br s, H-4), 4.48 (1H, br s, 1H, dd, J = 4 and 12 Hz, H-6)].

Methylation of 27 with dimethyl sulfate and anhydrous potassium carbonate in dry acetone gave the pentadecamethyl ether (27a), [M$^+$ m/z: 1148, field desorption mass spectrum (FD-MS)]. Alkaline hydrolysis of 27a, followed by methylation with diazomethane, afforded methyl 3,4,5-trimethoxybenzoate (27b) and dimethyl 4,4′,5,5′,6,6′-hexamethoxydiphenol (27c). The negative value [−32.9° (acetone)] of the specific optical rotation of 27c confirmed the chirality of the biphenyl bond to be in the S-series.

To determine the location of each acyl group in the glucose moiety, 27 was subjected to partial hydrolysis in boiling water, which yielded ellagid acid and 1,2,4-tri-O-galloyl-β-d-glucopyranose (27d). This result indicated the location of the HHDP group to be at the 3,6-positions. Thus, macarrangani was characterized as 1,2,4-tri-O-galloyl-3,6-(S) HHDP-β-d-glucopyranose (27).

Compound 28 (tanarinin) was obtained as a yellow amorphous powder. The 1H-NMR spectrum of 28, although duplicated, exhibited signals corresponding to three galloyl groups [δ 7.16, 7.20 and 7.32 (each 3/2H, s), 7.31,
apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. 1H- and 13C-NMR spectra were taken with a JEOL FX-100 spectrometer, with tetramethylsilane as an internal standard; chemical shifts are given on a δ (ppm) scale. FAB- and PD-MS were performed on JEOL JMS DX-300 and D-300 spectrometers. Column chromatography was carried out with Sephadex LH-20 (25–100 μM, Pharmacia Fine Chemical Co., Ltd.), MCII-gel CHP 20P (75–150 μM, Mitsubishi Chemical Industries, Ltd.), Fuji-gel ODS G-3 (43–65 μM, Fuji Gel Hanbai Co., Ltd.), Bondapak C18/Porasil B (37–75 mesh, Waters Associates, Inc.) and silica gel 60 (70–230 mesh, Merck), Avicel cellulose (Fukunoshi). Thin-layer chromatography (TLC) was performed on precoated Silica gel 60 F254 plates (0.2 mm thick, Merck) with benzene-ethyl formate-formic acid (1:7:1, 1:7:1.5 or 1:5:1.5) and precoated Cellulose F254 Plates (0.1 mm thick, Merck) with 2% acetic acid, and spots were detected by the use of ultraviolet (UV) light or by spraying 1% ferric chloride, 10% sulfuric acid or aniline–hydrogen–phthalate reagents. Isolation The dried leaves (6.0 kg) of M. tanarius, collected in February 1987, in Taipei, Republic of China, were extracted three times with 70% aqueous acetone at room temperature. The extract was concentrated under reduced pressure, and the resulting precipitates, consisting mainly of chlorophylls and waxes, were removed by filtration. The filtrate was applied to a column of Sephadex LH-20. Elution with H2O containing increasing amounts of MeOH, and finally with 50% aqueous acetone afforded fractions I (138 g), II (135 g), III (410 g) and IV (75 g).

Repeated chromatography of fr. I on Sephadex LH-20 (H2O–MeOH, EtOAc), MCII-gel CHP 20P (H2O–MeOH) and Fuji-gel ODS G-3 (H2O–MeOH) yielded 1-O-galloyl-β-D-glucose (1) (210 mg), 4-O-galloyl-g-t-glucose (2) (60 mg), 6-O-galloyl-β-D-glucose (3) (520 mg), 5-O-galloyl-l-tyrosine (4) (210 mg), 4-O-galloyl-β-D-glucosamine (5) (12) (390 mg), 3,6-(S)-HHDP-D-glucose (14) (160 mg), and rhamnusinic acid A (21) (210 mg).

Fraction II was repeatedly chromatographed over Sephadex LH-20 (EtOH–H2O–MeCO, EtOAc, 80% MeOH) and MCII-gel CHP 20P (H2O–MeOH) to give 2,3-di-O-galloyl-D-glucose (4) (40 mg), 2-O-galloyl-γ-D-glucuronide (5) (40 mg), 4,6-di-O-galloyl-D-glucoselinic acid (13) (60 mg), corosin (15) (1.28 g) and compounds 22 (320 mg), 23 (140 mg) and 26 (90 mg).

Fraction III yielded 1,2,6-tri-O-galloyl-β-D-glucose (5) (80 mg), 2,4,6-tri-O-galloyl-D-glucose (6) (230 mg), furosin (17) (490 mg), tercebin (18) (90 mg) and mallotusinic acid (20) (240 mg) on repeated chromatography over MCII-gel CHP 20P (H2O–MeOH), Sephadex LH-20 (EtOH–H2O–MeCO, EtOAc, 80% MeOH), Fuji-gel ODS G-3 (H2O–MeOH), Bondapak C18/Porasil B (H2O–MeOH) and Avicel cellulose (2% AcOH).

Similar repeated chromatography of fr. IV yielded 1,2,3,6-tetra-O-galloyl-β-D-glucose (7) (100 mg), 1,2,4,6-tetra-O-galloyl-D-glucose (8) (130 mg), 1,2,3,4,6-penta-O-galloyl-D-glucose (9) (200 mg), puniguloin (16) (690 mg), geraniin (19) (345 mg), and compounds 24 (280 mg), 25 (220 mg), 27 (1400 mg) and 28 (340 mg).

Compounds 21 A white powder (H2O, mp 195–197°C, [α]20° +37.3° (c=0.6, MeOH). Negative FAB-MS m/z: 483 [M–H–]. Anal. Calcd for C20H18O14·3H2O: C, 46.61; H, 4.87. Found: C, 44.73; H, 4.75. 1HNMR (acetone-d6, δ 3.58–3.62 (2H, m, H-6), 3.89 (1H, dd, J=4, 10 Hz, H-4), 4.08 (1H, d, J=10 Hz, H-3), 4.10 (1H, d, J=10 Hz, H-5), 7.19 (2H, s, H-8), 7.25 (2H, s, H-8). 13CNMR (acetone-d6, δ 66.1 (glc C-6), 70.0 (CC), 72.4, 73.7 (glc C-2, 3, 4, 5), 93.0 (glc C-1), 110.2 (4C, galloyl C-2,6), 120.7, 120.9 (galloyl C-1), 139.2, 139.5 (galloyl C-4, 145.9 (4C, galloyl C-3, 5), 166.1 167.0 (COO–)).

Acid Hydrolysis of 22 A solution of 22 (2 mg) in 1 N H2SO4 (1 ml) was heated at 90°C for 2 h. After cooling, the reaction mixture was extracted with ethyl acetate. TLC examination of the ethyl acetate layer showed the presence of gallic acid [silica gel/benzene-ethyl formate-formic acid (2:7:1); Rf: 0.69]. The aqueous layer was neutralized with Amberlite MB-3 ion exchange resins, and analyzed by cellulose TLC [solvent, n-BuOH–pyridine–H2O (6:4:3); detection, aniline–hydrogen–phthalate reagent]. A spot (Rf: 0.39) corresponding to glucose was detected.

Compound 23 An off-white amorphous powder, [α]20° +46.2° (c= 0.7, MeOH). Negative FAB-MS m/z: 483 [M–H–]. 1HNMR (270 MHz, acetone-d6, δ 3.40–3.41 (2H, m, H-3, m), (β-H-5), 4.18–4.28 (2H, m, (β-H-5), (β-H-3), 5.17 (1H, d, J=8 Hz, (β-H-5)), 5.17 (1H, d, J=8 Hz, (β-H-5)), 5.20 (2H, H-t), J=10 Hz, (β-H-3), 5.32 (2H, H, d, J=4 Hz, (α-H-1), 5.47 (1H, H, t, J=10 Hz, (β-H-3), 5.69 (2H, H-t, J=10 Hz, (β-H-3)), 7.03, 7.04, 7.10 (4H in total, each s, galloyl H).

Acid Hydrolysis of 23 23 (5 mg) was treated with 1 N H2SO4 (1 ml) as described for 22, and the spots of gallic acid and glucose were detected by TLC.

Methylation of 23 A mixture of 23 (58 mg), dimethyl sulfate (0.3 ml)
and anhydrous potassium carbonate (600 mg) in dry acetone (6 ml) was heated under reflux for 2 h. After removal of the inorganic precipitation by filtration, the filtrate was concentrated to dryness under reduced pressure.

The residue was separated by preparative TLC [solvent: CHCl₃-MeOH (19:1 v/v)] to afford 23a (5 mg) and 23b (2 mg). 23a: an off-white amorphous powder. M.p.: 88-90°C (MeOH); [α]₂₅° = -13.0° (c = 0.1, MeOH). 23b: an off-white amorphous powder, m.p.: 85-87°C (MeOH); [α]₂₅° = -4.1° (c = 0.1, MeOH).

**Compound 24** A tan amorphous powder, [α]₉₀° = -14.5° (c = 0.8, MeOH). Negative FAB-MS m/z: 1089 [M - H]⁺. 937 [M - galloyl]. Anal. Calc. for C₄₅H₃₁O₂₅Br: C, 53.7; H, 3.0; N, 1.6; S, 1.9. Found: C, 54.2; H, 2.9; N, 1.9; S, 1.9.

**Compound 25** A tan amorphous powder, [α]₉₀° = -104.4° (c = 0.6, MeOH). Negative FAB-MS m/z: 1103 [M - H]⁻. 951 [M - galloyl]. Anal. Calc. for C₄₄H₃₅O₂₃S: C, 71.4; H, 4.7; N, 1.4; S, 2.7. Found: C, 71.6; H, 4.9; N, 1.3; S, 2.8.

**Preparation of Phenazine Derivative 25a** A solution of 25 (70 mg) and o-phenylenediamine (10 mg) in 20% AcOH-EtOH (3 ml) was stirred at room temperature for 1 h. After removal of EtOH under reduced pressure, the aqueous solution was subjected to MCI-gel CHP 20 chromatography to give 25a as a tan amorphous powder, Anal. Calc. for C₄₅H₄₃N₅O₁₅OH₂C: C, 50.4; H, 3.7; N, 2.19. Found: C, 50.4; H, 3.6; N, 2.16.

**Acid Hydrolysis of 26** A solution of 26 (2 mg) in 1 M H₂SO₄ (0.2 ml) was heated at 95°C for 1 h. After cooling, the reaction mixture was neutralized with Amberlite MB-3. Spots corresponding to glucose, gallic acid and biphenoxyacetic acid [silica gel/benzene-ethyl formate] (1:7:1.5, Rf 0.65) were detected by TLC examination.
Compound 28 A yellow amorphous powder, [α]D = 83.7° (c = 0.5, MeOH). Negative FAB-MS m/z: 953 [M – H]–. Anal. Caled for Ca13-
H2O22·4H2O: C: 47.96; H: 3.73. Found: C: 47.76, H: 3.64. 1H-NMR (100 MHz, gypsum), δ: 0.40 (4H, t, J = 8 Hz, H-3), 4.30 (4H, s, galloyl H) (2H, s, galloyl H).

Reduction of 28 A solution of 28 (40 mg) in 10% aqueous Na2S2O4 (7 ml) was stirred at room temperature for 1 h. The reaction mixture was directly subjected to MCI-gel CHP 20P chromatography to afford punicafol (16) (20 mg).

Preparation of Phensane Derivative (28a) A solution of 28 (50 mg) in EtOH (3 ml) was treated with o-phenylenediamine (10 mg) in 20% AcOH–EtOH (3.5 ml). The mixture was stirred at room temperature for 1.5 h. After removal of EtOH under reduced pressure, the product was separated by MCI-gel CHP 20P chromatography to give 28a as a tan amorphous powder, [α]D = 17.4° (c = 0.4, acetone). Anal. Caled for Ca13-
H2O22·3H2O·2C: 53.11; H: 3.60; N: 2.65. Found: C: 53.01; H: 3.79; N: 2.53. 1H-NMR (acetone-d6, δ): 3.10 – 4.90 (6H, in total, H-5, 6, 6a, 6b, 12.01 (2C), 129.2 (DHPD C-3), 191.2 (DHPD C-4), 96.6 (DHPD C-5), 94.1 (DHPD C-6), 114.0, 114.8 (DHPD C-1′, 3′), 138.5 (DHPD C-2′), 143.0 (DHPD C-6′), 164.7, 165.3, 165.5, 165.7, 168.3 (COO–).

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References and Notes
24) The observation of four meta-coupled galloyl doublets suggested that at least two of these proximal galloyl groups possess the depside galloyl groups.