# New Stilbene, Benzofuran, and Coumarin Glycosides from *Morus alba*

by **Shu-Juan Piao**[a]), **Feng Qiu***[a]), **Li-Xia Chen**[a]), **Ya Pan**[a]), and **De-Qiang Dou**[b])

[a]) Department of Natural Products Chemistry 81#, Shenyang Pharmaceutical University, 103 Wenhua Avenue, Shenyang 110016, P. R. China (phone: +86-24-23986463; fax: +86-24-23993994; e-mail: fengqiu2000@tom.com)

[b]) School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Shenyang 110032, P. R. China

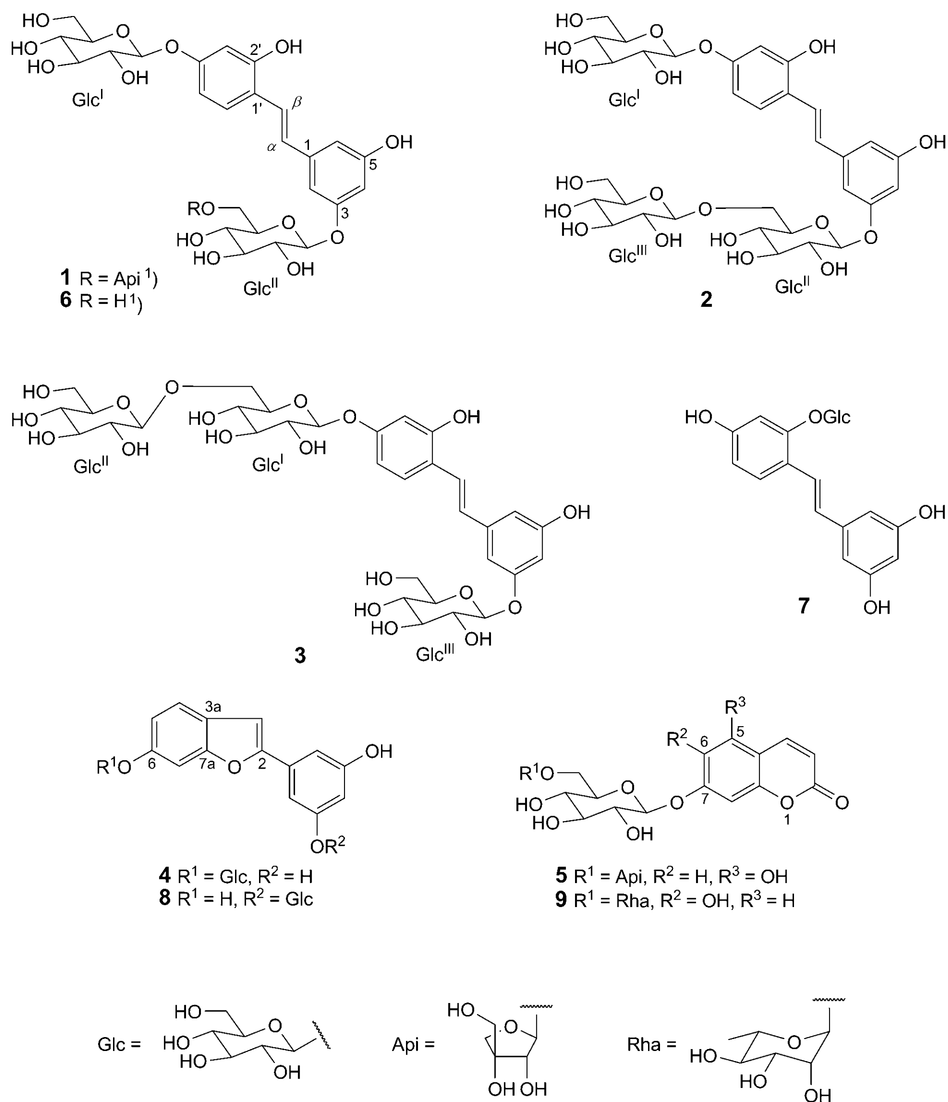
Five new compounds, including three new stilbene-derived glycosides, one novel arylbenzofuran derivative, and one new coumarin glycoside, along with ten known compounds were isolated from the $H_2O$ extract of the bark of *Morus alba* L. The new compounds were assigned to the structures of 4′-*O*-β-D-glucopyranosyl-2′-hydroxyresveratrol 3-(6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside) (**1**), 4′-*O*-β-D-glucopyranosyl-2′-hydroxyresveratrol 3-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside) (**2**), 3-*O*-β-D-glucopyranosyl-2′-hydroxyresveratrol 4′-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside) (**3**), moracin M 6-(β-D-glucopyranoside) (**4**), and 5,7-dihydroxycoumarin 7-(6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside) (**5**) by spectroscopic means, and acid and enzymatic hydrolyses. Three compounds, 2′-hydroxyresveratrol 2′-(β-D-glucopyranoside) (**7**), moracin M 3′-(β-D-glucopyranoside) (**8**), and 6,7-dihydroxycoumarin 7-(6-*O*-α-rhamnopyranosyl-β-D-glucopyranoside) (**9**), were found for the first time in *Morus alba*.

**Introduction.** – *Morus alba* L. (Moraceae) is widely distributed in the Anhui, Hunan, and Shanxi provinces in China. Its root bark is extensively used as an antitussive and antiasthmatic agent [1] in traditional Chinese medicines. Although many compounds such as stilbenes, flavonoids, benzofuran derivatives, and coumarins have been identified in *Morus alba* [2–6], there have been few reports on its pharmacological properties. In our previous study, we investigated the absorbed constituents in rats following oral administration of the $H_2O$ extract of the bark of *Morus alba* [7][8], and several stilbene derivatives were identified in the plasma, urine, and bile of rats. Further pharmacological studies showed that mulberroside A, a major stilbene constituent in the $H_2O$ extract, displayed a significant antitussive and antiasthmatic effect in animals [9]. In our ongoing search for the bioactive constituents from the $H_2O$ extract of *Morus alba* L., a systematic investigation on the chemical constituents of water extract of *Morus alba* was carried out, and resulted in the isolation of eight stilbene glycosides, three arylbenzofuran derivatives, and four coumarin glycosides. This article reports the isolation and structural elucidation of the three new stilbene glycosides **1**–**3**[1]), the novel arylbenzofuran derivative **4**, and the new coumarin glycoside **5**. Ten known compounds were identified as mulberroside A (**6**) [10], *cis*-mulberroside A [2], oxyresveratrol 3′-*O*-β-D-glucopyranoside (=2′-hydroxyresveratrol 3-(β-D-glucopyranoside)) [2], oxyresveratrol 2-*O*-β-D-glucopyranoside (=2′-hydroxy-

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

© 2009 Verlag Helvetica Chimica Acta AG, Zürich

resveratrol 2′-(β-D-glucopyranoside); **7**) [2], resveratrol 3,4′-*O*,*O*′-di-β-D-glucopyranoside (= resveratrol 3,4′-di(β-D-glucopyranoside)) [11], moracin M 3′-*O*-β-D-glucopyranoside (= moracin M 3′-(β-D-glucopyranoside); **8**) [11], moracin M 6,3′-di-*O*-β-D-glucopyranoside (= moracin M 3′,6-di(β-D-glucopyranoside)) [5], 6-hydroxycoumarin 7-*O*-α-rhamnopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside (= 6,7-dihydroxycoumarin 7-(6-*O*-α-rhamnosyl-β-D-glucopyranoside); **9**) [12], scopoletin (= 7-hydroxy-6-methoxy-2*H*-benzopyran-2-one) [13], and 6-hydroxycoumarin 7-*O*-β-D-glucopyranoside (= 6,7-dihydroxycoumarin 7-(β-D-glucopyranoside)) [14] by comparison with the reported data. Three compounds, *i.e.*, **7**–**9**, were found for the first time in *Morus alba*.



**Results and Discussion.** – Compound **1** was obtained as a white amorphous powder. The UV spectrum (MeOH) exhibited $\lambda_{max}$ (log $\varepsilon$) at 214 (4.49), 290 (4.28), 301 (4.30), and 324 (4.41) nm, similar to those of mulberroside A (**6**) and its derivatives obtained from mulberry root bark [10], suggesting that **1** is a stilbene derivative. HR-ESI-MS gave a quasimolecular-ion peak $[M + Na]^+$ at $m/z$ 723.2089 which, in combination with $^1$H- and $^{13}$C-NMR data (*Table 1*), established the molecular formula as $C_{31}H_{40}O_{18}$. Prominent ESI-MS fragment peaks occurred at $m/z$ 537 ($[M - 162 - H]^-$), 405 ($[M - 162 - 132 - H]^-$), and 243 ($[M - 162 - 132 - 162 - H]^-$), indicating the presence of two hexoses and one pentose in the structure. The $^1$H-NMR spectrum of **1** showed the signals ascribable to two *trans*-olefinic protons at $\delta$ 6.94 and 7.22 (each *d*, $J = 16.3$ Hz, 1 H), along with proton signals belonging to two independent aromatic rings at $\delta$ 7.45 (*d*, $J = 7.1$ Hz, 1 H), 6.49 (br. *d*, $J = 7.1$ Hz, 1 H), and 6.60, 6.59, 6.54, and 6.34 (4 br. *s* each 1 H), implying that **1** was an analogue of mulberroside A (= 3-{(1*E*)-2-[4-($\beta$-D-glucopyranosyloxy)-2-hydroxyphenyl]ethenyl}-5-hydroxyphenyl $\beta$-D-glucopyranoside; **6**). In the $^1$H-NMR spectrum, three anomeric-proton signals at $\delta$ 4.83 (*d*, $J = 3.1$ Hz), 4.79 (*d*, $J = 7.6$ Hz), and 4.78 (*d*, $J = 7.5$ Hz) substantiated the presence of three sugar moieties in the structure. The $^{13}$C-NMR spectrum showed two *trans*-substituted olefinic C-atoms, twelve aromatic C-atoms (6 CH, 2 C, 4 C–O), and seventeen sugar C-atoms. Assignments of the protons in the $^1$H-NMR spectrum as well as the C-atoms in the $^{13}$C-NMR spectrum (*Table 1*) were performed by HSQC, HMBC, NOESY, and HSQC-TOCSY experiments. Acid hydrolysis of **1** produced apiose and glucose as sugar residues [15]. Moreover, enzymatic hydrolysis of **1** by $\beta$-glucosidase (emulsin) yielded the aglycone oxyresveratrol (= 2′-hydroxyresveratrol = 4-[(1*E*)-2-(3,5-dihydroxyphenyl)ethenyl]benzene-1,3-diol), which indicates the $\beta$-D-configuration of the glucose residues[ 15]. The $\beta$-D-configuration of the apiose residue was attributed by $^{13}$C-NMR chemical shifts and the coupling constant ($J = 3.1$ Hz) of the anomeric proton [16], in combination with the NOE correlation H–C(2)(Api)/H–C(5)(Api). The HMBC between the anomeric-proton signal at $\delta$ 4.78 (H–C(1)(Glc$^{II}$)) and $\delta$ 158.4 (C(3)), together with the NOESY correlations $\delta$ 4.78 (H–C(1)(Glc$^{II}$))/H–C(2) and H–C(4) of the aglycone suggested that the Glc$^{II}$ residue was connected to C(3) of the aglycone. The HMBC between the anomeric-proton signal at $\delta$ 4.79 (H–C(1)(Glc$^{I}$)) and $\delta$ 157.9 (C(4′)), and the NOESY correlations $\delta$ 4.79 (H–C(1)(Glc$^{I}$))/H–C(3′) and H–C(5′) of the aglycone implied the linkage of the Glc$^{I}$ residue at C(4′) of the aglycone. Also in the HMBC spectrum, the correlation $\delta$ 4.83 (H–C(1)(Api))/$\delta$ 67.2 (C(6)(Glc$^{II}$)) demonstrated that the apiose residue was linked to C(6) of Glc$^{II}$. The NOESY correlations $\delta$ 3.87 (H–C(6)(Glc$^{II}$))/$\delta$ 4.83(H–C(1)(Api)) further corroborated the connective relationship of the two sugar residues. On the basis of the above evidences, the structure of **1** was deduced as 4′-*O*-$\beta$-D-glucopyranosyl-2′-hydroxyresveratrol 3-(6-*O*-$\beta$-D-apiofuranosyl-$\beta$-D-glucopyranoside)[1]).

Compound **3** was obtained as a white amorphous powder. HR-ESI-MS showed a quasimolecular-ion peak $[M + Na]^+$ at 753.2165, establishing the molecular formula as $C_{32}H_{42}O_{19}$, in combination with the $^1$H- and $^{13}$C-NMR data (*Table 1*). Prominent ESI-MS fragment peaks occurred at $m/z$ 567 ($[M - 162 - H]^-$), 405 $[M - (162 \times 2) - H]^-$, and 243 $[M - (162 \times 3) - H]^-$, showing the presence of three hexoses in the structure. Acid hydrolysis of **3** produced only glucose as sugar residue [15]. The values of the coupling constants of the three anomeric protons ($J = 7.4$, 7.6, and 7.4 Hz) suggested $\beta$-

Table 1. *$^1$H- and $^{13}$C-NMR* (600 and 150 MHz, resp.; (D$_6$)DMSO) *Data of Compounds* **1**–**3**[a]). $\delta$ in ppm, *J* in Hz.

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) |
| C(1) | 139.9 | | 139.9 | | 139.7 | |
| C(2) | 105.5 | 6.59 (br.*s*) | 105.8 | 6.60 (*br.* s) | 105.2 | 6.63 (br. *s*) |
| C(3) | 158.4 | | 158.5 | | 158.3 | |
| C(4) | 102.5 | 6.34 (br. *s*) | 102.6 | 6.42 (br. *s*) | 102.5 | 6.34 (br. *s*) |
| C(5) | 158.8 | | 158.9 | | 158.8 | |
| C(6) | 106.1 | 6.60 (br. *s*) | 106.3 | 6.62 (br. *s*) | 106.4 | 6.58 (br. *s*) |
| C(1′) | 117.7 | | 117.9 | | 117.8 | |
| C(2′) | 155.9 | | 156.0 | | 155.8 | |
| C(3′) | 103.8 | 6.54 (br. *s*) | 103.9 | 6.55 (br. *s*) | 103.6 | 6.61 (br. *s*) |
| C(4′) | 157.9 | | 158.1 | | 157.8 | |
| C(5′) | 107.4 | 6.49 (br. *d*, *J* = 7.1) | 107.3 | 6.52 (br. *d*, *J* = 7.7) | 107.6 | 6.57 (br. *d*, *J* = 8.5) |
| C(6′) | 127.1 | 7.45 (*d*, *J* = 7.1) | 127.3 | 7.48 (*d*, *J* = 7.7) | 127.3 | 7.46 (*d*, *J* = 8.5) |
| H−C($\alpha$) | 125.5 | 6.94 (*d*, *J* = 16.3) | 125.9 | 6.96 (*d*, *J* = 16.1) | 125.8 | 6.94 (*d*, *J* = 16.4) |
| H−C($\beta$) | 123.4 | 7.22 (*d*, *J* = 16.3) | 123.4 | 7.23 (*d*, *J* = 16.1) | 123.4 | 7.23 (*d*, *J* = 16.4) |
| Glc$^{I}$: | | | | | | |
| H−C(1) | 100.2 | 4.79 (*d*, *J* = 7.6) | 100.4 | 4.79 (*d*, *J* = 7.1) | 100.4 | 4.76 (*d*, *J* = 7.4) |
| H−C(2) | 73.1 | 3.14–3.22 (*m*) | 73.3 | 3.16–3.22 (*m*) | 73.2 | 3.19–3.25 (*m*) |
| H−C(3) | 76.9 | 3.24–3.28 (*m*) | 77.1 | 3.22–3.27 (*m*) | 76.4 | 3.24–3.29 (*m*) |
| H−C(4) | 69.4 | 3.16–3.20 (*m*) | 69.5 | 3.20–3.25 (*m*) | 69.5 | 3.15–3.20 (*m*) |
| H−C(5) | 76.3 | 3.16–3.20 (*m*) | 76.7 | 3.22–3.27 (*m*) | 76.0 | 3.50–3.54 (*m*) |
| CH$_2$(6)[a]) | 60.4 | 3.68 (br. *d*, *J* = 11.5), 3.50 (*dd*, *J* = 11.5, 4.4) | 60.6 | 3.69 (br. *d*, *J* = 11.2), 3.46–3.52 (o) | 68.0 | 4.01 (br. *d*, *J* = 11.2), 3.60–3.64 (o) |
| Glc$^{II}$: | | | | | | |
| H−C(1) | 100.6 | 4.78 (*d*, *J* = 7.5) | 100.8 | 4.79 (*d*, *J* = 7.1) | 103.1 | 4.26 (*d*, *J* = 7.6) |
| H−C(2) | 73.1 | 3.14–3.22 (*m*) | 73.3 | 3.16–3.22 (*m*) | 73.6 | 2.98–3.02 (*m*) |
| H−C(3) | 76.5 | 3.24–3.28 (*m*) | 76.7 | 3.22–3.27 (*m*) | 76.9 | 3.00–3.04 (*m*) |
| H−C(4) | 69.6 | 3.12–3.16 (*m*) | 69.6 | 3.15–3.20 (*m*) | 70.0 | 3.03–3.08 (*m*) |
| H−C(5) | 75.3 | 3.43–3.47 (*m*) | 75.8 | 3.50–3.54 (*m*) | 76.4 | 3.10–3.16 (*m*) |
| CH$_2$(6)[a]) | 67.2 | 3.87 (br. *d*, *J* = 9.0), 3.45 (br. *d*, *J* = 9.0) | 68.3 | 4.00 (br. *d*, *J* = 10.8), 3.60–3.64 (o) | 60.8 | 3.44 (br. *d*, *J* = 11.3), 3.65 (br. *d*, *J* = 11.3) |
| Api or Glc$^{III}$: | | | | | | |
| H−C(1) | 109.0 | 4.83 (*d*, *J* = 3.1) | 103.5 | 4.23 (*d*, *J* = 7.4) | 100.6 | 4.80 (*d*, *J* = 7.4) |
| H−C(2) | 75.9 | 3.77 (br. *s*) | 73.7 | 2.98–3.02 (*m*) | 73.1 | 3.19–3.25 (*m*) |
| C(3) or H−C(3) | 78.7 | | 77.0 | 3.02–3.06 (*m*) | 76.7 | 3.24–3.29 (*m*) |
| CH$_2$(4) or H−C(4) | 73.2 | 3.58 (*d*, *J* = 9.2), 3.87 (*d*, *J* = 9.2) | 70.1 | 3.15–3.20 (*m*) | 69.5 | 3.15–3.20 (*m*) |
| CH$_2$(5) or H−C(5) | 63.1 | 3.28–3.36 (o), 3.28–3.36 (o) | 76.5 | 3.12–3.14 (*m*) | 76.5 | 3.24–3.29 (*m*) |
| CH$_2$(6)[a]) | | | 61.0 | 3.43 (br. *d*, *J* = 9.3), 3.63–3.66 (o) | 60.5 | 3.69 (br. *d*, *J* = 9.6), 3.46–3.52 (o) |

[a]) 'o' stands for overlapping.

configuration for all the three sugars. Moreover, enzymatic hydrolysis of **3** by $\beta$-glucosidase (emulsin) yielded the aglycone oxyresveratrol, which indicate the $\beta$-D-configuration of the glucose residues [15]. The $^1$H- and $^{13}$C-NMR data of compound **3**

were very close to those of **1**, except for those of the sugar moieties, substantiating that **3** also is an analogue of mulberroside A. Assignments of the protons in the $^{1}$H-NMR spectrum as well as the C-atoms in the $^{13}$C-NMR spectrum (*Table 1*) were performed by HSQC, HMBC, NOESY, and HSQC-TOCSY experiments. In the HMBC spectrum of **3**, the correlation $\delta$ 4.26 (H−C(1)(Glc$^{II}$))/$\delta$ 68.0 (C(6)(Glc$^{I}$)) suggested that the Glc$^{II}$ residue was linked to C(6) of Glc$^{I}$. The correlations between the anomeric-proton signal at $\delta$ 4.76 (H−C(1)(Glc$^{I}$)) and $\delta$ 157.8 (C(4′)), and between another anomeric-proton signal at $\delta$ 4.80 (H−C(1)(Glc$^{III}$)) and $\delta$ 158.3 (C(3)) demonstrated that Glc$^{I}$ and Glc$^{III}$ were connected to C(4′) and C(3) of the aglycone, respectively; this was further supported by the correlations $\delta$ 4.76 (H−C(1)(Glc$^{I}$))/H−C(3′) and H−C(5′) and of $\delta$ 4.80 (H−C(1)(Glc$^{III}$))/H−C(2), H−C(4) in the NOESY plot of **3**. Therefore, the structure of **3** was deduced as 3-*O*-$\beta$-D-glucopyranosyl-2′-hydroxyresveratrol 4′-(6-*O*-$\beta$-D-glucopyranosyl-$\beta$-D-glucopyranoside)[1]).

Compound **2** was obtained as a white amorphous powder. HR-ESI-MS also showed a quasimolecular-ion peak $[M + Na]^{+}$ at 753.2277 which, by combining with the $^{1}$H- and $^{13}$C-NMR data (*Table 1*), established the molecular formula as $C_{32}H_{42}O_{19}$. Prominent ESI-MS fragment peaks occurred at *m/z* 567 ($[M - 162 - H]^{-}$), 405 ($[M - (162 \times 2) - H]^{-}$), and 243 ($[M - (162 \times 3) - H]^{-}$). Acid hydrolysis of **2** produced only glucose as sugar residue, and enzymatic hydrolysis of **2** with $\beta$-glucosidase yielded the aglycone [15], indicating the $\beta$-D-configuration of the glucose residues. The $^{1}$H- and $^{13}$C-NMR data of **2** were very similar to those of **3**, except for the sugar moieties, suggesting that **2** is an isomer of **3**. In the HMBC spectrum, the correlation $\delta$ 4.23 (H−C(1)(Glc$^{III}$))/$\delta$ 68.3 (C(6)(Glc$^{II}$)) of **2** suggested that Glc$^{III}$ was linked to C(6) of Glc$^{II}$. The HMBCs of the anomeric-proton signal at $\delta$ 4.79 (H−C(1)(Glc$^{I,II}$), overlapped) with $\delta$ 158.1 (C(4′)) and 158.5 (C(3)) established that the glucose moieties Glc$^{I}$ and Glc$^{II}$ were connected to C(4′) and C(3), respectively, which was further supported by the NOESY correlations $\delta$ 4.79 (H−C(1)(Glc$^{I,II}$)/H−C(3′), H−C(5′), H−C(2), and H−C(4). Therefore, the structure of **2** must be 4′-*O*-$\beta$-D-glucopyranosyl-2′-hydroxyresveratrol 3-(6-*O*-$\beta$-D-glucopyranosyl-$\beta$-D-glucopyranoside)[1]). Assignments of the protons in the $^{1}$H-NMR spectrum as well as the C-atoms in the $^{13}$C-NMR spectrum (*Table 1*) were performed by HSQC, HMBC, NOESY, and HSQC-TOCSY experiments.

Compound **4** was obtained as a brown amorphous powder. The UV spectrum exhibited absorption maxima at 207 nm (4.45) and 310 nm (4.22). The molecular formula was established as $C_{20}H_{20}O_{9}$ by HR-ESI-MS showing a quasimolecular-ion peak $[M + Na]^{+}$ at *m/z* 427.1038, in combination with $^{1}$H- and $^{13}$C-NMR data (*Table 2*). A prominent ESI-MS fragment peak occurred at *m/z* 241 ($[M - 162 - H]^{-}$), suggesting the presence of one hexose residue in the structure. Acid hydrolysis of **4** produced glucose as sugar residue, and enzymatic hydrolysis of **4** with $\beta$-D-glucosidase yielded the aglycone [15]. The $^{1}$H-NMR spectrum exhibited one anomeric-proton signal at $\delta$ 4.91 (*d*, $J = 7.3$ Hz), and seven aromatic-proton signals at $\delta$ 7.51 (*d*, $J = 8.5$ Hz, 1 H), 6.97 (*dd*, $J = 8.5$, 1.9 Hz, 1 H), 6.72 (*d*, $J = 1.9$ Hz, 2 H), and 7.31, 7.17, and 6.23 (3 br. *s* each 1 H). The $^{13}$C-NMR spectrum showed the presence of one glucopyranosyl residue and fourteen aromatic C-atoms for the aglycone moiety. The $^{1}$H- and $^{13}$C-NMR data of **4** were very similar to those of moracin M 3′-($\beta$-D-glucopyranoside) [11], except for the linkage between the sugar and the aglycone. The NOESY experiment exhibited the correlations between the anomeric-proton signal at $\delta$ 4.91 (H−C(1)(Glc)) and both $\delta$

6.97 (H–C(5)) and $\delta$ 7.31 (H–C(7)), confirming that the glucose residue was connected to C(6) of the aglycone. From the above evidences, the structure of **4** was identified as moracin M 6-($\beta$-D-glucopyranoside). Assignments of the protons in the $^{1}$H-NMR spectrum as well as the C-atoms in the $^{13}$C-NMR spectrum (*Table 2*) were performed by HSQC, HMBC, and NOESY experiments.

Table 2. *$^{1}$H- and $^{13}$C-NMR* (600 and 150 MHz, resp.; ($D_6$)DMSO) *Data for Compound* **4**[1]). $\delta$ in ppm, *J* in Hz.

| | $\delta$(C) | $\delta$(H) |
|---|---|---|
| C(2) | 154.8 | |
| H–C(3) | 103.1 | 7.17 (*s*) |
| C(3a) | 121.1 | |
| H–C(4) | 123.3 | 7.51 (*d*, *J* = 8.5) |
| H–C(5) | 113.7 | 6.97 (*dd*, *J* = 8.5, 1.9) |
| C(6) | 155.2 | |
| H–C(7) | 99.1 | 7.31 (br. *s*) |
| C(7a) | 155.8 | |
| C(1′) | 131.5 | |
| H–C(2′) | 102.6 | 6.72 (*d*, *J* = 1.9) |
| C(3′) | 158.9 | |
| H–C(4′) | 101.6 | 6.23 (br. *s*) |
| C(5′) | 158.9 | |
| H–C(6′) | 102.6 | 6.72 (*d*, *J* = 1.9) |
| Glc: | | |
| H–C(1) | 101.2 | 4.91 (*d*, *J* = 7.3) |
| H–C(2) | 73.4 | 3.15–3.19 (*m*) |
| H–C(3) | 77.2 | 3.25–3.31 (*m*) |
| H–C(4) | 69.9 | 3.15–3.19 (*m*) |
| H–C(5) | 76.7 | 3.25–3.31 (*m*) |
| $CH_2$(6) | 60.9 | 3.47 (*dd*, *J* = 11.8, 5.8), 3.73 (*dd*, *J* = 11.8, 4.0) |

Compound **5** was isolated as a yellow amorphous powder. The HR-ESI-MS gave a quasimolecular-ion peak $[M+Na]^+$ at *m/z* 495.1123, demonstrating, in combination with $^{1}$H- and $^{13}$C-NMR data (*Table 3*), the molecular formula as $C_{20}H_{24}O_{13}$. A prominent ESI-MS fragment peak occurred at *m/z* 177 ($[M-294-H]^-$), suggesting the presence of one hexose and one pentose in the structure. Acid hydrolysis of **5** produced glucose and apiose as sugar residues [15]. Moreover, enzymatic hydrolysis of **5** by $\beta$-glucosidase (emulsin) yielded the aglycone, which indicates the $\beta$-D-configuration of the glucose residue [15]. The $\beta$-D-configuration of the apiose residue was attributed by $^{13}$C-NMR chemical shifts and the coupling constant ($J$ = 3.1 Hz) of the anomeric proton [16], in combination with the NOE correlation H–C(2)(Api)/H–C(5)(Api). The $^{1}$H-NMR spectrum of **5** showed the presence of the coumarin skeleton at $\delta$ 6.04 (*d*, *J* = 9.5 Hz, H–C(3)) and 8.01 (*d*, *J* = 9.5 Hz, H–C(4)), with two '*meta*'-proton signals at $\delta$ 6.33 and 6.32 (*d*, *J* = 2.7 Hz) assigned to H–C(6) and H–C(8), respectively. The $^{13}$C-NMR data showed eleven C-atoms for two sugar residues and nine C-atoms for the aglycone. In the HMBC spectrum, the correlation $\delta$ 4.85 (H–C(1)(Api))/$\delta$ 67.2 (C(6)(Glc)) demonstrated that the apiose was linked to C(6) of the glucose residue. The HMBC between the anomeric-proton signat at $\delta$ 4.86

(H–C(1)(Glc)) and δ 161.2 (C(7)), in combination with the NOESY correlations δ 4.86 (H–C(1)(Glc))/H–C(6) and H–C(8) corroborated that the glucose residue was connected to C(7) of the aglycone. On the basis of the above evidences, the structure of **5** was determined to be 5,7-dihydroxycoumarin 7-(6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside). Assignments of the protons in the $^{1}$H-NMR spectrum as well as of the C-atoms in the $^{13}$C-NMR spectrum (*Table 3*) were performed by HSQC, HMBC, and NOESY experiments.

Table 3. *$^{1}$H- and $^{13}$C-NMR* (600 and 150 MHz, resp.; ($D_6$)DMSO) *of Data for Compound* **5**. δ in ppm, *J* in Hz.

| | δ(C) | δ(H) | | δ(C) | δ(H) |
|---|---|---|---|---|---|
| C(2) | 160.9 | | Glc: | | |
| H–C(3) | 108.9 | 6.04 (*d*, *J* = 9.5) | H–C(1) | 99.9 | 4.86 (*d*, *J* = 7.6) |
| H–C(4) | 140.1 | 8.01 (*d*, *J* = 9.5) | H–C(2) | 73.1 | 3.20–3.26 (*m*) |
| C(4a) | 104.3 | | H–C(3)[b]) | 76.4 | 3.22–3.30 (*m*) |
| C(5) | 156.3 | | H–C(4) | 69.7 | 3.09–3.14 (*m*) |
| H–C(6) | 99.6 | 6.33 (*d*, *J* = 2.7) | H–C(5) | 75.9 | 3.48–3.54 (*m*) |
| C(7) | 161.2 | | $CH_2$(6) | 67.2 | 3.86 (br. *d*, *J* = 10.1) |
| H–C(8) | 92.7 | 6.32 (*d*, *J* = 2.7) | | | 3.45 (*dd*, *J* = 10.1, 6.4) |
| C(8a) | 156.3 | | Api: | | |
| | | | H–C(1) | 109.2 | 4.85 (*d*, *J* = 3.1) |
| | | | H–C(2) | 75.9 | 3.76 (*d*, *J* = 3.1) |
| | | | C(3) | 78.7 | |
| | | | $CH_2$(4) | 73.4 | 3.57 (*d*, *J* = 9.3), 3.88 (*d*, *J* = 9.3) |
| | | | $CH_2$(5) | 63.3 | 3.35 (*d*, *J* = 7.2), 3.35 (*d*, *J* = 7.2) |

The authors thank Dr. *Zhong Yuan* of the Shenyang Pharmaceutical University for the identification of *Morus alba* L. (Moraceae) and the *National Natural Science Foundation of China* for financial support (No. 30371749).

## Experimental Part

*General.* All the anal. reagents were of anal. grade and purchased from *Shenyang Chemical Company* (Shenyang, China). MeOH was HPLC grade, and $H_2O$ was doubly distilled. Column chromatography (CC): polyamide (*Shanghai Wanteng Chemical Industrial Reagent Co. Ltd.*), *Sephadex LH-20* (*Pharmacia*), and *ODS* (40–75 μm; *Fuji Silysia Chemical Ltd.*, Japan) stationary phases. Prep. HPLC: *Waters 600* liquid chromatograph apparatus equipped with a *Waters-490* UV detector; *ODS* column (*C18*, 250 × 20 mm, 5 μm, *Inertsil*; *GL Sciences*); flow rate 10 ml/min; $t_R$ in min. Optical rotations: *Perkin-Elmer 241* polarimeter. UV Spectra: *Shimadzu UV-2201* spectrophotometer; $\lambda_{max}$ (log ε) in nm. NMR Spectra: *Bruker ARX-600* spectrometer; at 600 ($^{1}$H) and 150 MHz ($^{13}$C) in ($D_6$)DMSO with $SiMe_4$ as internal standard; chemical shifts δ in ppm and coupling constants *J* in Hz. HR-ESI-MS: *Bruker Apex-II* mass spectrometer; in *m/z*. ESI-MS: *Bruker Esquire-2000* spectrometer; in *m/z*.

*Plant Material.* The root bark of *Morus alba* L. was collected from Shanxi Province of China in June, 2003, and was identified by Dr. *Yuan Zhong* of the Shenyang Pharmaceutical University. A voucher specimen (No. 20030620) was deposited with the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, China.

*Extraction and Isolation.* The shade-dried powdered root bark (2 kg) was extracted three times with hot $H_2O$ (90°, 2 h, 10 l each) to give the $H_2O$ extract. The extract was concentrated: 320 g of residue. The residue was first subjected to CC (polyamide column (10 × 120 cm, 80–100 mesh), $H_2O$/EtOH 1 : 19, 1 : 9,

1:4, and 3:7): *Fractions I–IV*. *Fr. I* was recrystallized from MeOH: **6** (14.9 g). *Fr. II* was further subjected to CC (*Sephadex LH*-20 (150 g), MeOH/$H_2O$ 1:1, followed by prep. HPLC (MeOH/$H_2O$ 15:85): **1** (7.4 mg; $t_R$ 15.2), **2** (10.7 mg; $t_R$ 19.3), **3** (5.7 mg; $t_R$ 22.6), and *cis*-mulberroside A (370.3 mg; $t_R$ 33.1). *Fr. III* was subjected to CC (*Sephadex LH-20*, MeOH/$H_2O$ 1:1), and then to prep. HPLC (MeOH/$H_2O$ 25:75): **5** (65.4 mg; $t_R$ 12.8), resveratrol 3,4′-di-(β-D-glucopyranoside) (35.9 mg; $t_R$ 16.0), moracin M 3′,6-di(β-D-glucopyranoside) (37.6 mg; $t_R$ 19.4), and **9** (4.8 mg; $t_R$ 23.9). *Fr. IV* was subjected to MPLC (*ODS*, MeOH/$H_2O$ 30:70) and then further separated by prep. HPLC (MeOH/$H_2O$ 35:65): **4** (6.2 mg; $t_R$ 21.5), 2′-hydroxyresveratrol 3-(β-D-glucopyranoside) (85.3 mg; $t_R$ 24.4), **7** (85.7 mg; $t_R$ 27.7), **8** (13.7 mg; $t_R$ 30.7), scopoletin (14.1 mg; $t_R$ 33.5), and 6,7-dihydroxycoumarin 7-(β-D-glucopyranoside) (8.5 mg; $t_R$ 36.3).

*4′-O-β-D-Glucopyranosyl-2′-hydroxyresveratrol 3-(6-O-β-D-Apiofuranosyl-β-D-glucopyranoside) (=3-{(1E)-2-[4-(β-D-Glucopyranosyloxy)-2-hydroxyphenyl]ethenyl}-5-hydroxyphenyl 6-O-D-Apio-β-D-furanosyl-β-D-glucopyranoside*; **1**): White amorphous powder. $[\alpha]_D^{20} = -31.8$ ($c = 0.085$, DMSO). UV (MeOH): 214 (4.49), 290 (4.28), 301 (4.30), 324 (4.41). $^1$H- and $^{13}$C-NMR: *Table 1*. ESI-MS: 723 ([$M$ + Na]$^+$), 699 ([$M$ − H]$^-$), 537 ([$M$ − 162 − H]$^-$), 405 ([$M$ − 162 − 132 − H]$^-$), 243 ([$M$ − 162 − 132 − 162 − H]$^-$). HR-ESI-MS: 723.2089 ([$M$ + Na]$^+$, $C_{31}H_{40}NaO_{18}^+$; calc. 723.2112).

*4′-O-β-D-Glucopyranosyl-2′-hydroxyresveratrol 3-(6-O-β-D-Glucopyranosyl-β-D-glucopyranoside) (=3-{(1E)-2-[4-(β-D-Glucopyranosyloxy)-2-hydroxyphenyl]ethenyl}-5-hydroxyphenyl 6-O-β-D-Glucopyranosyl-β-D-glucopyranoside*; **2**): White amorphous powder. $[\alpha]_D^{20} = -73.9$ ($c = 0.033$, MeOH). UV (MeOH): 214 (4.51), 290 (4.30), 301 (4.32), 324 (4.42). $^1$H- and $^{13}$C-NMR: *Table 1*. ESI-MS: 753 ([$M$ + Na]$^+$), 729 ([$M$ − H]$^-$), 567 ([$M$ − 162 − H]$^-$), 405 ([$M$ − (162 × 2) − H]$^-$, 243 ([$M$ − (162 × 3) − H]$^-$). HR-ESI-MS: 753.2277 ([$M$ + Na]$^+$, $C_{20}H_{28}NaO_5^+$; calc. 753.2218).

*3-O-β-D-Glucopyranosyl-2′-hydroxyresveratrol 4′-(6-O-β-D-Glucopyranosyl-β-D-glucopyranoside) (=4-{(1E)-2-[3-(β-D-Glucopyranosyloxy)-5-hydroxyphenyl]ethenyl}-3-hydroxyphenyl 6-O-β-D-Glucopyranosyl-β-D-glucopyranoside*; **3**): White amorphous powder. $[\alpha]_D^{20} = -78.8$ ($c = 0.088$, MeOH). UV (MeOH): 214 (4.51), 290 (4.30), 301 (4.32), 324 (4.42). $^1$H- and $^{13}$C-NMR: *Table 1*. ESI-MS: 753 ([$M$ + Na]$^+$), 729 ([$M$ − H]$^-$), 567 ([$M$ − 162 − H]$^-$), 405 ([$M$ − (162 × 2) − H]$^-$), 243 ([$M$ − (162 × 3) − H]$^-$). HR-ESI-MS: 753.2165 ([$M$ + Na]$^+$, $C_{32}H_{42}NaO_{19}^+$; calc. 753.2218).

*Moracin M 6-(β-D-Glucopyranoside) (=2-(3,5-Dihydroxyphenyl)benzofuran-6-yl β-D-Glucopyranoside*; **4**): Brown amorphous powder. $[\alpha]_D^{20} = -23.3$ ($c = 00.1$, MeOH). UV (MeOH): 207 (4.45), 310 (4.22). $^1$H- and $^{13}$C-NMR: *Table 2*. ESI-MS: 427 ([$M$ + Na]$^+$), 403 ([$M$ − H]$^-$), 241 ([$M$ − 162 − H]$^-$). HR-ESI-MS: 427.1038 ([$M$ + Na]$^+$, $C_{20}H_{20}NaO_9^+$; calc. 427.1005).

*5,7-Dihydroxycoumarin 7-(6-O-β-D-Apiofuranosyl-β-D-glucopyranoside) (=7-[(6-O-D-Apio-β-D-furanosyl-β-D-glucopyranosyl)oxy]-5-hydroxy-2H-1-benzopyran-2-one*; **5**): Yellow amorphous powder. $[\alpha]_D^{20} = -56.0$ ($c = 0.1$, MeOH). UV (MeOH): 204 (4.68), 254 (3.97), 318 (4.25). $^1$H- and $^{13}$C-NMR: *Table 3*. ESI-MS: 495 ([$M$ + Na]$^+$), 471 ([$M$ − H]$^-$), 177 ([$M$ − 294 − H]$^-$). HR-ESI-MS: 495.1123 ([$M$ + Na]$^+$, $C_{20}H_{24}NaO_{13}^+$; calc. 495.1115).

*Acid Hydrolysis of* **1**–**5**. Each compound (1.0 mg) in 15% aq. HCl soln. (1.5 ml) was heated in a sealed ampoule at 110° for 2 h. The aglycone was extracted 3 × with AcOEt (analyzed by ESI-MS), and the aq. phase was concentrated. Then, pyridine (1 ml) and $NH_2OH \cdot HCl$ (2 mg) were added to the residue, and the mixture was heated at 100° for 1 h. After cooling, $Ac_2O$ (0.5 ml) was added, and the mixture was heated at 100° for 1 h. The mixture was concentrated, and the resulting aldononitrile peracetates were analyzed by GC/MS with standard aldononitrile peracetates as reference samples.

*Enzymatic Hydrolysis of* **1**–**5**. Each compound (0.5 mg) was dissolved in acetate buffer pH 5.0 (2 ml) and incubated for 48 h at 37° with 3.0 mg of β-D-glucosidase (emulsin (EC 3.2.1.21, *Sigma*)). Then, the soln. was extracted by the same volume of AcOEt. The aglycone was analyzed by ESI-MS.

## REFERENCES


[1] The 8th Committee of China Pharmacopeia, 'China Pharmacopeia, Section I', 2005, p. 209.
[2] F. Qiu, K. Kenichi, K. Kawasaki, K. Saito, X. S. Yao, Y. Kano, *Planta Med.* **1996**, *62*, 559.
[3] J. Du, Z. D. He, R. W. Jiang, W. C. Ye, H. X. Xu, P. P. But, *Phytochemistry* **2003**, *62*, 1235.

[4] A. N. Singab, H. A. El-Beshbishy, M. Yonekawa, T. Nomura, T. Fukai, *J. Ethnopharmacol.* **2005**, *100*, 333.
[5] S. H. Lee, S. Y. Choi, H. Kim, J. S. Hwang, B. G. Lee, J. J. Gao, S. Y. Kim, *Biol. Pharm. Bull.* **2002**, *25*, 1045.
[6] K. Doi, T. Kojima, M. Makino, Y. Kimura, Y. Fujimoto, *Chem. Pharm. Bull.* **2001**, *49*, 151.
[7] F. Qiu, K. Komatsu, K. Saito, K. Kawasaki, X. S. Yao, Y. Kano, *Nat. Med.* **1996**, *50*, 103.
[8] F. Qiu, K.-I. Komatsu, K.-I. Saito, K. Kawasaki, X. S. Yao, Y. Kano, *Biol. Pharm. Bull.* **1996**, *19*, 1463.
[9] Q. M. Kan, N. Kang, H. T. Tian, C. L. Li, F. Qiu, Y. L. Liu, *Shenyang Yaoke Daxue Xuebao* **2006**, *23*, 388.
[10] K. Hirakura, Y. Fujimoto, T. Fukai, *J. Nat. Prod.* **1986**, *49*, 218.
[11] T. Kanchanapoom, K. Suga, R. Kasai, K. Yamasaki, M. S. Kamel, M. H. Mohamed, *Chem. Pharm. Bull.* **2002**, *50*, 863.
[12] S. D. Sarker, P. G. Waterman, *J. Nat. Prod.* **1995**, *58*, 1109.
[13] S. Sibanda, B. Ndengu, G. Multari, V. Pompi, C. Galeffi, *Phytochemistry* **1989**, *28*, 1550.
[14] H. Kuwajima, M. Morita, K. Takaishi, K. Inoue, T. Fujita, Z. D. He, C. R. Yang, *Phytochemistry* **1992**, *31*, 1277.
[15] L. Qiu, F. Zhao, H. W. Liu, L. X. Chen, Z. H. Jiang, H. X. Liu, N. L. Wang, X. S. Yao, F. Qiu, *Chem. Biodiversity* **2008**, *5*, 758.
[16] T. Ishii, M. Yanagisawa, *Carbohydr. Res.* **1998**, *313*, 189.

*Received July 15, 2008*