Tannins of Euphorbiaceous Plants. XIII.[1] New Hydrolyzable Tannins Having Phloroglucinol Residue from *Glochidion rubrum* BLUME

Lih-Geeng CHEN,[a] Ling-Ling YANG,*[,a] Kun-Ying YEN,[a] Tsutomu HATANO,[b] Takashi YOSHIDA,[b] and Takuo OKUDA[b]

Graduate Institute of Pharmacognosy Science, Taipei Medical College,[a] Taipei, Taiwan, R.O.C. and Faculty of Pharmaceutical Sciences, Okayama University,[b] Tsushima, Okayama 700, Japan.
Received June 7, 1995; accepted August 14, 1995

Three hydrolyzable tannins of new types, glochiins M_1, M_2 and C_1, were isolated from dried leaves of *Glochidion rubrum* BLUME, and their structures were elucidated by spectral and chemical methods.

Key words *Glochidion rubrum*; Euphorbiaceae; tannin; glochiin M_1; glochiin M_2; glochiin C_1

Glochidion rubrum BLUME (Euphorbiaceae) is widely grown in Malaysia, the Ryukyus and Taiwan. Its leaves are used as folk medicine in Taiwan for stomach disorder, and another species of the same genus (*G. cordatum*) is also used in Fiji for stomach troubles and other ailments.[2] Although their medicinal value is ascribable to tannins, few of the polyphenols in the plants of this genus have been studied. We have now isolated three hydrolyzable tannins of new types, glochiin M_1 (**1**), glochiin M_2 (**2**) and glochiin C_1 (**3**), from the leaf extract of this plant. The former two tannins, **1** and **2** are gallates of phoroglucinol glucoside, and the third one, **3**, is a complextannin which might be biosynthesized *via* **1**.

Results and Discussion

The concentrated filtrate from the aqueous acetone homogenate of the dried leaves of *G. rubrum* was extracted with ether to remove non-polar components. The remaining water layer was applied to Diaion HP-20, Toyopearl HW-40(C) and ODS-AQ columns, yielding glochiin M_1 (**1**), glochiin M_2 (**2**) and glochiin C_1 (**3**).

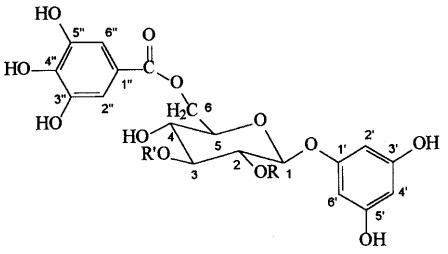
Glochiin M_1 (**1**), colorless fine crystals, $[\alpha]_D$ $-28°$ (MeOH), showed in the fast-atom bombardment mass spectrum (FAB-MS), the $(M+H)^+$ ion peak at *m/z* 593, corresponding to the molecular formula $C_{26}H_{24}O_{16}$. Its proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed two 2H singlets (δ 7.15, 7.11) and two *meta*-coupled peaks ($J=2$ Hz), (δ 6.02, 2H, d; δ 5.99, 1H, t) in the aromatic region. These signals are attributable to two galloyl groups and one phloroglucinol group in the molecule of **1**. The coupling pattern of the sugar proton signals assigned on the basis of the ^{1}H–^{1}H shift correlation spectrum (COSY) indicated that the glucose adopts the $^4C_1$ conformation. The chemical shifts of the glucose proton signals showed that the glucose C-3 and C-4 hydroxyl groups are free. The chemical shift of the anomeric proton signal (δ 5.23) of **1** compared with those of other C-1 acylated tannins (*e.g.*, isorugosin A δ 6.17)[3] indicated the presence of an ordinary glucoside linkage rather than an ester linkage. The ^{13}C-NMR spectral comparison with phloroglucinol-β-D-glucopyranoside[4] revealed the acylation at glucose C-2 and C-6 in **1**. The structure of **1**, thus elucidated to be phloroglucinol-(2,6-di-*O*-galloyl)-β-D-glucopyranoside, was supported by acid hydrolysis of **1** with hot 5% H_2SO_4, yielding gallic acid (**4**) and phloroglucinol (**5**).

Glochiin M_2 (**2**), $[\alpha]_D$ $-8°$ (MeOH), showed in the FAB-MS the $(M+Na)^+$ ion peak at *m/z* 615, corresponding to the molecular formula $C_{26}H_{24}O_{16}$. Its ^{1}H-NMR spectrum showed two 2H singlets (δ 7.15, 7.14) and two *meta*-coupled peaks ($J=2$ Hz), (δ 6.15, 2H, d; δ 6.03, 1H, t) in the aromatic region. Therefore **2** could be an isomer of **1**, differing only in the location of the galloyl group on the glucose core. The downfield shift of the glucose signals, assigned on the basis of the ^{1}H–^{1}H-COSY spectrum, showed that the C-3 and C-6 hydroxyl groups of the glucose are acylated. Glochiin M_2 is thus phloroglucinol-(3,6-di-*O*-galloyl)-β-D-glucopyranoside.

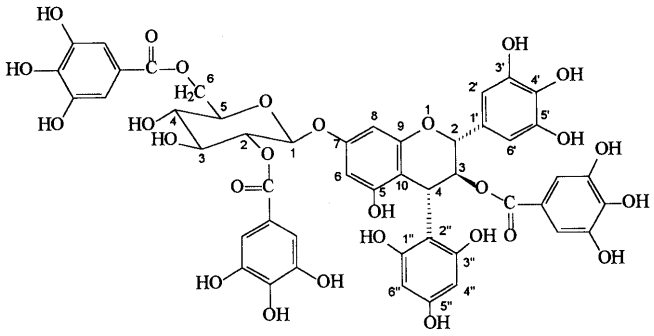
Glochiin C_1 (**3**), $[\alpha]_D$ $-66°$ (MeOH), exhibited in the FAB-MS the $(M+H)^+$, $(M+Na)^+$, and $(M+K)^+$ peaks at *m/z* 1049, 1071, and 1087, respectively, in accord with the molecular formula $C_{48}H_{40}O_{27}$. The ^{1}H-NMR spectrum showed that **3** consists of three galloyl groups [δ 7.30, 7.12, and 7.00 (2H each, s)], a glucose moiety in the $^4C_1$ conformation, a gallocatechin gallate (GCG) residue and a phloroglucinol residue (δ 5.77 and 5.75, each 1H, d, $J=2$ Hz). The chemical shifts of the glucose protons indicated that O-2 and O-6 of the glucose residue in **3** are acylated as in **1**. The long-range heteronuclear COSY spectrum of **3** showed a correlation of H-4 of GCG with C-1″ and C-3″ of phloroglucinol. It implied the presence of a C–C bond between GCG C-4 and phloroglucinol C-2″. The downfield shift of C-2 from that of the *cis* congener signal (δ 80.65) of GCG in the ^{13}C-NMR spectrum suggested the *trans* correlation of the two aromatic rings at C-2 and C-3 (β-orientation of the galloyloxy group).[5] This was supported by the ^{1}H-NMR spectrum, which showed large couplings ($J=10$ Hz) between H-2 and H-3, and also between H-3 and H-4 of GCG. The long-range heteronuclear COSY spectrum of **3** also showed a correlation between glucose H-1 and GCG C-7, in accord with the ether linkage formation between glucose C-1 and GCG C-7. The signal of glucose H-5 (δ 3.18) in **3** was shifted upfield from that of glucose H-5 (δ 3.92) in **1**, presumably due to the anisotropic effect of the aromatic rings in GCG. The non-equivalency of the NMR signals of the phloroglucinol residue is regarded as due to restricted rotation induced by the presence of polar and bulky substituents around the C-4—C-2″ bond.

Glochiin C_1 is a complextannin of a new type, which

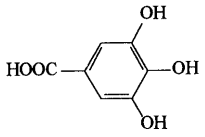
* To whom correspondence should be addressed.



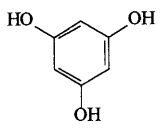
1 : R=galloyl, R'=H
2 : R=H, R'=galloyl



3[6]



4



5

is composed of gallocatechin glucoside acylated at both the gallocatechin and the glucose moieties. Compounds **1** and **3** are also hydrolyzable tannins of a new type. The structural correlation between **1** and **3** and their co-occurrence in a species of plant imply that **1** may be a precursor of **3**, if the A-ring in the flavan skeleton in **3** can be formed from the phloroglucinol residue in **1**. If so, this is a new pathway of flavonoid biosynthesis in which the flavan skeleton is produced after phloroglucinol glucoside formation. In this way, the phloroglucinol residue in **3** may be the precursor of the A-ring in the second flavan unit upon further biosynthetic elaboration.

Experimental

General UV spectra were taken on a Shimadzu UV-160, and optical rotations on a JASCO DIP-4 polarimeter. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (126 MHz) spectra were recorded on a Varian VXR 500 instrument, and chemical shifts are given in δ values (ppm) relative to tetramethylsilane. FAB-MS were recorded on a VG 70-SE mass spectrometer using 3-nitrobenzyl alcohol as the matrix agent. Normal-phase HPLC was conducted on a YMC-pack SIL-06 (4.6 × 250 mm) column with *n*-hexane–MeOH–THF–HCOOH (42 : 47 : 10 : 1) containing oxalic acid (450 mg/l) (flow rate, 1.0 ml/min; detection 280 nm). Reversed-phase HPLC was performed on a YMC-pack ODS-A (4.6 × 250 mm) column using the following solvent systems: (1) 0.1 M H_3PO_4–0.1 M KH_2PO_4–CH_3CN (42.5 : 42.5 : 15) (2) 0.1 M H_3PO_4–0.1 M KH_2PO_4–CH_3CN (45 : 45 : 10) (3) 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (10 : 10 : 2 : 1). Column chromatography was carried out on Toyopearl HW-40 (C) (Tosoh), ODS-AQ (YMC), Diaion HP-20 and MCI Gel CHP 20P (Mitsubishi Chemical Industry Co., Ltd.).

Isolation of Tannins from *G. rubrum* The dried leaves (2 kg) of *G. rubrum*, collected in Pintung, Taiwan, in summer 1993, were extracted with 70% aqueous acetone (15 l × 3). After removal of acetone by evaporation, the concentrated solution was extracted with ether, to yield the ether extract and water-soluble residue (140 g). A portion (40 g) of the residue was chromatographed over Diaion HP-20 (7.5 cm i.d. × 35 cm) with aqueous MeOH (20% → 30% → 40% → 60%). The 20% MeOH eluate was further purified by column chromatography over Toyopearl HW-40 (C) (2.5 cm i.d. × 55 cm) with 70% MeOH and on an ODS-AQ column to give glochiin C_1 (**3**) (20 mg). The 30% MeOH eluate was further chromatographed over Toyopearl HW-40 (C) with MeOH–H_2O–acetone (4 : 1 : 1) and on an ODS-AQ column to give glochiin M_1 (**1**) (11 mg) and glochiin M_2 (**2**) (9 mg).

Glochiin M_1 (1) Colorless fine crystals from water. mp 240—242 °C (dec.). $[\alpha]_D$ −28° (c = 1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 216 (4.89 sh), 274 (4.48). FAB-MS *m/z*: 593 $(M+H)^+$. ^{1}H-NMR (acetone-d_6 + D_2O) δ: 7.15 (2H, s), 7.11 (2H, s) (galloyl × 2); 6.02 (2H, d, J = 2 Hz, H-2′, H-6′), 5.99 (1H, t, J = 2 Hz, H-4′) (phloroglucinol). Glucose protons: 5.23 (1H, d, J = 9 Hz, H-1), 5.16 (1H, t, J = 9 Hz, H-2), 4.60 (1H, dd, J = 1.5 Hz, 12 Hz, H-6), 4.41 (1H, dd, J = 5 Hz, 12 Hz, H-6), 3.92 (1H, m, H-5), 3.88 (1H, t, J = 9 Hz, H-3), 3.72 (1H, t, J = 9 Hz, H-4). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 160.14, 159.70 (2C), 98.13, 96.42 (2C) (phloroglucinol), 145.97 (2C), 145.94 (2C), 139.03, 139.00, 121.24, 121.09, 109.87 (2C), 109.74 (2C) (galloyl), 166.94, 166.10 (ester carbonyl), 99.84 (Glc C-1), 75.47 (Glc C-3), 74.93 (Glc C-5), 74.44 (Glc C-2), 70.99 (Glc C-4), 64.04 (Glc C-6).

Acid Hydrolysis of 1 A solution of **1** (1 mg) in 5% H_2SO_4 (1 ml) was heated in a boiling-water bath for 3 h. After cooling, the reaction mixture was extracted with EtOAc. The EtOAc extract was analyzed by HPLC and TLC (cellulose plate developed with 6% HOAc) to detect peaks and spots identical with those of authentic gallic acid (**4**) and phloroglucinol (**5**).

Glochiin M_2 (2) An off-white amorphous powder. $[\alpha]_D$ −8° (c = 1.0, MeOH). FAB-MS *m/z*: 615 $(M+Na)^+$. ^{1}H-NMR (acetone-d_6 + D_2O) δ: 7.15 (2H, s), 7.14 (2H, s) (galloyl × 2); 6.15 (2H, d, J = 2 Hz, H-2, H-6), 6.03 (1H, t, J = 2 Hz, H-4) (phloroglucinol). Glucose protons: 5.29 (1H, t, J = 9.5 Hz, H-3), 5.10 (1H, d, J = 8 Hz, H-1), 4.60 (1H, dd, J = 2, 11.5 Hz, H-6), 4.43 (1H, dd, J = 4.5, 11.5 Hz, H-6), 3.97 (1H, ddd, J = 2, 4.5, 9.5 Hz, H-5), 3.86 (1H, t, J = 9.5 Hz, H-4), 3.75 (1H, dd, J = 8, 9.5 Hz, H-2). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 167.06, 166.97 (ester carbonyl), 160.21, 159.59 (2C), 97.87 (2C), 96.28 (phloroglucinol), 145.90 (2C), 145.84 (2C), 138.91, 138.77, 121.54, 121.05, 109.95 (2C), 109.77 (2C) (galloyl), 101.37 (Glc C-1), 78.40 (Glc C-3), 74.70 (Glc C-5), 72.62 (Glc C-2), 69.14 (Glc C-4), 64.08 (Glc C-6).

Glochiin C_1 (3) A pale yellow amorphous powder. $[\alpha]_D$ −66° (c = 1.0, MeOH). FAB-MS *m/z*: 1049 $(M+H)^+$, 1071 $(M+Na)^+$, 1087 $(M+K)^+$. ^{1}H-NMR (acetone-d_6 + D_2O) δ: 7.30 (2H, s), 7.12 (2H, s), 7.00 (2H, s) (galloyl × 3), 6.44 (2H, s, GCG H-2′, H-6′), 5.77, 5.75 (each 1H, d, J = 2 Hz), (phloroglucinol H-4″, H-6″). 6.34, 6.08 (each 1H, d, J = 2 Hz, GCG H-8 and H-6), 6.13 (1H, t, J = 10 Hz, GCG H-3), 4.53 (1H, d J = 10 Hz, GCG H-4), 4.13 (1H, d, J = 10 Hz, GCG H-2). Glucose protons: 5.26 (1H, br t, J = 9.5 Hz, H-2), 4.82 (1H, d, J = 8 Hz, H-1), 4.47 (1H, dd, J = 1.5, 12 Hz, H-6), 4.38 (1H, dd, J = 3.5, 12 Hz, H-6), 3.88 (1H, t, J = 9.5 Hz, H-3), 3.73 (1H, t, J = 9.5 Hz, H-4), 3.18 (1H, br d, J = 9.5 Hz, H-5). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 166.99, 166.93, 165.14 (ester carbonyl), 157.85, 157.54, 157.31, 157.13, 156.80 (phloroglucinol C-1″, C-3″, C-5″, and GCG C-5, C-7), 145.89, 145.76, 145.61 (galloyl C-3, C-5), 145.49 (GCG C-3′, C-5′), 138.89, 138.81, 138.65 (galloyl C-4), 133.23 (GCG C-4′), 129.98 (phloroglucinol C-2″), 121.71, 121.68, 121.33, 121.28 (galloyl C-1 and GCG C-1′), 110.47 (GCG C-9), 110.58, 110.24, 109.85 (galloyl C-2, C-6), 108.06 (GCG C-2′, C-6′), 104.58 (GCG C-10), 102.95 (Glc C-1), 101.23 (GCG C-8), 100.97 (GCG C-6), 97.47, 95.98 (phloroglucinol C-4″, C-6″), 80.65 (GCG C-2), 75.19 (Glc C-2), 74.96 (Glc C-5), 74.85 (Glc C-3), 72.48 (GCG C-3), 70.75 (Glc C-4), 63.35 (Glc C-6), 36.36 (GCG C-4).

Acknowledgements The authors are grateful to Mr. F.-C. Ho, Heng-Chun Tropical Botanic Garden, Taiwan, R.O.C., for identification of plant species, and also to the SC-NMR Laboratory of Okayama University for NMR experiments using a Varian VXR 500 instrument.

References

1) Part XII: Yoshida T., Namba O., Kurokawa K., Amakura Y., Liu

Y.-Z., Okuda T., *Chem. Pharm. Bull.*, **42**, 2005 (1994).
2) Siri V. R., Frank J., "New Plant Sources for Drugs and Foods from The New York Botanical Garden Herbarium," Harvard University Press, Cambridge, Massachusetts, London, 1982, p. 159.
3) Hatano T., Kira R., Yasuhara T., Okuda T., *Chem. Pharm. Bull.*, **36**, 3920 (1988).
4) Foo L. Y., Karchesy J. J., *Phytochemistry*, **28**, 1237 (1989).
5) Porter L. J., Newman R. H., Foo L. Y., Wong H., Hemingway R. W., *J. Chem. Soc., Perkin Trans. 1*, 1217 (1982).
6) Absolute configurations are undetermined.