New Laxative Constituents of Rhubarb. Isolation and Characterization of Rheinosides A, B, C and D¹⁾

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Four new laxative constituents, rheinosides A, B, C and D, were isolated from commercial rhubarb (*Rhei Rhizoma*). On the basis of high-resolution nuclear magnetic resonance spectral data and chemical correlation with rhein, the structures were characterized as stereoisomers of 8-*O*- β -D-glucosyl-10-hydroxy-10-*C*- β -D-glucosyl rhein-9-anthrone (rheinosides A and B), and 8-*O*- β -D-glucosyl-10-*C*- β -D-glucosyl rhein-9-anthrone (rheinosides C and D).

Keywords—rhubarb; *Rhei Rhizoma*; Polygonaceae; rheinoid; C-glucosyl anthrone; rhein anthrone; laxative activity

Rhubarb (*Rhei Rhizoma*) has been used as an important traditional crude drug in China and Japan, and as a laxative medicine in European countries. Many investigators have studied the constituents of rhubarb, and it has been shown to contain anthraquinones,³⁾ dianthrones (sennosides),⁴⁾ naphthalenes,⁵⁾ stilbenes,^{6,7)} phenylbutanones,⁷⁻⁹⁾ chromones,¹⁰⁾ *etc.* Recently, we have reported the isolation and characterization of highly water-soluble polyphenolic compounds (rhatannin)⁸⁾ which decreased urea nitrogen concentration in rat serum, and other tannin-related compounds.^{8,9,11,12)} Chemical and pharmacological studies of rhubarb have revealed that sennosides A—F are active principles.⁴⁾ Our systematic investigation on the water-soluble fraction of rhubarb, however, has resulted in the isolation of four new laxative C-glucosides. In this paper, we report the characterization and laxative activity of these new principles.

A commercial rhubarb (Gaoh, produced in Sichuan province in China) obtained in Osaka market was extracted with 50% aqueous acetone, and the extract was partitioned between ethyl acetate and water. The water-soluble portion containing a mixture of

Fig. 1. High-Performance Liquid Chromatogram of Rheinosides

Conditions: column, Nucleosil 5C₁₈ (4 i.d. $\times$ 250 mm); solvent, CH₃CN:H₂O:HCOOH = 12:88:1; flow rate, 1.2 ml/min; detection, 350 nm.

1, rheinoside A; 2, rheinoside B; 3, rheinoside C; 4, rheinoside D.

polyphenolics, sennosides and *C*-glucosides, was applied to a Sephadex LH-20 column. The column was eluted with water, and then 50% aqueous acetone. The polyphenolic compounds were efficiently adsorbed on the Sephadex column, and a mixture of *C*-glycosides was obtained from the water eluate. High-performance liquid chromatographic (HPLC) analysis of the mixture of *C*-glycosides showed four major peaks in the chromatogram (Fig. 1). These *C*-glycosides, named rheinosides A (**1**), B (**2**), C (**3**) and D (**4**), were isolated by repeated preparative liquid chromatography on a reversed-phase column using two solvent systems (acetonitrile–water–acetic acid and methanol–water–acetic acid), as pale yellow amorphous powders.

All of these compounds showed blue colorations with the FeCl_3 reagent and were soluble in dilute aqueous NaOH, giving yellow solutions which showed intense yellow fluorescence under ultraviolet (UV) light (360 nm). The infrared (IR) spectra of these compounds showed absorptions at 1640 cm^{-1} (ketone) and 1700 cm^{-1} (carboxylic acid), and the UV spectra showed absorption maxima around 268, 295 and 330 nm which closely resemble those of barbaloin (aloe-emodin anthrone 10-*C*- β -D-glucoside).¹³⁾

The field desorption mass spectra (FD-MS) of **1** and **2** showed the same $(\text{M} + \text{Na})^+$ ion peak at m/z 633, and the elemental analyses established the molecular formulae to be

TABLE I. ^{13}C -NMR Data

	1	2	3	4
Anthrone moiety				
C-1	160.8 s	161.4 s	160.9 s	161.3 s
C-2	120.4 d	120.4 d	119.1 d	116.6 d
C-3	137.9 s	138.6 s	137.8 s	137.9 s
C-4	119.7 d	120.5 d	121.6 d	122.9 d
C-4a	147.6 s	149.7 s	145.9 s	147.8 s
C-5	123.3 d	123.0 d	126.2 d	125.8 d
C-6	138.0 d	138.4 d	137.8 d	138.4 d
C-7	118.4 d	120.2 d	117.4 d	119.6 d
C-8	159.3 s	159.0 s	159.5 s	159.8 s
C-8a	124.5 s ^{a)}	123.5 s ^{a)}	125.2 s	125.4 s ^{a)}
C-9	191.6 s	192.0 s	192.7 s	193.0 s
C-9a	123.6 s ^{a)}	123.5 s ^{a)}	125.2 s	125.1 s ^{a)}
C-10	77.6 s	77.7 s	47.0 d	46.7 d
C-10a	147.8 s	146.0 s	144.0 s	141.9 s
COOH	171.5 s	175.7 s	171.4 s	171.9 s
<i>C</i> -Glucose moiety				
C-1'	84.7 d	84.6 d	85.6 d	85.4 d
C-2'	75.6 d	74.0 d	73.1 d	72.9 d
C-3'	80.0 d	79.0 d	80.4 d	80.4 d
C-4'	71.8 d	71.8 d ^{b)}	72.0 d	72.2 d ^{b)}
C-5'	82.0 d	82.1 d	82.0 d	82.1 d
C-6'	63.7 t	63.7 t	63.5 t	63.7 t
<i>O</i> -Glucose moiety				
C-1''	103.3 d	104.5 d	103.1 d	105.0 d
C-2''	75.6 d	75.7 d	75.6 d	75.7 d
C-3''	78.3 d	77.7 d	78.4 d	77.9 d
C-4''	74.1 d	72.0 d ^{b)}	72.0 d	72.1 d ^{b)}
C-5''	78.8 d	79.0 d	78.9 d	79.0 d
C-6''	63.2 t	63.3 t	63.2 t	63.4 t

a, b) Assignments in each column may be interchanged. Multiplicities were determined by the INEPT method. Assignments in rheinoside A (**1**) were made by ^1H - ^{13}C shift correlation spectroscopy (^1H - ^{13}C COSY).

$C_{27}H_{30}O_{16}$. The carbon-13 nuclear magnetic resonance (^{13}C -NMR) spectra of **1** and **2** showed the presence of two aromatic rings, a carbonyl carbon, a carboxyl carbon, and a quaternary carbon bearing an oxygen function, together with two sugar moieties (see Table I). The proton nuclear magnetic resonance (1H -NMR) spectra showed the presence of 14 protons assignable to sugar moieties and 5 aromatic protons.

On hydrolysis with 0.5 N H_2SO_4 , **1** afforded a sugar and a pale yellow compound (**1a**), mp $230^\circ C$, $[\alpha]_D^{25} + 34^\circ$ ($c=0.50$, methanol). EI-MS m/z : 448 (M^+). The sugar was identified as D-glucose by HPLC on Nucleosil 5NH₂ and from its optical rotation. On the other hand, oxidative degradation of **1** with 2 N H_2SO_4 in the presence of $FeCl_3$ afforded rhein, D-glucose and D-arabinose. Hay and Haynes¹³⁾ have already reported that treatment of barbaloin with $FeCl_3$ in 50% H_2SO_4 gave aloe-emodin and D-arabinose, and that D-arabinose was artificially formed from the C-glucosyl moiety by the retro-Prince reaction.¹⁴⁾ Our results, combined with these findings, suggested that **1** was a compound analogous to barbaloin, having a rhein anthrone skeleton with both O- and C-glucosyl moieties.

All signals in the 1H -NMR spectrum of **1** were assigned by measurement of two-dimensional shift correlation (COSY, Fig. 2) and two-dimensional J -resolved (2D- J) spectra. The anomeric protons of O-glucosyl and C-glucosyl moieties were observed at δ 5.19 ($J=7.9$ Hz) and δ 3.34 ($J=9.8$ Hz), respectively, and there was no signal due to the C-10 proton. In the ^{13}C -NMR spectrum of **1**, a quaternary carbon bearing a hydroxy group was observed at δ 77.6 which was assigned to the C-10 carbon of the rhein anthrone moiety. These

Fig. 2. Two-Dimensional Shift Correlation (COSY) Spectrum of Rheinioside A (Sugar Proton Region)

1, $C_{1''}$ -H; 2, $C_{6''}$ -H; 3, $C_{6''}$ -H'; 4, $C_{2''}$ -H; 5, $C_{5''}$ -H; 6, $C_{4''}$ -H; 7, $C_{3''}$ -H; 8, $C_{6''}$ -H; 9, $C_{6''}$ -H'; 10, $C_{1''}$ -H; 11, $C_{3''}$ -H; 12, $C_{5''}$ -H; 13, $C_{4''}$ -H; 14, $C_{3''}$ -H.

Fig. 3. Nuclear Overhauser Effect (NOE) Differential Spectra of **1a** and **2**

(A) NOE differential spectrum of **1a** irradiated at C_5 -H (7.35 ppm).
(B) NOE differential spectrum of **1a** irradiated at C_4 -H (7.75 ppm).
(C) Normal spectrum of **1a**.
(D) NOE differential spectrum of **2** irradiated at C_4 -H (7.82 ppm).
(E) NOE differential spectrum of **2** irradiated at C_5 -H (7.65 ppm).
(F) Normal spectrum of **2**.

Fig. 4. NOE Differential Spectrum of **1** Irradiated at $C_{1''}$ -H (5.19 ppm)

Fig. 5. CD Spectra of **1** (—), **2** (-----), **3** (- - - -) and **4** (-----)

observations suggested that the *C*-glucosyl linkage was located at the C-10 position of the rhein anthrone moiety. This was further confirmed by nuclear Overhauser effect (NOE) experiments on **1** and **1a**. In the NOE differential spectra of **1a**, irradiation of the C-4 and C-5 protons of the rhein anthrone moiety showed NOE effects at the C-1' and C-2' protons, respectively (Fig. 3). The NOE effect was also observed at the C-7 proton of the rhein anthrone moiety in **1** when the C-1'' proton was irradiated (Fig. 4). Moreover, the fact that the signal (δ 6.95, d, $J=8.4$ Hz) of the C-7 proton in **1a** appeared at 0.39 ppm higher field compared with that of **1** indicated that the *O*-glucosyl group was attached to the C-8 hydroxy group of the rhein anthrone moiety. Therefore, **1** was characterized as 8-*O*- β -D-glucosyl-10-*C*- β -D-glucosyl-10-hydroxy rhein-9-anthrone.

The spectral and chemical properties of rheinoside B (**2**) resembled those of **1** except for the results of NOE experiments and circular dichroism (CD) data. The CD spectrum of **1** showed a negative Cotton effect at 374 nm ($[\theta]=3.60 \times 10^3$) and positive one at 321 nm ($[\theta]=3.96 \times 10^3$), whereas that of **2** showed a positive Cotton effect at 355 nm ($[\theta]=11.0 \times 10^3$) and negative one at 321 nm ($[\theta]=17.2 \times 10^3$) as shown in Fig. 5. In the NOE differential spectra of **2** obtained by irradiation of the C-5 and C-4 protons, the NOE effects were observed at the C-1' and C-2' protons, respectively (Fig. 3), which was opposite to those in **1**. These observations indicated that **1** and **2** were configurational isomers with respect to the C-10 position of the rhein anthrone framework.

Rheinosides C (**3**) and D (**4**) showed the same $(M+Na)^+$ ion peak at m/z 617 in their FD-MS, which was 16 mass units less than that of **1** and **2**. Combined with this data, the molecular

formulae of **3** and **4** were determined to be $C_{27}H_{30}O_{15}$ by elemental analyses. The 1H -NMR spectra of **3** and **4** resembled those of **1** and **2** except for the presence of doublets at δ 4.24 ($J=1.8$ Hz for **3**) and δ 4.12 ($J=0.5$ Hz for **4**). The ^{13}C -NMR spectra of **3** and **4** also resembled those of **1** and **2**, although methine carbon signals were observed at δ 47.0 (**3**) and δ 46.7 (**4**) instead of the quaternary carbons (δ 77.6 for **1** and δ 77.7 for **2**). These results implied the absence of the hydroxy group at C-10 of the rhein anthrone framework.

On oxidative degradation in the presence of $FeCl_3$, **3** gave rhein, D-glucose and D-arabinose. In the NOE differential spectra of **3**, irradiation of the C-1'' proton showed an NOE effect at the C-7 proton of rhein anthrone moiety. Irradiation of the C-1' proton showed NOE effects at the C-4 and C-10 protons, and irradiation of the C-2' proton showed NOE effects at the C-5 and C-10 protons. These results indicated that the O- and C-glucosyl moieties were located at the C-8 and C-10 positions of rhein anthrone, respectively.

The NOE experiment on **4** also showed the presence of the O-glucosyl moiety at the C-8 position. In the NOE differential spectra of **4**, irradiation of C-1' proton showed NOE effects at the C-5 and C-10 protons, and irradiation of the C-2' proton showed NOE effects at the C-4 and C-10 protons. In the CD spectra, **3** and **4** showed almost opposite Cotton effects around 365 and 320 nm (Fig. 5), indicating that **3** and **4** differ only in the configuration at the C-10 position. Therefore, **3** and **4** were characterized as a pair of stereoisomers at the C-10 position of 8-O- β -D-glucosyl-10-C- β -D-glucosyl rhein-9-anthrone.

Chart 1

The laxative effect of rheinoside A was tested in Wistar rats, and ED_{50} was determined to be 15.6 mg/kg. In some commercial rhubarbs, the total rheinosides content exceeded 10 mg/g, being comparable to that of sennosides. Therefore, rheinosides may also play an important role in the laxative activity of rhubarb. Elucidation of the absolute structures of the rheinosides is in progress.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus (model MS-S3) and are uncorrected. UV and IR spectra were obtained with Hitachi model 200-10 and model 260-10 spectrometers, respectively. EI and FD-MS were measured with a Shimadzu 2000-DF spectrometer. 1H - and ^{13}C -NMR spectra were taken with JEOL GX-500 and FX-200 spectrometers using sodium 3-trimethylsilylpropane sulfonic acid (DSS) as an internal standard, and chemical shifts are given in δ (ppm). Optical rotations were determined with a JASCO DIP-4 digital polarimeter and CD spectra with a JASCO CD J-500 spectropolarimeter in methanol solution (1.0 mg/20 ml). HPLC was performed on a Hitachi model 655 liquid chromatograph equipped with a Nucleosil 5C₁₈ (Macherey-Nagel) column (4 mm i.d. $\times$ 250 mm) and a variable-wavelength spectrophotometric detector operated at 280 and 350 nm. The mobile phase was prepared by mixing 1 ml of formic acid with 100 ml of water containing acetonitrile (10–20%). Column chromatographies were carried out on Sephadex LH-20 (20–100 μ , Pharmacia Fine Chemical Co., Ltd.) with a water–acetone system, and Fuji gel ODS-Q3 (30–50 μ , Fuji gel Hanbai Co., Ltd.) with a solvent system similar to that employed for HPLC.

Isolation of Rheinosides—The powdered Rhei Rhizoma (710 g, Gaoh [雅黄] produced in Sichuan province of

China) was extracted twice with 2 l each of 50% aqueous acetone at room temperature. The extracts were concentrated under reduced pressure below 40 °C, and then extracted with 1 l of ethyl acetate. The aqueous layer was concentrated to 500 ml. Acidification with 10 ml of acetic acid afforded dark brown precipitates, which were removed by decantation after standing at room temperature for 12 h. The supernatant was, after concentration, passed through a column of Sephadex LH-20 (24 i.d. × 200 mm) with water and then 50% aqueous acetone. The water eluate was repeatedly chromatographed on Sephadex LH-20 with the same solvent system, yielding a fraction (38.4 g) containing a mixture of rheinosides, which was treated with methanol. The methanol-soluble portion was dissolved in water and applied to a column of ODS-Q3 with a solvent system of 5–15% acetonitrile in water containing acetic acid (10 ml/l), to give rheinosides A (14.18 g), B (3.64 g), C (2.64 g) and D (4.56 g).

Rheinoside A (1)—A pale yellow amorphous powder, $[\alpha]_D^{25} -53^\circ$ ($c=0.5$, MeOH), IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 3600–3100 (OH), 1700 (COOH), 1640 (C=O). UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 267 (6300), 296 (8800), 346 (7000). FD-MS m/z : 633 ($M+Na$)⁺. CD ($c=0.005$, MeOH) $[\theta]^{25}$ (nm): -3.6×10^3 (374), 4.0×10^3 (321), 9.6×10^3 (292). ¹H-NMR (D_2O) δ : 7.88 (s, C₄-H), 7.75 (dd, $J=7.2$, 7.6, C₆-H), 7.67 (d, $J=7.6$, C₅-H), 7.38 (s, C₂-H), 7.34 (d, $J=7.3$, C₇-H), 5.19 (d, $J=7.9$, C₁-H), 3.98 (d, $J=12.5$, C₆-H), 3.82 (dd, $J=5.5$, 12.5, C₆-H'), 3.79 (dd, $J=7.9$, 9.1, C₂-H), 3.71 (m, C₅-H), 3.65 (dd, $J=8.9$, 9.1, C₃-H), 3.60 (dd, $J=8.9$, 9.1, C₄-H), 3.60 (brd, $J=12.2$, C₆-H), 3.43 (dd, $J=5.5$, 12.2, C₆-H'), 3.34 (d, $J=9.8$, C₁-H), 3.33 (t, $J=9.2$, C₃-H), 3.03 (m, C₅-H), 2.95 (t, $J=9.2$, C₄-H), 2.94 (dd, $J=9.2$, 9.8, C₂-H). Anal. Calcd for C₂₇H₃₀O₁₆·2H₂O: C, 50.15; H, 4.99. Found: C, 50.19; H, 4.96.

Hydrolysis of Rheinoside A—A solution of rheinoside A (300 mg) in 5 ml of 0.5 N H₂SO₄ was refluxed for 2 h. After cooling, the reaction mixture was extracted with 50 ml each of ethyl acetate three times. The combined ethyl acetate layers were washed with water, dried over Na₂SO₄, and then concentrated to dryness. The product was purified on a column of ODS-Q2 using acetonitrile (15–20%)–water–acetic acid (1%) as an eluent to yield the C-glucoside **1a** (114 mg, 52%). After neutralization with Ba(OH)₂, the aqueous layer was passed through a Sep-pak ODS (Waters Associates Co., Ltd.). D-Glucose ($[\alpha]_D^{25} +33.5^\circ$, $c=0.8$ water) was separated from the water eluate by preparative HPLC on a Nucleosil 10 NH₂ column.

1a: Pale yellow crystals, mp 230 °C, $[\alpha]_D^{25} +34^\circ$ ($c=0.5$, MeOH). EI-MS m/z : 448 (M^+). ¹H-NMR (D_2O) δ : 7.75 (d, $J=1.2$, C₄-H), 7.63 (dd, $J=8.1$, 8.4, C₆-H), 7.39 (d, $J=1.2$, C₂-H), 7.35 (d, $J=8.1$, C₅-H), 6.95 (d, $J=8.4$, C₇-H), 3.66 (dd, $J=1.8$, 12.1, C₆-H), 3.46 (dd, $J=6.2$, 12.1, C₆-H'), 3.52 (d, $J=9.5$, C₁-H), 3.22 (dd, $J=8.8$, 9.2, C₃-H), 3.08 (m, C₅-H), 2.88 (dd, $J=8.8$, 9.5, C₄-H), 2.63 (dd, $J=9.2$, 9.5, C₂-H). ¹³C-NMR (D_2O) δ : 194.7 (C₉, C=O), 171.1 (COOH), 163.2 (s), 162.3 (s), 148.2 (s), 147.1 (s), 139.5 (d), 138.8 (s), 121.2 (d, 2C), 120.6 (s), 120.6 (d), 120.1 (d), 118.2 (s), 86.1 (d), 82.1 (d), 80.0 (d), 77.0 (s), 73.7 (d), 71.9 (s), 63.8 (t). Anal. Calcd for C₂₁H₂₀O₁₁·1.5H₂O: C, 53.05; H, 4.88. Found: C, 52.84; H, 4.58.

Oxidative Degradation of Rheinoside A—A solution of 10 mg of rheinoside A and 50 mg of FeCl₃ in 5 ml of 2 N H₂SO₄ was refluxed for 30 min. After cooling, the reaction mixture was diluted with water (50 ml) and extracted with ethyl acetate (30 ml × 3). The combined ethyl acetate layers were dried over Na₂SO₄ and concentrated. HPLC showed a peak identical with that of rhein. The aqueous layer was neutralized with Ba(OH)₂ and passed through a Sep-pak ODS. The fraction eluted with water was analyzed by HPLC on a column of Nucleosil 10NH₂ (mobile phase: CH₃CN:H₂O=70:30), and showed peaks corresponding to glucose and arabinose.

Rheinoside B (2)—A pale yellow amorphous powder, $[\alpha]_D^{25} -43^\circ$ ($c=0.5$, MeOH). IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 3600–3100 (OH), 1700 (C=O), 1640 (COOH). UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 269 (6200), 298 (9100), 345 (6600). FD-MS m/z : 633 ($M+Na$)⁺. CD ($c=0.005$, MeOH) $[\theta]^{25}$ (nm): $+11.0 \times 10^3$ (355), -17.2×10^3 (321), -5.6×10^3 (275). ¹H-NMR (D_2O) δ : 7.82 (d, $J=1.2$, C₄-H), 7.71 (dd, $J=7.6$, 8.2, C₆-H), 7.65 (d, $J=7.6$, C₅-H), 7.42 (d, $J=1.2$, C₂-H), 7.37 (d, $J=8.2$, C₇-H), 5.20 (d, $J=7.6$, C₁-H), 3.96 (dd, $J=1.8$, 12.5, C₆-H), 3.80 (dd, $J=5.5$, 12.5, C₆-H'), 3.74 (dd, $J=7.6$, 9.2, C₂-H), 3.68 (dd, $J=9.2$, 9.5, C₃-H), 3.66 (ddd, $J=1.8$, 5.5, 9.5, C₅-H), 3.58 (t, $J=9.2$, C₄-H), 3.56 (brd, $J=12.2$, C₆-H), 3.44 (dd, $J=5.5$, 12.2, C₆-H'), 3.38 (d, $J=9.8$, C₁-H), 3.34 (t, $J=9.2$, C₃-H), 3.03 (ddd, $J=1.9$, 7.9, 9.8, C₅-H), 2.93 (t, $J=9.5$, C₄-H), 2.89 (dd, $J=9.2$, 9.8, C₂-H). Anal. Calcd for C₂₇H₃₀O₁₆·H₂O: C, 51.59; H, 5.27. Found: C, 51.27; H, 5.13.

Rheinoside C (3)—A pale yellow amorphous powder, $[\alpha]_D^{25} -40^\circ$ ($c=0.5$, MeOH). IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 3600–3100 (OH), 1700 (C=O), 1640 (COOH). UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 268 (7500), 292 (10500), 335 (7500). FD-MS m/z : 617 ($M+Na$)⁺. CD ($c=0.005$, MeOH) $[\theta]^{25}$ (nm): -5.6×10^3 (378), $+6.3 \times 10^3$ (325), -14.0×10^3 (298). ¹H-NMR (D_2O) δ : 7.60 (dd, $J=7.6$, 8.2, C₆-H), 7.24 (d, $J=8.2$, C₅-H), 7.23 (d, $J=1.5$, C₄-H), 7.08 (d, $J=7.6$, C₇-H), 7.03 (d, $J=1.5$, C₂-H), 5.21 (d, $J=7.9$, C₁-H), 4.24 (d, $J=1.8$, C₁₀-H), 3.99 (dd, $J=2.2$, 12.5, C₆-H), 3.833 (dd, $J=5.5$, 12.5, C₆-H'), 3.827 (d, $J=7.9$, 9.5, C₂-H), 3.731 (m, C₅-H), 3.727 (t, $J=9.5$, C₄-H), 3.63 (t, $J=9.5$, C₃-H), 3.49 (dd, $J=1.8$, 12.2, C₆-H), 3.38 (dd, $J=5.2$, 12.2, C₆-H'), 3.31 (t, $J=8.9$, C₃-H), 3.26 (dd, $J=1.8$, 9.8, C₁-H), 2.88–2.98 (m, C₂-H, C₄-H, C₅-H). Anal. Calcd for C₂₇H₃₀O₁₅·2H₂O: C, 51.43; H, 5.44. Found: C, 51.71; H, 5.31.

Oxidative Degradation of Rheinoside C—A solution of 10 mg of rheinoside C and 50 mg of FeCl₃ in 5 ml of 2 N H₂SO₄ was refluxed for 30 min, and treated as mentioned above. The HPLC analyses showed peaks corresponding to rhein, glucose and arabinose.

Rheinoside D (4)—A pale yellow amorphous powder, $[\alpha]_D^{25} -56^\circ$ ($c=0.5$, MeOH). IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 3600–3100 (OH), 1700 (C=O), 1640 (COOH). UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 270 (7100), 295 (9800), 327 (7600). FD-MS m/z : 617 ($M+Na$)⁺. CD ($c=0.005$, MeOH) $[\theta]^{25}$ (nm): $+9.6 \times 10^3$ (361), -19.4×10^3 (319), -4.5×10^3 (275). ¹H-NMR

(D₂O) δ : 7.65 (t, $J=8.5$, C₆-H), 7.34 (d, $J=8.5$, C₅-H), 7.25 (d, $J=1.6$, C₄-H), 7.17 (d, $J=1.6$, C₂-H), 7.16 (d, $J=8.5$, C₇-H), 5.09 (d, $J=7.6$, C₁'-H), 4.12 (d, $J=0.5$, C₁₀-H), 4.02 (brd, $J=12.0$, C₆'-H), 3.88 (dd, $J=5.2$, 12.0, C₆'-H'), 3.73 (dd, $J=7.6$, 8.9, C₂'-H), 3.61—3.68 (m, C₃'-H, C₄'-H, C₅'-H), 3.46 (brd, $J=12.2$, C₆-H), 3.32 (brd, $J=9.5$, C₁-H), 3.31 (dd, $J=5.5$, 12.2, C₆-H'), 2.85—2.93 (m, C₃-H, C₄-H, C₅-H), 2.81 (t, $J=9.5$, C₂-H). *Anal.* Calcd for C₂₇H₃₀O₁₅·2.5H₂O: C, 50.70; H, 5.52. Found: C, 50.70; H, 5.23.

Bioassay—Laxative activity of rheinoside A was determined according to Ishii *et al.*¹⁵ Doses of 21.0, 14.2, 10.2 and 7.1 mg/kg of rheinoside A were administered to groups of five Wistar rats with an average body weight of 120 g, and the activities were measured after 20 h. The ED₅₀ value was calculated by the Litchfield–Wilcoxon method.

References and Notes

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