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# NEW GALLIC ACID GLYCOSIDES FROM *MALLOTUS PLICATUS*

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**Abstract** – From the stem bark of *Mallotus plicatus* (Müll.Arg.) Airy Shaw, two new gallic acid glycosides, namely bergenin-8-*O*-$\alpha$-L-rhamnoside (**1**) and *seco*-bergenin-8-*O*-$\alpha$-L-rhamnoside (**2**) were isolated along with seven known compounds (**3**-**9**). The structures of the new isolates (**1** and **2**) were elucidated through analysis of their 1D- and 2D-NMR, and MS spectral properties, as well as chemical evidence. *seco*-Bergenin-8-*O*-$\alpha$-L-rhamnoside (**2**) and protocatechuic acid (**7**) showed weak inhibitory activity against *Herpes simplex* virus, whereas the other compounds were devoid of such activity.

*Mallotus plicatus* (Müll.Arg.) Airy Shaw (Euphorbiaceae) is a shrub to small tree widely found in Southeast Asia, including Myanmar, Thailand, Laos, Cambodia, and Vietnam.[1,2] The plant is also known by several synonyms, such as *Coccoceras plicatum* Müll.Arg., *C. anisopodum* Gagnep., *Mallotus eriocarpoides* Wall. Ex Müll.Arg., *M. wallichianus* Müll.Arg. and *M. anisopodus* (Gagnep.) Airy Shaw.[1] A previous phytochemical study on this plant (described as *Mallotus anisopodus* from Vietnam) revealed the presence of megastigmane sulphonoglucosides and phenolic glucosides.[3] As a continuation of our study on antiherpetic plants in the genus *Mallotus*,[4] EtOAc and MeOH extracts prepared from the stem bark of *Mallotus plicatus* were evaluated for inhibitory effects on *Herpes simplex* virus types 1 and 2, (HSV-1 and HSV-2) and found to have significant antiviral potential (see the Experimental). This prompted us to investigate these extracts to identify the active principles. As a result, nine pure compounds (**1**–**9**) were isolated and characterized (Figure 1). **1** and **2** were structurally elucidated as new compounds with structures related to bergenin (**3**). The other compounds were identified as bergenin (**3**),[5] 11-*O*-acetylbergenin (**4**),[6] aleuritolic acid acetate (**5**),[7] daucosterol (**6**),[8] protocatechuic acid (**7**),[9] scopoletin

(**8**),[10] and blumenol A(**9**)[11] through comparison of their physical and spectroscopic properties with reported values.

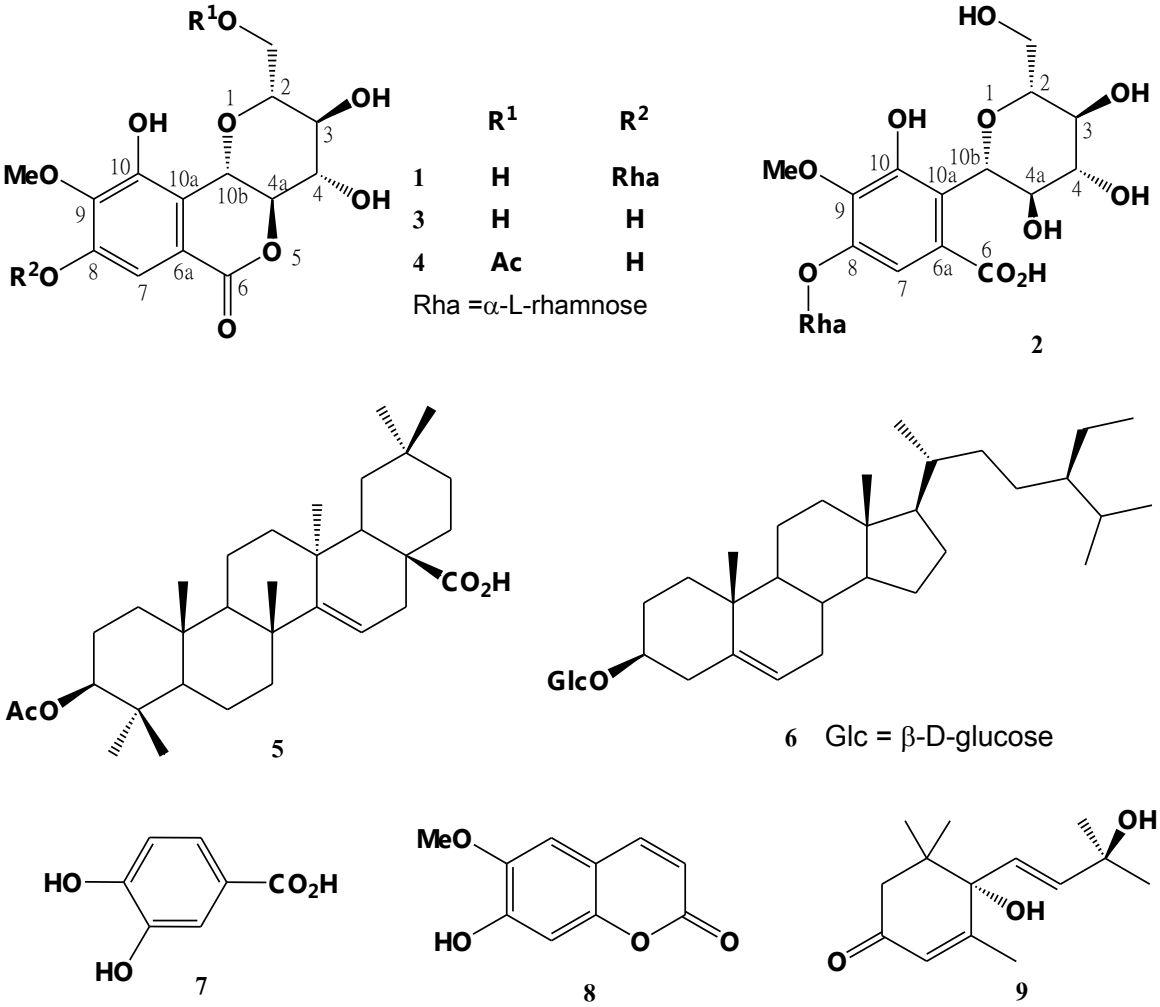


**Figure 1.** Structures of **1**-**9** from *Mallotus plicatus*

Compound **1** was obtained as colorless needles. The HR-ESI mass spectrum of **1** showed $[M+Na]^+$ at *m/z* 497.1287 (calcd. for $C_{20}H_{26}O_{13}Na$ 497.1271), suggesting the molecular formula $C_{20}H_{26}O_{13}$. The UV maximal absorptions at 223 and 274 nm and the IR bands at 1721 (lactone) and 3000-3200 (aromatic ring) $cm^{-1}$ of **1** were similar to those of bergenin (**3**), a gallic acid *C*-glucoside.[5] In the $^1H$ NMR spectrum (Table 1), **1** exhibited characteristic proton resonances for the bergenin skeleton at $\delta$ 4.07 (1H, t, $J$ = 10.1 Hz, H-4a) and $\delta$ 5.04 (1H, d, $J$ = 10.1 Hz, H-10b).[5] This was further supported by correlation peaks for these two protons at $\delta$ 79.7 (C-4a) and $\delta$ 72.1 (C-10b) in the HSQC spectrum. Further comparison of the $^1H$ and $^{13}C$ NMR data, and the molecular formula of **1** with those of **3** suggested that **1** should be a derivative of **3** with an additional sugar moiety with the composition $C_6H_{11}O_4$. This second sugar unit was suggested to be rhamnose by six HSQC correlation peaks that represented one acetal methine [C-1′, $\delta_H$ 5.33 (1H, br s)/$\delta_C$ 99.7], four oxymethines [C-2′, $\delta_H$ 3.87 (1H, m)/$\delta_C$ 70.2; C-3′, $\delta_H$ 3.64 1(1H, m)/$\delta_C$ 70.5; C-4′, $\delta_H$ 3.30 (1H,

t, d, $J$ = 9.2 Hz)/$\delta_C$ 71.7; C-5′, $\delta_H$ 3.53 (1H, m)/$\delta_C$ 70.1] and one methyl carbon [C-6′, $\delta_H$ 1.11 (3H, d, $J$ = 6.0 Hz)/$\delta_C$ 18.0].

**Table 1.** $^{1}$H and $^{13}$NMR data of compounds **1** and **2**

| **Position** | **1** (DMSO-$d_6$) | | **2** (methanol-$d_4$) | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | 3.59 (m) | 81.9 d | 3.40 (m) | 82.8 d |
| 3 | 3.21 (t, 9.0) | 70.7 d | 3.50 (t, 9.6) | 80.7 d |
| 4 | 3.67 (m) | 73.7 d | 3.43 (t, 9.6) | 73.9 d |
| 4a | 4.07 (t, 10.1) | 79.7 d | 3.69 (m) | 74.3 d |
| 6 | – | 163.3 s | – | 176.1 s |
| 6a | – | 119.2 s | – | 137.3 s |
| 7 | 7.24 (s) | 110.0 d | 7.02 (s) | 109.3 d |
| 8 | – | 149.8 s | – | 150.4 s |
| 9 | – | 142.8 s | – | 140.2 s |
| 10 | – | 148.3 s | – | 150.4 s |
| 10a | – | 118.3 s | – | 117.8 s |
| 10b | 5.04 (d, 10.1) | 72.1 d | 5.14 (d, 9.9) | 80.1 d |
| 11 | 3.44 (m), 3.85 (m) | 61.2 t | 3.83 (2H, m) | 62.1 t |
| MeO | 3.83 (3H, s) | 60.4 q | 3.83 (3H, s) | 61.4 q |
| 1′ | 5.33 (br s) | 99.7 d | 5.48 (br s) | 100.5 d |
| 2′ | 3.87 (m) | 70.2 d | 4.06 (dd, 3.3, 1.7) | 72.2 d |
| 3′ | 3.64 (m) | 70.5 d | 3.9 (dd, 9.6, 3.3) | 72.4 d |
| 4′ | 3.30 (t, 9.2) | 71.7 d | 3.54 (m) | 71.1 d |
| 5′ | 3.53 (m) | 70.1 d | 3.71 (m) | 71.0 d |
| 6′ | 1.11 (d, 6.0) | 18.0 q | 1.23 (d, 6.2) | 18.1 q |

($\delta$ in ppm and $J$ in Hz).

A NOESY experiment was then performed to locate the point of attachment of this rhamnose unit to the aglycone structure. In the NOESY spectrum (Figure 2), a correlation peak was observed between H-7 ($\delta$ 7.24, s) and H-1′ ($\delta$ 5.33, br s), indicating that the rhamnose should be attached to C-8 of bergenin through an ether [(C-1′)-*O*-(C-8)] linkage. In addition, in the rhamnopyranose moiety H-1′ displayed a NOESY cross peak with H-2′, in agreement with their di-equatorial relation.

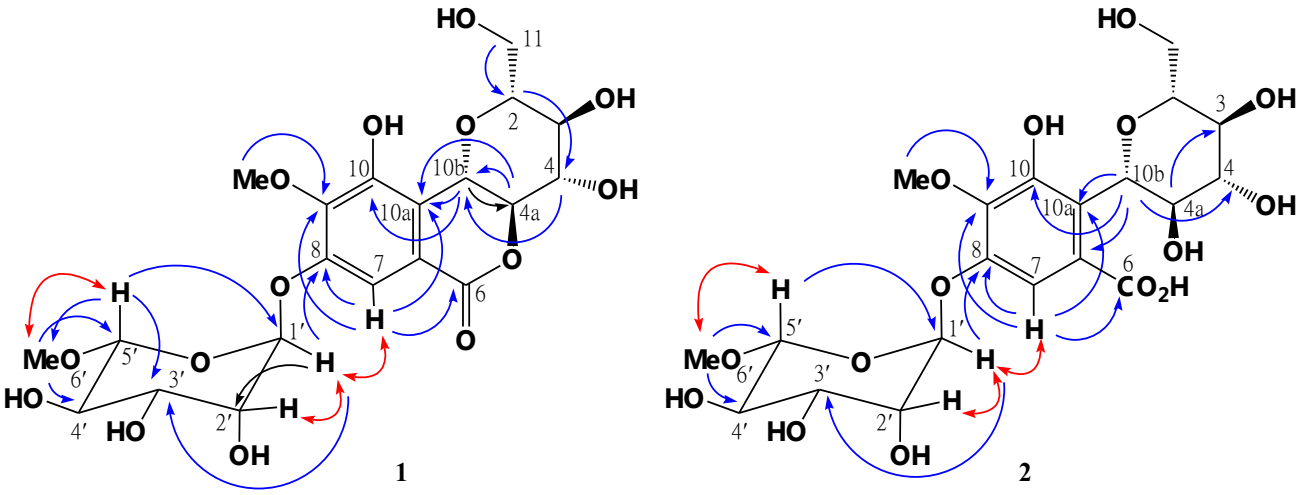


**Figure 2.** NOESY ($^1$H↔$^1$H) and HMBC ($^1$H→$^{13}$C) correlations for **1** and **2**

The presence of the ether bridge that connected the two structural parts was further corroborated by the 3-bond HMBC connectivity from H-1′ to C-8, which was already assigned through its a 2-bond coupling with H-7 (Figure 2). Finally, to confirm the proposed structure, **1** was subjected to acid hydrolysis with 2N HCl under reflux. The reaction was completed after 3 h, and the products were identified by TLC to be bergenin (**3**) and L-rhamnose. It should be noted here that under this reaction condition, the structure of the alycone bergenin was still intact, meaning that both the *C/C*-glycosidic bond and the lactone functionality were resistant to acid hydrolysis. Thus, based on the above-discussed spectroscopic data and chemical evidence, together with the known fact that natural rhamnose occurs in L-form, **1** was concluded to be bergenin-8-*O*-$\alpha$-L-rhamnoside.

Compound **2** was isolated as colorless cubic crystals. The $^1$H and $^{13}$C NMR spectra obtained for **2** (Table 1) showed strong resemblance to those obtained for **1**. However, substantial differences were observed for the $^{13}$C NMR resonances of the three carbons that were supposed to be part of the lactone ring, i.e. C-4a, C-6 and C-6a (Table 1). In **2**, C-4a was moved to a more upfield position, showing at $\delta$ 74.3, whereas C-6 and C-6a were downfield shifted, resonating at $\delta$ 176.1 and 137.3, respectively. It could be inferred from the downfield shift of C-6 that in **2**, the lactone functionality was missing, and in its place, an open carboxyl group and a free hydroxyl appeared. This was also reflected by the higher resonance frequency of C-10b of **2** ($\delta$ 80.1) as compared with that of **1** ($\delta$ 72.1), which was due to the dissapearance of γ-steric interaction with C-6. The fact that the molecular mass of **2**, which was deduced from the $[M–H]^-$ at *m/z* 491.1399 (calcd. for $C_{20}H_{27}O_{14}$ 491.1406) in the HR-ESIMS, was 18 amu higher than that of **1** further supported this argument. A NOESY correlation peak observed between H-7 and H-1′(Figure 2) indicated that **2** had the deoxyhexosyl moiety attached to C-8 of the alycone through an ether bridge, which was similar to that of **1**. In support of this, a three-bond coupling was observed between H-1′ and C-8 in the HMBC spectrum

(Figure 2). Upon complete acid hydrolysis, compound **2** gave bergenin (**3**) and L-rhamnose. It is important to note that the final aglycone product was bergenin (**3**), not *seco*-bergenin. This means that during the reaction, the initial product *seco*-bergenin underwent cyclization rapidly and spontaneously to produce **3**. At present, there are no reports on the isolation of *seco*-bergenin from natural sources. Hence, **2** was characterized as a new naturally occurring compound, and named *seco*-bergenin-8-*O*-$\alpha$-L-rhamnoside.

All of the compounds (**1**-**9**) were evaluated for their inhibitory activity against *Herpes simplex* virus types 1 and 2, using established protocols.[4] Compound **2** showed weak activity against HSV-1 ($IC_{50}$ 193.1 $\mu$M) but no activity against HSV-2, whereas **7** exhibited only marginal activity against both types of virus ($IC_{50}$ 616.8 $\mu$M) (positive control acyclovir $IC_{50}$ 1.5 and 2.9 $\mu$M). The other compounds were devoid of activity at a concentration of 100 $\mu$g/mL. It appears that a free carboxyl group was required for the activity of these aromatic compounds since antiviral activity was observed only in **2** and **7**, but not in **1**, **3** or **4**.

## EXPERIMENTAL

### *General*

Melting points were recorded on a Fisher-Johns melting point apparatus. Optical rotations were measured on a Perkin-Elmer 341 polarimeter, and the CD spectra were recorded on a JASCO J-715 spectropolarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra on a Perkin-Elmer FT-IR 1760X spectrophotometer. Mass spectra were recorded with a Bruker micro TOF mass spectrometer (ESI-TOF). NMR spectra were obtained on a Bruker Avance DPX-300 FT-NMR spectrometer. Vacuum-liquid column chromatography (VLC) and column chromatography (CC) were performed on silica gel 60 (Merck, Kieselgel 60, 70-320 mesh), silica gel 60 (Merck, Kieselgel 60, 230-400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.).

### *Plant Material*

The stem bark of *Mallotus plicatus* was collected from the Botanical Garden of Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, in June 2009. Authentication was performed by comparison with herbarium specimens at the Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resource and Environment.

### *Extraction and Isolation*

The dried and powdered stem bark of *Mallotus plicatus* (7.5 kg) was successively extracted with EtOAc and MeOH to give an EtOAc (56 g) and a MeOH (890 g) extract after removal of the solvent. The EtOAC extract showed $IC_{50}$ values of 87.5 and 68.8 μg/mL against HSV-1 and HSV-2, respectively, whereas and

the MeOH extract had $IC_{50}$ values of 43.8 and 31.3 μg/mL, correspondingly. The EtOAc extract was then fractionated by VLC (silica gel, *n*-hexane–$CH_2Cl_2$ gradient) to obtain 14 fractions (I–XIV). Fraction VIII (2.2 g) was further separated on a silica gel column (*n*-hexane–$CH_2Cl_2$ polarity gradient) to give 17 fractions. Fractions 12–17 were combined and dried, and the obtained residue was recrystallized from petroleum ether to give aleuritolic acid acetate (**5**) as colorless needles (188 mg). Fraction XII (3.1 g) was subjected to CC (silica gel, $CH_2Cl_2$–MeOH gradient) to yield 9 subfractions. Fraction 7 from this column, after drying, was purified by recrystallization from acetone to afford daucosterol (**6**) as a white powder (31 mg). Fraction 9, upon standing, gave brown crystals. The crystals were collected, dried and purified by washing with acetone to furnish colorless needles of bergenin (**3**) (62 mg). The mother liquor, after drying, was separated by preparative TLC (silica gel, $CH_2Cl_2$–acetone 4:6) to afford protocatechuic acid (**7**) (8 mg). The MeOH extract was partitioned between $H_2O$ and *n*-BuOH to give an *n*-BuOH extract (38 g) and a water extract (54 g) after evaporation of the solvents. Separation of the *n*-BuOH extract by CC (silica gel, $CH_2Cl_2$–MeOH gradient) gave 7 fractions (A–G). Fraction B was fractionated on a silica gel column ($CH_2Cl_2$–acetone gradient) to provide 26 fractions (B1–B26). Fractions B6–B9 were pooled and dried, and further purified by high performance liquid chromatography (ODS, $H_2O$–MeCN 6:4) to give scopoletin (**8**, 2 mg). Fraction B24, after purification by HPLC using similar stationary and mobile phases, presented blumenol A (**9**) as a yellow gum (17 mg). Purification of fraction B26 by recrystallization from $CH_2Cl_2$ afforded 11-*O*-acetylbergenin (**4**) as colorless needles (28 mg). Separation of the water extract on an MCI column ($H_2O$-MeOH gradient) gave 5 fractions (1–5). Fraction 2 was separated on a silica gel column (EtOAc–MeOH gradient) to give 8 fractions (W1–W8). Fraction W2 was further separated by CC ($CH_2Cl_2$–MeOH gradient) and then by preparative TLC (silica gel, $CHCl_3$–MeOH 8:2) with double development to give compound **1** as colorless needles (42 mg). Fraction W8 was separated by prep. TLC (silica gel, *n*-BuOH–AcOH–$H_2O$ 8:1:1) and then purified by CC (silica gel, $CH_2Cl_2$–MeOH 1:1) to furnish compound **2** as colorless cubic crystals (6 mg).

**Compound 1 (bergenin-8-*O*-α-L-rhamnoside)**: colorless needles; mp 198-203 °C; $[\alpha]_D^{20}$ –62.3 (*c* 0.001, MeOH); UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$): 223 (4.42), 274 (3.67); IR (KBr) $\nu_{max}$: 3400, 1722, 1612, 1510, 1460, 1374, 1337, 1093 $cm^{-1}$; HR-ESI-MS: $[M+Na]^+$ at *m/z* 497.1287 (calcd. for $C_{20}H_{26}O_{13}Na$, 497.1271); $^1H$ (300 MHz) and $^{13}C$ (75 MHz) NMR spectral data see Table 1.

**Compound 2 (*seco*-bergenin-8-*O*-α-L-rhamnoside)**: cubic crystals; mp 146-149 °C; $[\alpha]_D^{20}$ –10.4 (*c* 0.0003, MeOH); UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$): 219 (3.12); IR (KBr) $\nu_{max}$: 3434, 1623, 1573, 1402, 1384, 1094 $cm^{-1}$; HR-ESI-MS: $[M-H]^-$ at *m/z* 491.1399 (calcd. for $C_{20}H_{27}O_{14}$, 491.1406); $^1H$ (300 MHz) and $^{13}C$ (75 MHz) NMR spectral data see Table 1.

*Hydrolysis of **1** and **2***

The hydrolysis reactions of **1** and **2** were carried out as follows. Each compound (2 mg) was dissolved in 2 N HCl (2 mL) and refluxed at 100 °C for 3 h. Then, the solvent was removed from the hydrolysate, and $H_2O$ (10 mL) was added to redissolve the residue. The solution was then partitioned with EtOAc (3 x 10 mL). Each fraction was dried and the residue was redissolved in MeOH to yield a sample solution for comparative examination by TLC (silica gel, $CHCl_3$–MeOH–$H_2O$ 5:4:1) with the authentic samples bergenin and L-rhamnose. Spot visualization was done by spraying the developed TLC plate with 10% sulfuric acid followed by heating at 120 °C for 2 min. Both **1** and **2** presented L-rhamnose as the sugar unit, and bergenin (**3**) as the aglycone product.

*Evaluation of antiherpetic activity*

Antiviral activities against HSV-1 (KOS) and HSV-2 (186) were determined with the aid of the so-called inactivation method, as previously described.[4] Briefly, the virus (30 PFU/25 $\mu$L) was mixed with 25 $\mu$L of complete medium containing various concentrations of test compound, and then incubated at 37 °C in 5% humidified $CO_2$ for 1 h. After incubation, the mixtures were added to Vero cells (6×105 cells/mL) in 96-well microtiter plates (50 $\mu$L/well), and incubated at 37 °C in 5% humidified $CO_2$ for 2 h. The overlay medium containing the test compound (100 $\mu$L/well) in various concentrations was then added to the mixtures, which were incubated at 37 °C in 5% humidified $CO_2$ for 2 d. Then, the virus-growth inhibition was evaluated by counting the virus plaque forming on the Vero cells, as compared to the control. The percent plaque inhibition was determined, and the $IC_{50}$ value was calculated for the test compounds that showed more than 50% inhibition at 100 $\mu$g/mL. Acyclovir was used as positive control.

## ACKNOWLEDGEMENTS

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