# Water-Soluble Glycosides from *Ruta graveolens*

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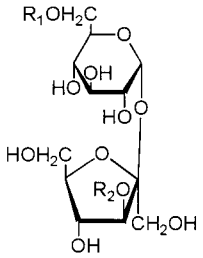
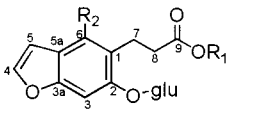
An EtOH extract of the dried aerial parts of *Ruta graveolens* was suspended in water and then partitioned with EtOAc. Three new glycosides, 3′-sinapoyl-6-feruloylsucrose (**4**), methylcnidioside A (**5**), and methylpicraquassioside A (**6**), together with four known glycosides, 3′,6-disinapoylsucrose (**1**), cnidioside A (**2**), rutin, and picraquassioside A (**3**), were isolated from the water-soluble part. Their structures were elucidated by interpretation of IR, MS, and 1D and 2D NMR spectra and comparison with literature data.

*Ruta graveolens* L. (Rutaceae) has been cultivated in many regions of the world because of its medicinal properties. The plant is reputedly used as an abortifacient or emmenagogue in many countries.[1] In Taiwan, the fresh aerial part of this plant is widely used to treat palpitation of the heart and circulatory disorders. Quinoline alkaloids,[2,3] coumarins,[4,5] lignans,[6] and flavonoids[1,7,8] have been isolated from *R. graveolens* and exhibit a variety of pharmacological activities. The present paper reports the isolation and structure elucidation of three new glycosides from the water-soluble part of *R. graveolens*.

An EtOH extract of the dried aerial parts of *R. graveolens* was suspended in $H_2O$ and then partitioned with EtOAc. The $H_2O$-soluble part, successively chromatographed using Diaion HP-20, silica gel, Sephadex LH-20, and HPLC, afforded four known glycosides, 3′,6-disinapoylsucrose (**1**),[9] cnidioside A (**2**),[10] rutin, and picraquassioside A (**3**),[11] and three new glycosides, 3′-sinapoyl-6-feruloylsucrose (**4**), methylcnidioside A (**5**), and methylpicraquassioside A (**6**).



**1** $R_1 = R_2$ = sinapoyl
**4** $R_1$ = feruloyl, $R_2$ = sinapoyl



**2** $R_1 = R_2 = H$
**3** $R_1 = H$, $R_2 = OCH_3$
**5** $R_1 = OCH_3$, $R_2 = H$
**6** $R_1 = OCH_3$, $R_2 = OCH_3$

Compound **4** was obtained as yellow powder. Its molecular formula ($C_{33}H_{40}O_{18}$) was deduced from ESIMS and $^{13}C$ NMR. The IR spectrum showed absorption bands due to hydroxyl (3386 $cm^{-1}$) and carbonyl (1701 $cm^{-1}$) groups. The $^1H$ NMR spectrum showed signals for two 1,2,3,5-tetrasubstituted aromatic protons [$\delta$ 7.04 (2H, s)] and three ABX-type aromatic protons [$\delta$ 6.78 (1H, d, $J$ = 8.5 Hz), 7.02 (1H, dd, $J$ = 2.0 and 8.5 Hz), and 7.17 (1H, d, $J$ = 2.0 Hz)]. The $^1H$ NMR also showed two pairs of *trans* double bond protons at $\delta$ 7.59 (1H, d, $J$ = 16.0 Hz) and 6.44 (1H, d, $J$ = 16.0 Hz); $\delta$ 7.67 (1H, d, $J$ = 16.0 Hz) and 6.43 (1H, d, $J$ = 16.0 Hz), and three methoxyls at $\delta$ 3.87 (6H, 2 × $OCH_3$) and 3.88 (3H, $OCH_3$), indicating *trans*-sinapoyl and *trans*-feruloyl moieties. $^{13}C$ NMR yielded $\delta$ 107.1 (d), 115.4 (d), 126.6 (s), 139.4 (s), 147.9 (d), 149.4 (s), 168.2 (s), and 56.9 (2 × $OCH_3$), indicating the *trans*-sinapoyl group, and $\delta$ 111.6 (d), 115.5 (d), 116.4 (d), 124.3 (d), 127.7 (s), 147.1 (d), 149.4 (s), 150.6 (s), 169.2 (s), and 56.5 ($OCH_3$) for the *trans*-feruloyl group, and 12 other carbons indicating sucrose.[12] Sucrose was confirmed using $^1H$ NMR by the characteristic doublet signal with a small coupling constant at $\delta$ 5.50 (1H, $J$ = 3.0 Hz) assignable to the anomeric proton in the α-glucopyranose unit. The linkage of *trans*-sinapoyl and *trans*-feruloyl moieties with sucrose was confirmed by the HMBC correlations (Figure 1). On the basis of the above data, compound **4** is 3′-sinapoyl-6-feruloylsucrose.

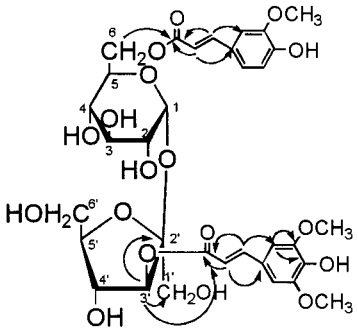


**Figure 1.** HMBC correlations of **4**.

Compound **5** was isolated as colorless powder. Its molecular formula ($C_{18}H_{22}O_9$) was deduced from HRFABMS and $^1H$ and $^{13}C$ NMR data. The IR spectrum showed absorption bands due to hydroxyl (3378 $cm^{-1}$) and carbonyl (1720 $cm^{-1}$) groups. The $^1H$ and $^{13}C$ NMR spectra of **5** resembled those of cnidioside A (**2**)[10] except for an additional methoxyl. $^1H$ NMR and $^1H-^1H$ COSY spectra showed signals due to an anomeric proton at $\delta$ 4.96 and four aromatic protons at $\delta$ 7.36, 7.37, 6.72, and 7.64, indicating that **5** was a 1,2-disubstituted benzofuran glycoside. This was also supported by the $^{13}C$ NMR spectrum. Acid hydrolysis of **5** yielded glucose, which was identical with an authentic sample using the HPAEC system.[13] $^1H$ and $^{13}C$ NMR data also revealed two connected methylenes, one methoxyl, and one carbonyl, indicating that **5** possessed a methyl propionate moiety. An HMBC experiment and observation of NOEs between H-5 and H-6, between $H_2$-7 and H-6, and between H-3 and H-1′ placed the substituent groups on the benzofuran ring. Thus, this compound was assigned structure **5** and accorded the trivial name methylcnidioside A.

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**Table 1.** $^1$H NMR Data of Compounds **1** and **4** in $CD_3OD$

| H | **1**[a] | **4**[a] | H | **1**[a] | **4**[a] |
|---|---|---|---|---|---|
| | sugar moiety | | | sugar moiety | |
| Glc-1 | 5.51 (d, 3.0) | 5.50 (d, 3.0) | Fru-1 | 3.59 (d, 12.5) | 3.58 (d, 12.0) |
| 2 | 3.49 (dd, 4.0, 9.5) | 3.47 (dd, 4.0, 9.5) | | 3.63 (d, 12.5) | 3.63 (d, 12.0) |
| 3 | 3.68 (t, 9.5) | 3.67 (t, 9.5) | 3 | 5.51 (d, 8.0) | 5.49 (d, 8.0) |
| 4 | 3.33 overlapped | 3.33 overlapped | 4 | 4.50 (t, 8.0) | 4.48 (t, 8.0) |
| 5 | 4.27 (br t, 9.5) | 4.26 (br t, 9.5) | 5 | 3.99 (ddd, 2.5, 7.0, 7.5) | 3.98 (ddd, 2.5, 7.0, 7.5) |
| 6 | 4.22 (dd, 7.5, 11.0) | 4.23 (dd, 7.0, 11.0) | 6 | 3.84 overlapped | 3.83 (dd, 3.5, 12.0) |
| | 4.67 (br d, 11.0) | 4.64 (br d, 11.0) | | 3.91 (dd, 7.0, 12.0) | 3.90 (dd, 7.0, 12.0) |
| | acid moiety (at C-6 of Glc) | | | acid moiety (at C-3 of Fru) | |
| 2 | 6.86 (s) | 7.17 (d, 2.0) | 2 | 6.91 (s) | 6.92 (s) |
| 5 | | 6.78 (d, 8.5) | 5 | | |
| 6 | 6.86 (s) | 7.02 (dd, 2.0, 8.5) | 6 | 6.91 (s) | 6.92 (s) |
| 7 | 7.58 (d, 16.0) | 7.59 (d, 16.0) | 7 | 7.66 (d, 16.0) | 7.67 (d, 16,0) |
| 8 | 6.46 (d, 16.0) | 6.44 (d, 16.0) | 8 | 6.44 (d, 16.0) | 6.43 (d, 16.0) |
| OMe | 3.83 (s) | 3.88 (s) | OMe | 3.86 (s) | 3.87 (s) |

Compound **6** had the molecular formula $C_{19}H_{24}O_{10}$ as deduced by HRFABMS, $^1$H and $^{13}$C NMR, 30 amu more than **5**. The additional mass corresponded to a methoxyl group, as was confirmed by $^1$H NMR. $^{13}$C NMR data of **6** were very similar to those of **5**, except for the presence of an extra methoxyl group (C-6) and disappearance of H-6. NOEs between the methoxyl group ($\delta$ 4.05) and $H_2$-7, H-5 confirmed the methoxyl group at C-6. Thus, **6** was a glucoside of a benzofuran derivative and was named methylpicraquassioside A.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. $^1$H and $^{13}$C NMR spectra were taken on a Varian Unity INOVA 500 spectrometer. UV spectra were measured on a Hitachi U-3200 spectrophotometer. Mass spectra were obtained on Finnigan LCQ and Finnigan MAT 95S spectrometers. Optical rotations were taken with a JASCO DIP-370 digital polarimeter. Analyses of sugars were carried out using a high-performance anion exchange chromatography (HPAEC) system (Dionex BioLC) equipped with a gradient pump, a pulsed amperometric detector (PAD-II), and an anion-exchange column (Carbopac PA-10, 4.6 × 250 mm).

**Plant Material.** The fresh aerial parts of *Ruta graveolens* L. were obtained from a market in Taipei, in June 1998, and were identified by Mr. Jun-Chi Ou, associate investigator of the National Research Institute of Chinese Medicine (NRICM). A voucher specimen (NRICM-98-048) is maintained in the herbarium of NRICM, Taipei, Taiwan.

**Extraction and Isolation.** The dried aerial parts of *R. graveolens* (6.5 kg) were extracted with EtOH (50 L × 3). The extract was concentrated in vacuo to yield a dark brown mass, which was suspended in $H_2O$ (10 L) and then partitioned with EtOAc (10 L). The $H_2O$-soluble portion was subjected to Diaion HP-20 chromatography, successively eluting with $H_2O$/MeOH at 100:0, 80:20, 50:50, and 0:100. The fraction eluted with 50% MeOH was separated over Sephadex LH-20 ($H_2O$/MeOH = 1:1) to afford six fractions. Fraction 1 was repeatedly chromatographed over Sephadex LH-20 ($H_2O$/MeOH = 2:1) and silica gel (EtOAc/MeOH/$H_2O$ = 20:5:5) columns to give rutin (1.5 g). Fraction 3 was repeatedly chromatographed over silica gel (EtOAc/MeOH = 10:1) and Sephadex LH-20 (MeOH) columns to give several fractions. Further HPLC purification ($CH_3CN$/$H_2O$ = 20:80, 2.5 mL/min; UV detector, 254 nm) afforded **1** (20.3 mg, $t_R$ 11.8−15.7 min), **2** (8.5 mg, $t_R$ 15.7−19.0 min), rutin ($t_R$ 19−22.6 min), **3** (4.2 mg, $t_R$ 22.6−26.2 min), **4** (13.3 mg, $t_R$ 26.2−29.0 min), **5** (19.7 mg, $t_R$ 36.1−38.3 min), and **6** (17.9 mg, $t_R$ 42.9−45.7 min).

**3′,6-Disinapoylsucrose (1):** yellow prisms, mp 133−135 °C (MeOH); $^1$H NMR ($CD_3OD$, 500 MHz) see Table 1; $^{13}$C NMR ($CD_3OD$, 125 MHz) see Table 2; ESIMS (positive-ion model) *m*/*z* 777 ($MNa^+$).

**Table 2.** $^{13}$C NMR Data ($\delta$) of Compounds **1** and **4** in $CD_3OD$

| C | **1**[a] | **4**[a] | C | **1**[a] | **4**[a] |
|---|---|---|---|---|---|
| | sugar moiety | | | sugar moiety | |
| Glc-1 | 92.6 | 92.7 | Fru-1 | 65.7 | 65.7 |
| 2 | 73.1 | 73.1 | 2 | 104.8 | 104.9 |
| 3 | 75.1 | 75.1 | 3 | 79.3 | 79.4 |
| 4 | 71.9 | 71.9 | 4 | 74.1 | 74.2 |
| 5 | 72.5 | 72.5 | 5 | 84.3 | 84.4 |
| 6 | 65.6 | 65.5 | 6 | 63.8 | 63.8 |
| | acid moiety (at C-6 of Glc) | | | acid moiety (at C-3 of Fru) | |
| 1 | 126.5 | 127.7 | 1 | 126.6 | 126.6 |
| 2 | 106.9 | 111.6 | 2 | 107.0 | 107.1 |
| 3 | 149.3 | 149.4 | 3 | 149.4 | 149.4 |
| 4 | 139.4 | 150.6 | 4 | 139.6 | 139.4 |
| 5 | 149.3 | 116.4 | 5 | 149.4 | 149.4 |
| 6 | 106.9 | 124.3 | 6 | 107.0 | 107.1 |
| 7 | 147.3 | 147.1 | 7 | 147.9 | 147.9 |
| 8 | 115.8 | 115.5 | 8 | 115.4 | 115.4 |
| 9 | 169.1 | 169.2 | 9 | 168.3 | 168.2 |
| OMe | 56.9 | 56.5 | OMe | 56.8 | 56.9 |

[a] Assigned with the aid of HMQC and HMBC spectra.

**Table 3.** $^{13}$C NMR Data ($\delta$) of Compounds **2**, **3**, **5**, and **6**

| C | **2**[a] | **3**[a] | **5**[b] | **6**[b] |
|---|---|---|---|---|
| 1 | 126.6 | 119.0 | 127.2 | 116.6 |
| 2 | 153.5 | 155.9 | 155.0 | 155.6 |
| 3 | 98.4 | 95.2 | 99.9 | 94.7 |
| 3a | 153.5 | 155.9 | 155.9 | 157.0 |
| 4 | 145.0 | 145.2 | 146.0 | 144.7 |
| 5 | 106.3 | 106.3 | 107.4 | 105.6 |
| 5a | 120.8 | 113.8 | 123.3 | 114.0 |
| 6 | 120.6 | 151.8 | 122.2 | 152.3 |
| 7 | 26.3 | 21.9 | 27.4 | 20.5 |
| 8 | 35.7 | 39.2 | 35.6 | 35.2 |
| 9 | 176.0 | 178.5 | 176.0 | 176.4 |
| 1′ | 101.7 | 104.1 | 103.2 | 103.2 |
| 2′ | 73.4 | 74.8 | 75.0 | 75.0 |
| 3′ | 77.1 | 78.6 | 78.3 | 78.2 |
| 4′ | 69.9 | 71.5 | 71.4 | 71.4 |
| 5′ | 76.6 | 78.0 | 78.2 | 78.2 |
| 6′ | 60.8 | 62.4 | 62.6 | 62.6 |
| $OCH_3$ | | 61.1 | 52.0 | 52.1 |
| | | | | 62.6 |

[a] In DMSO-$d_6$. [b] In $CD_3OD$.

**Cnidioside A** (**2**): amorphous powder, spectroscopic data agreed with those reported;[10] $^{13}$C NMR (DMSO-$d_6$, 125 MHz), Table 3; ESIMS *m*/*z* 367 $[M - H]^-$, 205 $[M - Glu\text{-}H]^-$.

**Picraquassioside A** (**3**): amorphous powder, spectroscopic data agreed with those reported;[11] $^{13}$C NMR (DMSO-$d_6$, 125 MHz), Table 3; ESIMS *m*/*z* 397 $[M - H]^-$, 235 $[M - Glu\text{-}H]^-$.

**3′-Sinapoyl-6-feruloylsucrose** (**4**)**:** amorphous powder; $[\alpha]_D^{25}$ −69.11° (*c* 0.34, MeOH); IR $\nu_{max}$ (in MeOH) 3386, 1701,

1630, 1597, 1517, 1282, 1055 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 328 (4.43), 238 (4.32), 202 (4.53) nm; $^1H$ NMR ($CD_3OD$, 500 MHz), Table 1; $^{13}C$ NMR ($CD_3OD$, 125 MHz), Table 2; HMBC correlations, Figure 1; ESIMS *m/z* 724 $[M]^+$.

**Methylcnidioside A (5):** glassy powder, mp 146−147 °C; $[\alpha]_D^{25}$ −46.87° (*c* 0.32, MeOH); IR $\nu_{max}$ (KBr) 3540, 3378, 1720, 1629, 1070 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 278 (3.81), 251 (4.12), 244 (4.13), 206 (4.59) nm; $^1H$ NMR ($CD_3OD$, 500 MHz) $\delta$ 2.70 (2H, m, $H_2$-8), 3.07 (2H, m, $H_2$-7), 3.44 (1H, m, H-4′), 3.53 (3H, m, H-2′, H-3′, H-5′), 3.65 (3H, s, 9-$OCH_3$), 3.74 (1H, dd, $J$ = 5.5 and 12.0 Hz, H-6′), 3.94 (1H, dd, $J$ = 2.0 and12.0 Hz, H-6′), 4.96 (1H, d, $J$ = 7.0 Hz, H-1′), 6.72 (1H, d, $J$ = 2.2 Hz, H-5), 7.36 (1H, s, H-3 or H-6), 7.37 (1H, s, H-6 or H-3), 7.64 (1H, d, $J$ = 2.2 Hz, H-4); $^{13}C$ NMR ($CD_3OD$, 125 MHz), Table 3; HMBC correlations 9-$OCH_3$/C-9; H-7/C-9, C-6, C-2, C-1, C-8; H-8/C-9, C-1, C-7; H-1′/C-2; H-5/C-3a, C-4,C-5a; ESIMS *m/z* 405 ($MNa^+$); FABMS *m/z* 405 ($MNa^+$), 383 ($MH^+$); HRFABMS *m/z* 383.1342 ($MH^+$, calcd for $C_{18}H_{23}O_9$).

**Methylpicraquassioside A (6):** glassy powder, mp 67−69 °C; $[\alpha]_D^{25}$ −58.82° (*c* 0.34, MeOH); IR $\nu_{max}$ (KBr) 3650, 3384, 1701, 1623, 1074 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 278 (3.37), 251 (4.08), 214 (4.59) nm; $^1H$ NMR ($CD_3OD$, 500 MHz) $\delta$ 2.51−2.58 (2H, m, $H_2$-8), 3.04 (2H, m, $H_2$-7), 3.40 (1H, m, H-4′), 3.50 (4H, m, H-2′, H-3′, H-5′), 3.65 (3H, s, 9-$OCH_3$), 3.71 (1H, dd, $J$ = 5.5 and 12.5 Hz, H-6′), 3.90 (1H, dd, $J$ = 2.0 and 12.5 Hz, H-6′), 4.05 (3H, s, 6-$OCH_3$), 4.91 (1H, d, $J$ = 7.5 Hz, H-1′), 6.95 (1H, d, $J$ = 2.5 Hz, H-5), 7.10 (1H, s, H-3), 7.67 (1H, d, $J$ = 2.5 Hz, H-4); $^{13}C$ NMR ($CD_3OD$, 125 MHz), Table 3; HMBC correlations 9-$OCH_3$/C-9; H-7/C-9, C-6, C-2, C-1, C-8; H-8/C-9, C-1, C-7; 6-$OCH_3$/C-6; H-1′/C-2; H-3/C-2, C-3a, C-1, C-5a; H-5/C-3a, C-4,C-5a; ESIMS *m/z* 413 ($MH^+$), 395, 358, 316, 275, 251; FABMS *m/z* 413 ($MH^+$), 391; HRFABMS *m/z* 413.1448 ($MH^+$, calcd for $C_{19}H_{25}O_{10}$).

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