Chemical Studies on Crude Drug Processing. VII. 1) On the Constituents of Rehmanniae Radix. (1): Absolute Stereostructures of Rehmaglutins A, B, and D Isolated from Chinese Rehmanniae Radix, the Dried Root of *Rehmannia glutinosa* LIBOSCH.

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An iridoid alcohol, rehmaglutin A, and two chlorinated iridoids, rehmaglutins B and D, were isolated from the less polar fraction of Chinese Rehmanniae Radix [the dried root of *Rehmannia glutinosa* LIBOSCH. (Kan-jio in Japanese)], together with rehmaglutinin C, rehmaionoside C, jio-cerebroside, and acetoside. The absolute configurations of rehmaglutins A, B, and D were established on the basis of chemical and spectral evidence which included the chemical derivations of rehmaglutins from the known iridoid glycoside catalpol and the application of the benzote chirality method.

Keywords *Rehmannia glutinosa*; Scrophulariaceae; crude drug processing; iridoid alcohol; iridoid chlorinated; rehmaglutinin A; rehmaglutinin B; rehmaionoside D

Rehmanniae Radix, which is a crude drug prepared from the roots of various *Rehmannia* spp. (Scrophulariaceae) is listed as an upper grade drug (上藥) in Shen Nung’s Herbal (神農本草經) and is one of the most important traditional Chinese medicines. Depending upon the kind of processing method, Rehmanniae Radix is classified into three types named in Japanese as Shô-jîô (生地黄 fresh root), Kan-jîô (乾地黄 dried root), and Juku-jîô (熟地黄 variously treated root), which have quite distinct applications in herbal formulae of Chinese traditional medicine. In recent years, due to the poor supply of Japanese Rehmanniae Radix, which is prepared from the root of *Rehmannia glutinosa* LIBOSCH. var. purpurea MAKINO (Akaya-jîô in Japanese) or *R. glutinosa* LIBOSCH. forma *hueichingensis* HSIAO (Kaikôi-jîô in Japanese), Chinese Rehmanniae Radix has been imported and commonly used in Chinese medicinal treatment in Japan.

In regard to chemical studies on the constituents of Rehmanniae Radix, we first reported in 1971 the isolation of catalpol (1) as the major iridoid glycoside from the fresh root of *R. glutinosa* LIBOSCH. forma *hueichingensis* HSIAO. 2) Since then, several chemical investigations of Japanese Rehmanniae Radix have been carried out to discover more iridoid glycosides such as aucubin, leonuride, melittoside, rehmammiosides A, B, C, and D, and various carbohydrate and amino acids. 3) However, no work on the chemical constituents of Chinese Rehmanniae Radix has been reported. 4)

In our continuing chemical studies on the processing of crude drugs, 5,11 we have compared the chemical constituents of differently processed Japanese, Chinese, and Korean Rehmanniae Radices. 9) From Chinese Rehmanniae Radix (Chinese Kan-jîô), the botanical origin of which was identified as the dried root of *R. glutinosa* LIBOSCH., 12) we have isolated various then-new constituents, namely four iridoids designated rehmaglutins A (3), B (4), 5) C, 7) and D (5), 6) a chlorinated iridoid glycoside, glutinoside, 7) three ionone glucosides, rehmaionosides A, B, and C, 8) a monoterpen glucoside, rehmagricoside, 5) and jio-cerebroside, together with a phenethylalcohol glycoside, actoside (2), and six known iridoid glycosides, catalpol (1), leonuride, monomelittoside, melittoside, rehmaionoside D, and dihydrocortexin. 9)

In this paper, we present a full account of the structure elucidation of rehmaglutins A (3), B (4), and D (5), which were isolated from the less polar fraction of the constituents of Chinese Rehmanniae Radix. 13)

After some preliminary examinations to identify optimal extraction conditions, it was found that the extraction of the dried root with 50% aqueous acetone below 25°C seemed a promising. The extract thus obtained was subjected to fractionation and purification procedures as shown

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**Chemical Diagram**

**Chinese Rehmanniae Radix**  
(*Rehmannia glutinosa* LIBOSCH., dried roots)

- Acetone-H₂O (1:1, r.t.)
- Acetone-H₂O ext.
- H₂O phase
- Charcoal column
- H₂O eluate (carbohydrate fr.)
- MeOH eluate (glycoside fr.)

**Chart 1**

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in Chart 1. Chromatographic purification of the ethyl acetate-soluble portion furnished acetoside (2),14 rehmaglutins A (3), B (4), C,7 and D (5), jio-cerebroside, and rehmaionoside C.8) The water soluble portion, after charcoal column chromatography, provided six known iridoid glycosides as mentioned above, together with eight carbohydrates: fructose, glucose, galactose, mannitol, sucrose, mannaiturose, raffinose, stachyose, and verbascose.

Rehmaglutin A (3) Rehmaglutin A (3) was obtained as colorless needles of mp 134—136°C. The molecular formula C19H24O3 was confirmed from the molecular ion peak in the mass spectrum (MS) and by high-resolution mass (high MS) measurement. The infrared (IR) spectrum of 3 showed a hydroxyl absorption band at 3450 cm⁻¹. The proton nuclear magnetic resonance (1H-NMR) and the carbon-13 nuclear magnetic resonance (13C-NMR) spectra of 3 showed signals assignable to an acetal moiety [δ 5.19 (d, J = 5 Hz); δC 101.0], two secondary hydroxyl groups [δ 3.79 (dd, J = 10, 10 Hz), 3.91 (dd, J = 1, 10 Hz); δC 75.4, 85.0] and a tertiary hydroxyl group [δC 85.2. Acetylation of 3 with acetic anhydride and pyridine afforded the triacetate (3a), the 1H-NMR data of which were assigned as shown in Table I on the basis of detailed decoupling experiments. Comparisons of the 1H- and 13C-NMR data (Table II) for 3a with those for 3 led us to presume the presence of 6-, 7-, and 8-hydroxyl groups and a 1,10-oxide ring in the tricyclic iridoid structure of 3. Furthermore, the relative configuration of 3a was clarified by nuclear Overhauser effect (NOE) experiments as depicted in Fig. 1 and also by comparison of the 1H–1H coupling constants with those reported for related iridoids.15)

In order to elucidate the absolute configuration of rehmaglutin A (3), the dibenzoyl chiralitaity method10 was applied to 3c which was prepared from 3. Namely, 3 was first converted to the 8-O-acetate 3b through the following procedures: i) silylation with 1,3-dichloro-1,1,3,3-tetraisopropylsiloxiane (TIPDISCl) in pyridine to protect the 6,7-trans diol moiety, ii) acetylation of the 8-hydroxy group with acetic anhydride in pyridine and dimethylamino-

pyridine (DMAP), and then iii) removal of the tetraisopropylsilyle group with tetra-n-butylammonium fluoride (n-Bu4NF) in tetrahydrofuran (THF). The 1H-NMR spectrum of 3b exhibited signals due to two hydroxy-bearing methines [δ 3.78 (dd, J = 9, 10 Hz), 4.16 (dd, J = 2, 9 Hz)], and an acetylation shift around C-8 was observed in the 13C-NMR spectrum of 3b (Table II). The 8-O-acetate 3b was then subjected to benzylation with benzoyl chloride in pyridine to furnish 3c. The 1H-NMR data for 3c showed the presence of two benzyloxyl-bearing methines at C-6 and C-7 [δ 5.91 (dd, J = 10, 10 Hz), 6.36 (brd, J = ca. 10 Hz)]. The circular dichroism (CD) spectrum of 3c gave a split Cotton curve ([θ]222 +61600 and [θ]222 −24200), indicating the 6S,7R configurations of 3c.

Finally, the absolute stereostructure of rehmaglutin A (3) was further confirmed by chemical derivation from catalp (1). Thus, catalytic hydrogenation of 1 gave dihydrocatalpol (6)17) which was converted to rehmaglutin A (3) in 15% overall yield, by alkaline treatment with 10% aqueous NaOH, cleaving the 7,8-epoxide ring to give the 7,8-diol derivative (presumably expressed as 7), and by subsequent methanalysis of this derivative to construct the 1,10-oxide ring with concomitant removal of the glucosylxoy moiety.

Rehmaglutin B (4) Rehmaglutin B (4), obtained as colorless prisms of mp 152–153°C, was shown to possess a chlorine atom by the positive Beilstein test. The chemical ionization mass spectrum (CI-MS) of 4 showed pairs of isotope ion peaks at m/z 237 (25%) and 239 (9%) due to a quasimolecular ion (M+H)⁺ at m/z 219 (100%) and 221 (33%) due to (M+H–H2O)⁺. The high MS measurement of 4 revealed the molecular formula to be C20H26O5. The 1H- and 13C-NMR data for 4, which resembled those for 3, suggested the presence of two acetal moieties, two secondary alcohols and a tertiary alcohol in the tricyclic iridoid structure. Ordinary acetylation of 4 gave the 3,6-di-O-acetate (4a) and the 3,6,8-tri-O-acetate (4b).

Detailed 1H-NMR decoupling experiments enabled us to make complete assignments of the signals of 4a and 4b (Table I). In the 1H-NMR spectrum of 4b, the signals of

Chart 2
Table I. $^1$H-NMR Data for Rehmaglutin Acetates

<table>
<thead>
<tr>
<th></th>
<th>3a (d, J = 5.2)</th>
<th>4a (d, J = 4.9)</th>
<th>4b (d, J = 4.9)</th>
<th>5a (d, J = 5.2)</th>
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<tr>
<td>H-1</td>
<td>5.34</td>
<td>5.59</td>
<td>5.55</td>
<td>5.46</td>
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<tr>
<td>H-3</td>
<td>3.63 (d, J = 4.9, 11.9, β-H)</td>
<td>6.41 (d, J = 6.4, 7.3)</td>
<td>6.44 (d, J = 6.7, 7.9)</td>
<td>3.62 (d, J = 5.2)</td>
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<td>4.07 (d, J = 2.4, 11.9, 12.8, α-H)</td>
<td>1.63 (d, J = 4.9, 7.3, 14.7, α-H)</td>
<td>1.58 (d, J = 4.9, 7.9, 14.3, α-H)</td>
<td>1.47 (br d, J = 14.3, α-H)</td>
<td>4.06 (dd, J = 2.1, 12.0, 12.2, β-H)</td>
</tr>
<tr>
<td>H-4</td>
<td>1.46 (br d, J = ca. 14.6, α-H)</td>
<td>2.11 (d, J = 3.8, 6.4, 14.7, β-H)</td>
<td>2.12 (d, J = 2.5, 6.7, 14.3, β-H)</td>
<td>1.77 (dd, J = 4.6, 5.2, 12.2, 14.3, β-H)</td>
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<tr>
<td>1.78 (dd, d, J = 4.9, 5.2, 12.8, 14.6, β-H)</td>
<td>2.47 (dd, J = 3.8, 4.9, 10.1, 10.5)</td>
<td>2.77 (dd, J = 2.5, 4.9, 9.8, 10.4)</td>
<td>2.56 (dd, J = 4.6, 9.8, 10.4)</td>
<td>3.45 (d, J = 10.4, 10.4)</td>
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<tr>
<td>H-5</td>
<td>2.64 (dd, J = 5.2, 9.8, 11.0)</td>
<td>2.47 (dd, d, J = 10.1, 10.1)</td>
<td>2.52 (d, J = 9.8, 10.4)</td>
<td>2.59 (dd, J = 10.4, 10.4)</td>
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<tr>
<td>H-6</td>
<td>5.44 (d, J = 9.5, 11.0)</td>
<td>4.25 (d, J = 10.1)</td>
<td>4.95 (d, J = 9.8)</td>
<td>4.81 (d, J = 1.5, 10.4)</td>
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<td>H-7</td>
<td>5.85 (d, J = 1.5, 9.5)</td>
<td>3.16 (dd, J = 4.9, 9.8)</td>
<td>2.85 (dd, J = 5.2, 9.8)</td>
<td>3.74 (dd, J = 1.5, 10.7, β-H)</td>
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<tr>
<td>H-9</td>
<td>2.74 (d, J = 5.2, 9.8)</td>
<td>3.86 (β-H, 4.34 (α-H) (both d, J = 11.0)</td>
<td>4.12 (β-H, 4.37 (α-H) (both d, J = 11.0)</td>
<td>4.61 (d, J = 10.7, α-H)</td>
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<td>H-10</td>
<td>3.59 (d, J = 1.5, 10.5, β-H)</td>
<td>4.59 (d, J = 10.5, α-H)</td>
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<tr>
<td>0.20 (d, J = 10.5, α-H)</td>
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</table>

* Measured at 500 MHz in CDCl$_3$. Chemical shifts are in δ and J values are in Hz.

Table II. $^{13}$C-NMR Data for Rehmaglutins A (3), B (4), and D (5) and Their Derivatives (22.5 MHz, δ$c$)

<table>
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<th></th>
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<th>3a (δ)</th>
<th>3c (δ)</th>
<th>3d (δ)</th>
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<th>4b (δ)</th>
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<th>5a (δ)</th>
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<td>100.5</td>
<td>99.1</td>
<td>98.9</td>
<td>99.5</td>
<td>102.1</td>
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<td>C-3</td>
<td>56.4</td>
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<td>55.5</td>
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<tr>
<td>C-6</td>
<td>75.4</td>
<td>74.2</td>
<td>73.1</td>
<td>75.2</td>
<td>73.6</td>
<td>74.8</td>
<td>79.0</td>
<td>78.1</td>
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<tr>
<td>C-7</td>
<td>85.0</td>
<td>79.1</td>
<td>78.0</td>
<td>82.8</td>
<td>78.3</td>
<td>78.1</td>
<td>70.1</td>
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<td>C-8</td>
<td>85.2</td>
<td>89.7</td>
<td>86.8</td>
<td>92.8</td>
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<td>89.9</td>
<td>90.8</td>
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<td>92.5</td>
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<tr>
<td>C-9</td>
<td>44.9</td>
<td>42.6</td>
<td>41.3</td>
<td>42.6</td>
<td>41.6</td>
<td>48.4</td>
<td>52.8</td>
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<td>C-10</td>
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<td>68.4</td>
<td>67.5</td>
<td>67.6</td>
<td>67.8</td>
<td>74.3</td>
<td>76.6</td>
<td>76.3</td>
<td>74.8</td>
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</table>

* The characterization of each carbon signal was made by INEPT (insensitive nuclei enhanced by polarization) and off-resonance experiments. b) Measured in d$_6$-acetone solution. c) Measured in CDCl$_3$ solution.

Fig. 1. NOE (%) of 3a, 4a, and 5a [1$^1$H-NMR (500 MHz, CDCl$_3$)]. The 7.9,10β-protons were observed with remarkable downfield shifts of 0.70, 0.45, and 0.26 ppm, respectively, as compared with those of 4a. These downfield shifts were ascribable to the paramagnetic effect of the acetyl-carbonyl group attached to the 8-hydroxyl group which indicated a cis-relationship of the 7.9,10β-protons and the 8-acetoxy group. The absolute configuration of rehmaglutin B (4) was determined as described for rehmaglutin A (3). First, the relative configuration of 4 was clarified by the NOE examinations as depicted in Fig. 1 and by comparison of 1$^1$H-1$^1$H coupling constants. Then, the aromatic chirality method was applied. Silylation of 4 with TIPPSiCl$_2$ under the same conditions as for 3 and subsequent treatment with methanol gave an unstable 3-O-silylated product (4c) which was further subjected to bezoylation followed by desilylation finally to furnish the 6.8-di-O-benzoyl derivative (4d). The CD spectrum of 4d showed a split Cotton curve with a small [θ] value ([θ]$^{234}$ + 50000, [θ]$^{255}$ - 10000), which indicated the presence of a long-range dibenzoate chirality in 4d. 16

Furthermore, the absolute stereostructure of rehmaglutin B (4) was substantiated by chemical correlation with catalpol (1). Thus, treatment of 1 with 0.6% HCl-dry methanol gave a chlorinated product (8) manufactured via cleavage of the 7,8-epoxide ring and acetal-formation between C-3 and C-10. Subsequent hydrolysis of the product with 10% aqueous HCl provided rehmaglutin B (4) in 45% overall yield.

Rehmaglutin D (5) Rehmaglutin D (5) was also a chloride-containing iodoid of mp 132—133°C (colorless prisms) as shown from the Beilstein test and the isotope ion peaks [m/z 221 (100%), 223 (33%) for (M + H) $^+$] observed in the CI-MS. Rehmaglutin D (5) was less polar than rehmaglutin B (4) and the molecular formula C$_{23}$H$_{33}$ClO$_4$ was determined by high-MS. The 1$^1$H- and 1$^{13}$C-NMR (Table II) spectra of 5 indicated a close similarity of its structure to those of rehmaglutins A (3) and B (4). Ordinary acetylation of 5 furnished the 6,8-di-O-acetate (5a) and the 1$^1$H- (Table I) and 1$^{13}$C- (Table II) NMR data for 5a showed the presence of 6,8-acetoxy and 7-chloro functions and also a 1,10-oxide moiety in its tricyclic iodoid structure. The
relative configuration of 5 was substantiated by detailed NOE examinations (Fig. 1) and comparisons of \(^1\)H \text{-} H coupling constants as carried out for 3a and 4a. Finally, methanolation of dihydrocatalpol (6) with 3% HCl in dry methanol furnished 5 in 53% yield. Thus, the absolute stereostructure of rehmaglutin D (5) was determined to be as shown.

**Experimental**

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.\(^{13}\)

**Isolation of Rehmaglutins A (3), B (4), C, and D (5), Rehmannioside C, Jio-cerebroside, and Acteoside (2)**

The air-dried roots of Chinese Rehmanniae Radix (3kg, imported from China, purchased from Tochimoto-Tenkaido, Osaka) were cut finely and extracted with 50% aqueous acetone three times (151 each) with occasional stirring at room temperature (below 25°C). After removal of the organic solvent from the aqueous acetone extract under reduced pressure, the remaining aqueous solution was extracted with AcOEt. Removal of the solvent from the organic phase under reduced pressure gave the residue (10.1 g), which was fractionated by column chromatography [SiO\(_2\), 200 g, CHCl\(_3\)-MeOH-\(\text{H}_2\)\(\text{O}\) (10:3:1→7:3:1→65:35:10, using the lower phase in each case) as the eluent] to furnish four fractions. Evaporation of the solvent under reduced pressure gave fr. 1 (lipsids etc., 2.4 g), fr. 2 (1.8 g), fr. 3 (0.7 g), and fr. 4 (1.4 g).

Fraction 2 (1.8 g) was purified by reverse-phase silica gel column chromatography [Bondapak C\(_18\), 200 g with \(\text{H}_2\text{O}-\text{MeOH} (3:1→2:1)\) gradient elution] to give rehmaglutin D (5, 147 mg) and jio-cerebroside (435 mg). Fraction 3 (0.7 g) was subjected successively to reverse-phase silica gel column chromatography [Bondapak C\(_18\), 100 g, \(\text{H}_2\text{O}-\text{MeOH} (3:1)\)] and ordinary-phase column chromatography [SiO\(_2\), 50 g, CHCl\(_3\)-\(\eta\)-BuOH (1:1)] to give rehmaglutins A (3, 108 mg), B (4, 87 mg), and C (27 mg). Fraction 4 (1.4 g) was purified by reverse-phase silica gel column chromatography [Bondapak C\(_18\), 200 g, elution with \(\text{H}_2\text{O}\) and \(\text{H}_2\text{O}-\text{MeOH} (10:1→2:1)\)] to furnish acteoside (2, 410 mg) and rehmannioside C (25 mg). Acteoside (2) was obtained as a white powder and was identified by comparing its physical data, \([\lambda]_D^{20} = -80° (c=1.1, \text{MeOH}),\) ultraviolet (UV), IR, secondary ion mass spectrometry (SIMS), \(^1\)H- and \(^{13}\)C-NMR, with those reported.\(^{19}\) The water-soluble portion (1.9kg), obtained after removal of the solvent from the aqueous phase under reduced pressure, was subjected to active charcoal column chromatography [charcoal 2kg-Celite 2kg, elution with \(\text{H}_2\text{O}, \text{H}_2\text{O}-\text{MeOH} (1:1)\), and then MeOH] to give a glycoside mixture (45g). The procedure for separation of the glycoside mixture will be reported in detail in our forthcoming paper.

Rehmaglutin A (3): mp 134°-136°C (colorless needles from MeOH), \([\lambda]_D^{20} +43.6° (c=0.28, \text{MeOH}),\) High MS: Found 202.085. Caled for \(\text{C}_{21}\text{H}_{22}\text{O}_{5} (\text{M}^+)\) 202.084. IR \(\nu_{max}\) cm\(^{-1}\) : 3450, 2950, 1035. \(^1\)H-NMR (90 MHz, \(\delta_6\)-acetone) \(\delta: 1.48-1.61 (2\text{H, m, 4-H}), 2.00-2.18 (2\text{H, m, 5, 9-H}), 3.22 (1\text{H, dd, } J=1, 10\text{Hz, 10p-H}), 3.60-3.82 (2\text{H, m, 3-H}), 3.79 (1\text{H, dd, } J=10, 10\text{Hz, 6-H}), 3.91 (1\text{H, dd, } J=1, 10\text{Hz, 7-H}), 4.35 (1\text{H, d, } J=10\text{Hz, 10a-H}), 5.19 (1\text{H, d, } J=5\text{Hz, 1-H}).\) \(^{13}\)C-NMR: see Table II. CI-MS \(m/z\): 203 \([(\text{M}+\text{H})^+\), 98], 185 \([(\text{M}+\text{H}−\text{H}_2\text{O})^+]\), 100, 167 (45).

Rehmaglutin B (4): mp 152°-153°C (colorless prisms from MeOH), \([\lambda]_D^{20} +33.8° (c=0.79, \text{MeOH}),\) High MS: Found 236.035. Caled for \(\text{C}_{19}\text{H}_{18}\text{O}_{4} (\text{M}^+)\) 236.035. IR \(\nu_{max}\) cm\(^{-1}\) : 3280, 2920, 1049, 1031. \(^1\)H-NMR (90 MHz, \(\delta_6\)-acetone) \(\delta: 1.38-1.63 (1\text{H, m, 4a-H}), 1.92-2.08 (1\text{H, m, 4b-H}), 2.23-2.57 (1\text{H, m, 5-H}), 2.43 (1\text{H, dd, } J=5, 10\text{Hz, 9-H}), 3.42 (1\text{H, dd, } J=1, 10\text{Hz, 10p-H}), 3.81 (1\text{H, dd, } J=9, 10\text{Hz, 6-H}), 4.12 (1\text{H, dd, } J=1, 10\text{Hz, 7-H}), 4.21 (1\text{H, dd, } J=10\text{Hz, 10a-H}), 5.25 (1\text{H, dd, } J=4, 9\text{Hz, 3-H}), 5.48 (1\text{H, d, } J=5\text{Hz, 1-H}).\) \(^{13}\)C-NMR: see Table II. CI-MS \(m/z\): 239 (239), 237 (25) \([(\text{M}+\text{H})^+\), 221 (34), 219 (100) \([(\text{M}+\text{H}−\text{H}_2\text{O})^+]\).

Rehmaglutin D (10): mp 132°-133°C (colorless prisms from Et\(_3\)O), \([\lambda]_D^{20} +60.6° (c=0.19, \text{MeOH}),\) High MS: Found 220.035. Caled for \(\text{C}_{18}\text{H}_{17}\text{O}_{4} (\text{M}^+)\) 220.050. IR \(\nu_{max}\) cm\(^{-1}\) : 3400, 2940, 1045, 1025. \(^1\)H-NMR (90 MHz, \(\delta_6\)-acetone) \(\delta: 1.62-1.81 (2\text{H, m, 4-H}), 2.10-2.30 (1\text{H, m, 5-H}), 2.35 (1\text{H, dd, } J=5, 10\text{Hz, 9-H}), 3.43 (1\text{H, dd, } J=2, 10\text{Hz, 10p-H}), 3.60-4.00 (2\text{H, m, 3-H}), 3.81 (1\text{H, dd, } J=9, 10\text{Hz, 6-H}), 4.18 (1\text{H, dd, } J=2, 10\text{Hz, 7-H}), 4.36 (1\text{H, d, } J=10\text{Hz, 10a-H}), 5.28 (1\text{H, d, } J=5\text{Hz, 1-H}).\) \(^{13}\)C-NMR: see Table II. CI-MS \(m/z\): 223 (33), 221 (100) \([(\text{M}+\text{H})^+\), 205 (7), 207 (18) \([(\text{M}+\text{H}−\text{H}_2\text{O})^+]\), 187 (2), 185 (5).
SIMS m/z (%) \(223 (20), 221 (66), 205 (10), 203 (31), 169 (10), 167 (30), 55 (10)\).

Solution of Rehmaglutin A (3) A solution of 3 (12 mg) in pyridine (1.0 ml) was treated with \(\text{Ac}_2\text{O} (1.0 \text{ ml})\) and the mixture was stirred at room temperature (20°C) for 8 h, then poured into ice-water. The water was extracted with AcOEt. The AcOEt extract was washed with diluted aqueous HCl, aqueous saturated NaHCO\(_3\), and brine, and then dried over MgSO\(_4\). After removal of the solvent from the AcOEt extract under reduced pressure, the product was purified by column chromatography \([\text{SO}_4 \text{Ig}, \text{n-} \text{hexane-} \text{AcOEt (2:1)}]\) to furnish the triacetate (3a, 19 mg).

3a: mp 128—130°C (colorless needles from Et\_O\_2), \([\alpha]_D^{2.6} +3.6 (c=0.35, \text{MeOH}),\) High MS: Found 328.117. Calc. for \(\text{C}_{13}\text{H}_{20}\text{O}_4\) (M\(^+\)) 328.116. IR \(\nu_{\text{cm}^{-1}}\): 1740, 1240, 1035. \(1^H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta\): 2.04, 2.33, 2.60 (3H each, all, s, OAc \times 3), and other signals as given in Table I. NOE (%): as shown in Fig. 1. 13C-NMR \(\delta\) \((\text{AcOEt})\): 21.1 (2C), 21.8, 171.0, 171.3, 171.6; (CDCl\(_3\)) 20.2 (2C), 21.5, 170.2 (2C), 170.7, and other data as given in Table II. CI-MS m/z (%): 329 \([M + H]^+\), 1, 269 \([M + H - \text{AcOEt}]^+\), 209, 40 (49).

Conversion of 3 to 3b A solution of 3 (14 mg) in pyridine (1.5 ml) was treated with TIPDDS (30 mg) and the mixture was stirred at room temperature (20°C) under \(N_2\) atmosphere for 5 h, then poured into ice-water. The whole was extracted with AcOEt. The AcOEt extract was washed successively with 2N HCl, aqueous saturated NaHCO\(_3\), and brine, then dried over MgSO\(_4\). Removal of the solvent under reduced pressure gave a product, which was dissolved in pyridine (1.0 ml), and the solution was stirred at room temperature (20°C) and DMF (a catalyst amount). The reaction mixture was stirred at room temperature (20°C) under \(N_2\) atmosphere for 2 h and then poured into ice-water. The whole was extracted with AcOEt and the AcOEt extract was worked up in the same manner as described above to give the product, which was dissolved in THF (2.0 ml). The solution was treated with \(\text{Bu}_3\text{NF} (104 \text{ mg})\) and the mixture was stirred at room temperature (20°C) under \(N_2\) atmosphere for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated NaHCO\(_3\), and brine, then dried over MgSO\(_4\). Removal of the solvent under reduced pressure gave a product, which was purified by column chromatography \([\text{SO}_4 \text{Ig}, \text{n} \text{-hexane-} \text{AcOEt (2:1)}]\) to furnish 3b (9 mg).

3b: \([\alpha]_D^{2.6} +50.9 (c=0.6, \text{MeOH}),\) MeOH. High MS: Found 453.157. Calc. for \(\text{C}_{17}\text{H}_{25}\text{O}_5\) (M\(^+\)) 453.156. UV \(\lambda_{	ext{max}}^{\text{nm}}\) (c): 229 (4900), 24200. CD (MeOH): \([\theta]_{222}^{\text{nm}}\) +6100 (pos. max.), \([\theta]_{222}^{\text{nm}}\) -11200 (neg. max.). IR \(\nu_{\text{cm}^{-1}}\): 1720, 1600, 1278, 1091. \(1^H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta\): 1.58—1.73 (2H, m, 4-H), 2.02 (3H, s, OAc), 2.17—2.32 (1H, m, 5-H), 2.61 (1H, dd, J = 6, 10 Hz, 9-H), 3.35—3.54 (1H, m, 3-H), 3.40 (1H, dd, J = 2, 10 Hz, 10-H), 3.72—3.90 (1H, m, 3-H), 3.78 (1H, dd, J = 9, 10 Hz, 6-H), 4.16 (1H, dd, J = 2, 9 Hz, 7-H), 4.51 (1H, m, 4-H), 5.10 (1H, dd, J = 10, 10 Hz), 5.35 (1H, dd, J = 6, 10 Hz, 1-H). 13C-NMR (CDCl\(_3\)) \(\delta\): 20.3, 79.6, 120.0, and others as given in Table II. CI-MS m/z (%): 245 \([M + H]^+\), 227 \([M + H - \text{H}_2\text{O}]^+\), 183, 185 (3) \([M + H - 3\text{AcOEt}]^+\), 100.

Benzoylation of 3b Giving 3c A solution of 3b (9 mg) in pyridine (1.5 ml) was treated with benzoyl chloride (0.05 ml) and the mixture was stirred at room temperature (20°C) for 3 h, then poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the product was purified by column chromatography \([\text{SO}_4 \text{Ig}, \text{n} \text{-hexane-} \text{AcOEt (4:1)}]\) to furnish 3c (11 mg).

3c: A colorless oil, \([\alpha]_D^{2.4} +53.2 (c=0.59, \text{MeOH}),\) High MS: Found 453.157. Calc. for \(\text{C}_{17}\text{H}_{26}\text{O}_6\) (M\(^+\)) 453.156. UV \(\lambda_{	ext{max}}^{\text{nm}}\) (c): 229 (12000). CD (MeOH): \([\theta]_{222}^{\text{nm}}\) +6100 (pos. max.), \([\theta]_{222}^{\text{nm}}\) -11000 (neg. max.). IR \(\nu_{\text{cm}^{-1}}\): 1720, 1600, 1278, 1091. \(1^H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta\): 1.58—1.72 (2H, m, 4-H), 2.10 (3H, s, OAc), 2.80—3.00 (2H, m, 5-H), 3.60—3.80 (1H, m, 3-H), 3.67 (1H, dd, J = 1, 10 Hz, 10-H), 4.08—4.41 (1H, m, 3-SH), 4.73 (1H, dd, J = 11, 10 Hz, 10a-H), 5.42 (1H, dd, J = 4, 10 Hz, 10a-H), 5.91 (1H, dd, J = 10, 10a Hz), 6.36 (1H, brd, J = 7, 10 Hz, 7a-H), 7.30—7.60 (6H), 7.95—8.09 (4H) \(\text{(both m, aromatic protons).} \)

13C-NMR (CDCl\(_3\)) \(\delta\): 21.5, 82.5 (12C), 84.6 (12C), 130.0 (2CC), 133.4 (2C), 165.8 (2C), 170.6, and others as given in Table II. CI-MS m/z (%): 453 \([M + H]^+\), 239 \([M + H - \text{H}_2\text{O}]^+\), 100, 271 (4).
reaction mixture was stirred at room temperature (19°C) for 14 h, then neutralized with Dowex 1 × 2 (OH− form) and filtered. Removal of the solvent from the filtrate under reduced pressure gave the product (crude 8). Acetylation of the product, which was stirred at room temperature (19°C) for 24 h, then neutralized with Dowex 1 × 2 (OH− form), and filtered. After removal of the solvent from the filtrate under reduced pressure, the product was purified by column chromatography [SiO2, 10 g, CHCl3–MeOH (10:1)] to furnish 4 (31 mg).

5 Thus obtained was shown to be identical with authentic rehmannitin B, which was isolated above from Rehmannia Radix, by TLC [CHCl3–MeOH–H2O (7:3:1, lower phase), CHCl3–MeOH (10:1), benzenoacetone (2:1), IR (KBr), and 1H-NMR (d4-acetone) comparisons. Acetylation of Rehmannitin D (5) A solution of 5 (12 mg) in pyridine (1.0 ml) was treated with Ac2O (1.0 ml) and the mixture was stirred at room temperature (21°C) for 3 h, then poured into ice-water. The whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave the product, which was purified by column chromatography [SiO2, 1 g, n-hexane–AcOEt (2:1)] to furnish 5a (16 mg).

5a mp 96–97°C (colorless prisms from Et2O), [α]D28 +28.0° (c = 0.48, MeOH), High MS: Found 304.071. Calc for C16H27O5 (M+) 304.071. IR (CHCl3) cm−1: 1733, 1235, 1039. 1H-NMR (500 MHz, CDCl3): δ: 2.10, 2.13 (3H each, both s, OAc × 2), and others as given in Table I. NOE (%): as shown in Fig. 1. 13C-NMR δc (d4-acetone) 22.2, 22.2, 171.3 (C2); (CDCl3) 20.8, 21.7, 170.3, 170.4, and others as given in Table II. CI-MS m/z (%): 307 (1), 305 (5) [M+H]+, 247 (13), 245 (40) [M+H−AcO]−, 187 (32), 185 (100) [(M+H−2AcO)]+. Comparison of the product (6) with Rehmannitin D (5) A solution of 6 (231 mg) in dry MeOH (2.0 ml) was treated with 9% HCl–dry MeOH (1.0 ml) and the mixture was stirred at 34°C for 3 h, then neutralized with Dowex 1 × 2 (OH− form), and filtered. After removal of the solvent from the filtrate, the product was purified by column chromatography [SiO2, 50 g, benzene-acetone (2:1)] to furnish 5 (74 mg). 8 thus obtained was shown to be identical with authentic rehmannitin D, isolated above from Rehmannia Radix, by mixed melting point determination, and by δc (d4-acetone) +60.0). TLC [CHCl3–MeOH–H2O (10:3:1, lower phase), CHCl3–MeOH (10:1), benzene-acetone (2:1)], IR (KBr), and 1H-NMR (d4-acetone) comparisons.

References and Notes
5) After we reported the chemical elucidation of many new constituents of Chinese Rehmanniae Radix1−8 and published a review,9 several additional chemical constituents of Chinese Rehmanniae Radix were reported.10
12) The botanical identification was kindly undertaken by Dr. Wang Baogui, National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Temple of Heaven, Beijing, China, to whom the authors' thanks are due.
18) The structure of this product was presumed to be 8, which is identical with the structure of glutinoside.10 Without further purification, the product was subjected to aqueous HCl treatment to furnish 4. The conversion of glutinoside to 4 has been achieved and will be reported in our forthcoming paper on the structure elucidation of glutinoside.10