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 rehmaglutin C, rehmaionoside C, jio-cerebroside, and acteoside. 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 benzoate chirality method.

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

Rehmanniae Radix, which is a crude drug prepared from the roots of various *Rehmannia* spp. (Scrophulariaceae) is listed as an upper grade drug (上薬) in Shen Nung's Herbal (神農本草経) 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 Shō-jiō (生地黄 fresh root), Kan-jiō (乾地黄 dried root), and Juku-jiō (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-jiō in Japanese) or *R. glutinosa* LIBOSCH. forma *hueichingensis* HSIAO (Kaikei-jiō in Japanese), Chinese Rehmanniae Radix has been imported and commonly used in Chinese medicinal treatment in Japan.[2]

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.[3] Since then, several chemical investigations of Japanese Rehmanniae Radix have been carried out to discover more iridoid glycosides such as aucubin, leonuride, melittoside, rehmanniosides A, B, C, and D, and various carbohydrates and amino acids.[4] However, no work on the chemical constituents of Chinese Rehmanniae Radix has been reported.[5]

In our continuing chemical studies on the processing of crude drugs,[1,11] we have compared the chemical constituents of differently processed Japanese, Chinese, and Korean Rehmanniae Radices.[9] From Chinese Rehmanniae Radix (Chinese Kan-jiō), 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**),[6] C,[7] and D (**5**),[6] a chlorinated iridoid glycoside, glutinoside,[7] three ionone glucosides, rehmaionosides A, B, and C,[8] a monoterpene glucoside, rehmapicroside,[8] and jio-cerebroside, together with a phenethylalcohol glycoside, acteoside (**2**), and six known iridoid glycosides, catalpol (**1**), leonuride, monomelittoside, melittoside, rehmannioside D, and dihydrocornin.[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

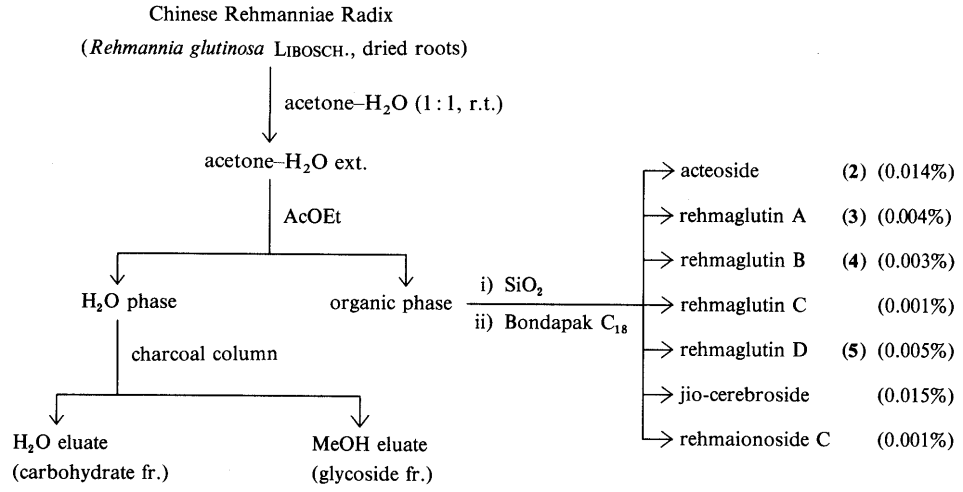

Chart 1

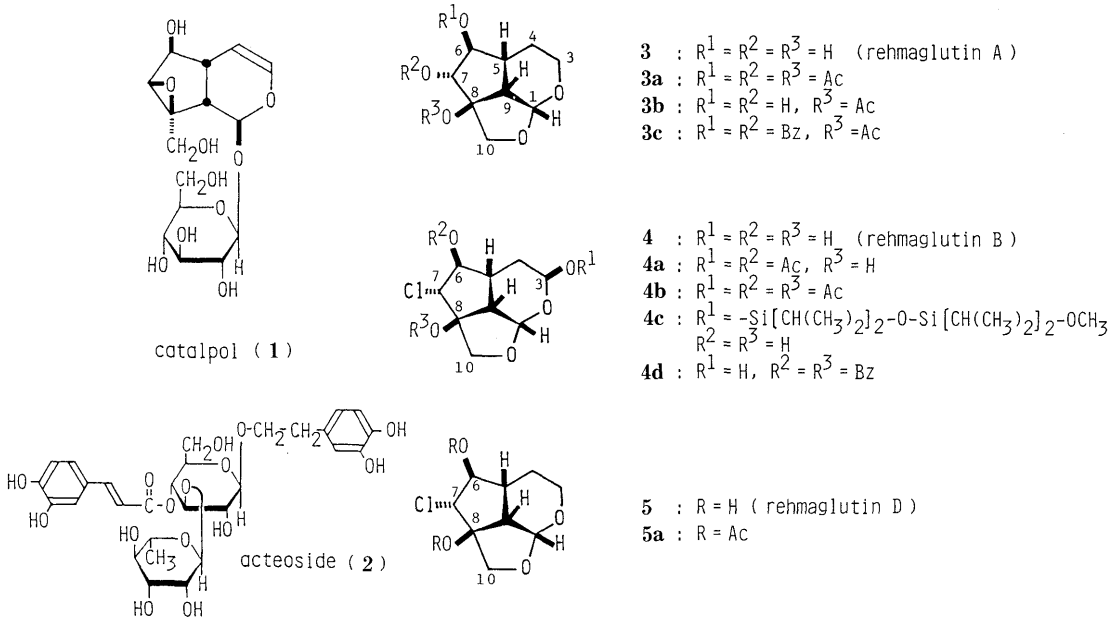

Chart 2

in Chart 1. Chromatographic purification of the ethyl acetate-soluble protion furnished acteoside (**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, manninotriose, raffinose, stachyose, and verbascose.

Rehmaglutin A (3) Rehmaglutin A (**3**) was obtained as colorless needles of mp 134—136 °C. The molecular formula $C_9H_{14}O_5$ 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 (^{1}H-NMR) and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **3** showed signals ascribable to an acetal moiety [δ 5.19 (d, J=5 Hz); δ_C 101.0], two secondary hydroxyl groups [δ 3.79 (dd, J=10, 10 Hz), 3.91 (dd, J=1, 10 Hz); δ_C 75.4, 85.0] and a tertiary hydroxyl group [δ_C 85.2]. Acetylation of **3** with acetic anhydride and pyridine afforded the triacetate (**3a**), the ^{1}H-NMR data of which were assigned as shown in Table I on the basis of detailed decoupling experiments. Comparisons of the ^{1}H- and ^{13}C-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 ^{1}H–^{1}H coupling constants with those reported for related iridoids.[15]

In order to elucidate the absolute configuration of rehmaglutin A (**3**), the dibenzoate chirality method[16] 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-tetraisopropyldisiloxane (TIPDSiCl$_2$) in pyridine to protect the 6,7-*trans* diol moiety, ii) acetylation of the 8-hydroxyl group with acetic anhydride in pyridine and dimethylaminopyridine (DMAP), and then iii) removal of the tetraisopropylsilyl group with tetra-*n*-butylammonium fluoride (*n*-Bu$_4$NF) in tetrahydrofuran (THF). The ^{1}H-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 ^{13}C-NMR spectrum of **3b** (Table II). The 8-*O*-acetate **3b** was then subjected to benzoylation with benzoyl chloride in pyridine to furnish **3c**. The ^{1}H-NMR data for **3c** showed the presence of two benzoyloxy-bearing methines at C-6 and C-7 [δ 5.91 (dd, J=10, 10 Hz), 6.36 (br d, J=*ca.* 10 Hz)]. The circular dichroism (CD) spectrum of **3c** gave a split Cotton curve ([θ]$_{237}$ +61600 and [θ]$_{222}$ −24200), indicating the 6*S*,7*R* configurations of **3c**.

Finally, the absolute stereostructure of rehmaglutin A (**3**) was further confirmed by chemical derivation from catalpol (**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 methanolysis of this derivative to construct the 1,10-oxide ring with concomitant removal of the glucosyloxy 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 $(M+H)^+$ and at *m/z* 219 (100%) and 221 (33%) due to $(M+H-H_2O)^+$. The high MS measurement of **4** revealed the molecular formula to be $C_9H_{13}ClO_5$. The ^{1}H- and ^{13}C-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 ^{1}H-NMR decoupling experiments enabled us to make complete assignments of the signals of **4a** and **4b** (Table I). In the ^{1}H-NMR spectrum of **4b**, the signals of

TABLE I. ^{1}H-NMR Data for Rehmaglutin Acetates[a]

	3a	4a	4b	5a
H-1	5.34 (d, J=5.2)	5.59 (d, J=4.9)	5.55 (d, J=4.9)	5.46 (d, J=5.2)
H-3	3.63 (dd, J=4.9, 11.9, β-H) 4.07 (ddd, J=2.4, 11.9, 12.8, α-H)	6.41 (dd, J=6.4, 7.3)	6.44 (dd, J=6.7, 7.9)	3.62 (dd, J=5.2, 12.0, α-H) 4.06 (ddd, J=2.1, 12.0, 12.2, β-H)
H-4	1.46 (br d, J=*ca.* 14.6, α-H) 1.78 (dddd, J=4.9, 5.2, 12.8, 14.6, β-H)	1.63 (ddd, J=4.9, 7.3, 14.7, α-H) 2.11 (ddd, J=3.8, 6.4, 14.7, β-H)	1.58 (ddd, J=4.9, 7.9, 14.3, α-H) 2.12 (ddd, J=2.5, 6.7, 14.3, β-H)	1.47 (br d, J=14.3, α-H) 1.77 (dddd, J=4.6, 5.2, 12.2, 14.3, β-H)
H-5	2.64 (ddd, J=5.2, 9.8, 11.0)	2.47 (dddd, J=3.8, 4.9, 10.1, 10.5)	2.77 (dddd, J=2.5, 4.9, 9.8, 10.4)	2.56 (ddd, J=4.6, 9.8, 10.4)
H-6	5.44 (dd, J=9.5, 11.0)	5.20 (dd, J=10.1, 10.1)	5.25 (dd, J=9.8, 10.4)	5.39 (dd, J=10.4, 10.4)
H-7	5.85 (dd, J=1.5, 9.5)	4.25 (d, J=10.1)	4.95 (d, J=9.8)	4.81 (dd, J=1.5, 10.4)
H-9	2.74 (dd, J=5.2, 9.8)	2.71 (dd, J=4.9, 10.5)	3.16 (dd, J=4.9, 9.8)	2.85 (dd, J=5.2, 9.8)
H-10	3.59 (dd, J=1.5, 10.5, β-H) 4.59 (d, J=10.5, α-H)	3.86 (β-H), 4.34 (α-H) (both d, J=11.0)	4.12 (β-H), 4.37 (α-H) (both d, J=11.0)	3.74 (dd, J=1.5, 10.7, β-H) 4.61 (d, J=10.7, α-H)

a) 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)[a]

	3[b]	3a[b]	3a[c]	3b[c]	3c[c]	4[b]	4a[b]	4a[c]	4b[b]	4b[c]	5[b]	5a[b]	5a[c]
C-1	101.0 (d)	100.5	99.1 (d)	98.9 (d)	99.5 (d)	102.1 (d)	100.6	98.9 (d)	100.6	98.7 (d)	101.3 (d)	101.2	99.9 (d)
C-3	56.4 (t)	56.4	55.5 (t)	55.8 (t)	56.0 (t)	85.8 (d)	86.2	85.9 (d)	90.8	89.7 (d)	56.3 (t)	56.3	55.6 (t)
C-4	22.4 (t)	22.2	21.1 (t)	21.2 (t)	21.6 (t)	32.4 (t)	26.7	25.8 (t)	26.5	25.4 (t)	22.3 (t)	21.1	21.2 (t)
C-5	34.9 (d)	33.8	32.5 (d)	33.7 (d)	33.1 (d)	38.9 (d)	36.4	35.1 (d)	37.0	35.4 (d)	37.0 (d)	35.8	34.6 (d)
C-6	75.4 (d)	74.2	73.1 (d)	75.2 (d)	73.6 (d)	74.8 (d)	79.0	78.1 (d)	78.8	77.6 (d)	73.0 (d)	75.6	74.7 (d)
C-7	85.0 (d)	79.1	78.0 (d)	82.8 (d)	78.3 (d)	78.1 (d)	70.1	67.7 (d)	64.9	62.6 (d)	75.3 (d)	67.5	65.4 (d)
C-8	85.2 (s)	89.7	88.6 (s)	92.8 (s)	88.9 (s)	89.9 (s)	90.8	89.9 (s)	92.5	91.4 (s)	85.5 (s)	90.5	89.5 (s)
C-9	44.9 (d)	42.6	41.3 (d)	42.6 (d)	41.6 (d)	48.4 (d)	52.8	51.4 (d)	50.1	48.9 (d)	46.2 (d)	42.7	41.5 (d)
C-10	71.0 (t)	68.4	67.5 (t)	67.6 (t)	67.8 (t)	74.3 (t)	76.6	76.3 (t)	74.8	74.0 (t)	76.4 (t)	69.5	68.9 (t)

a) 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.

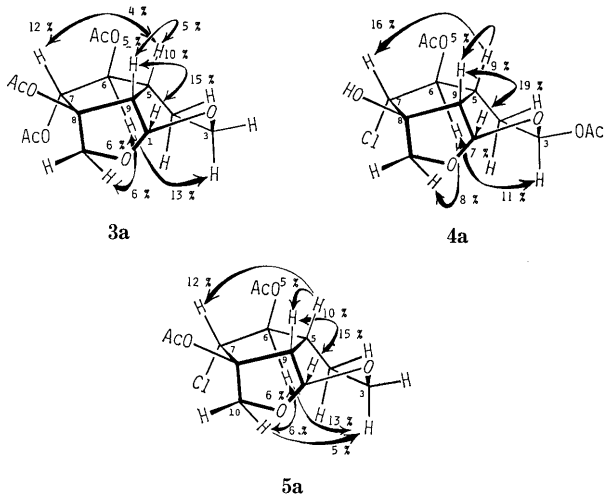

Fig. 1. NOE (%) of **3a**, **4a**, and **5a** [^{1}H-NMR (500 MHz, $CDCl_3$)]

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-acetoxyl 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}H–^{1}H coupling constants. Then, the aromatic chirality method was applied. Silylation of **4** with $TIPDSiCl_2$ 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 $[\theta]$ value ($[\theta]_{234}$ +50000, $[\theta]_{225}$ −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**)[18] formed *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 chlorine-containing iridoid 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 $C_9H_{13}ClO_4$ was determined by high-MS. The ^{1}H- and ^{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}H- (Table I) and ^{13}C- (Table II) NMR data for **5a** showed the presence of 6,8-acetoxyl and 7-chloro functions and also a 1,10-oxide moiety in its tricyclic iridoid structure. The

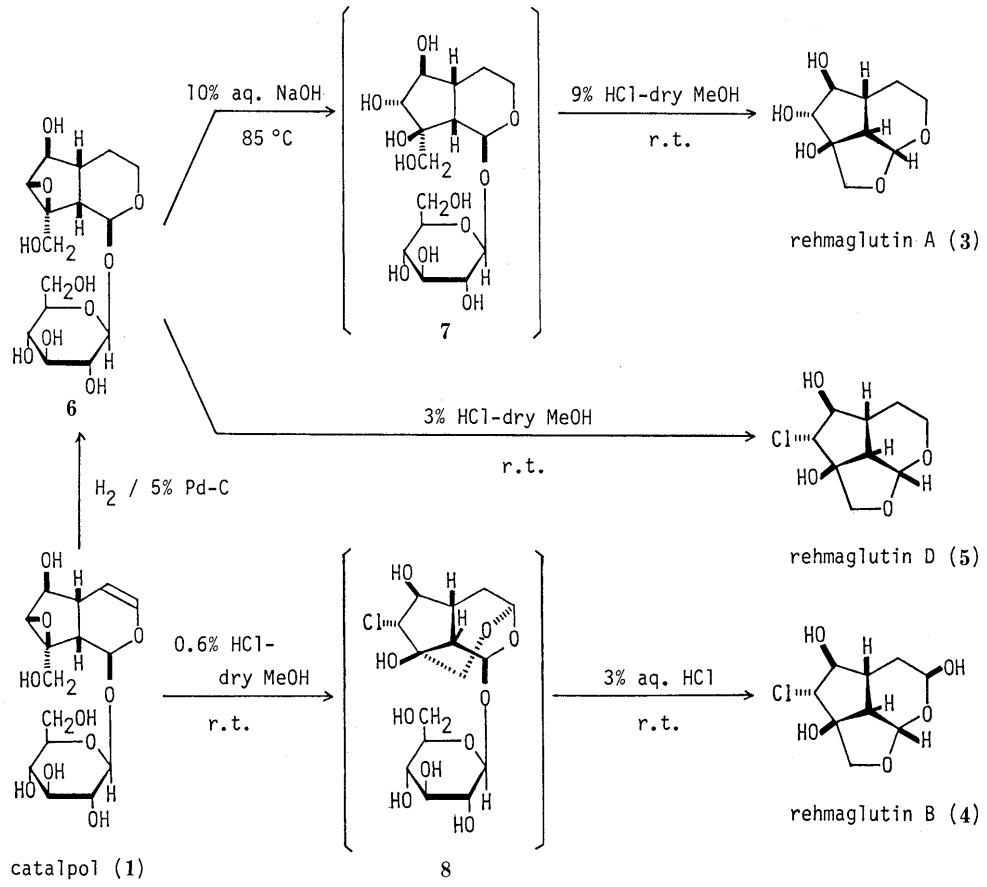

Chart 3

relative configuration of **5** was substantiated by detailed NOE examinations (Fig. 1) and comparisons of ^1H–^1H coupling constants as carried out for **3a** and **4a**. Finally, methanolysis 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.[1)]

Isolation of Rehmaglutins A (3), B (4), C, and D (5), Rehmaionoside C, Jio-cerebroside, and Acteoside (2) The air-dried roots of Chinese Rehmanniae Radix (3 kg, imported from China, purchased from Tochimoto-Tenkaido, Osaka) were cut finely and extracted with 50% aqueous acetone three times (15 l 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–H_2O (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 (lipids *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 reversed-phase silica gel column chromatography [Bondapak C_{18} 200 g with H_2O–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 reversed-phase silica gel column chromatography [Bondapak C_{18} 100 g, H_2O–MeOH (3:1)] and ordinary-phase column chromatography [SiO_2 50 g, $CHCl_3$–*n*-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 reversed-phase silica gel column chromatography [Bondapak C_{18} 200 g, elution with H_2O and H_2O–MeOH (10:1→2:1)] to furnish acteoside (**2**, 410 mg) and rehmaionoside C (25 mg). Acteoside (**2**) was obtained as a white powder and was identified by comparing its physical data, $[\alpha]_D^{20}$ −80° (c=1.1, MeOH), ultraviolet (UV), IR, secondary ion mass spectrometry (SIMS), ^1H- and ^{13}C-NMR, with those reported.[19)] The water-soluble portion (1.9 kg), obtained after removal of the solvent from the aqueous phase under reduced pressure, was subjected to active charcoal column chromatography [charcoal 2 kg–Celite 2 kg, elution with H_2O, H_2O–MeOH (1:1), and then MeOH] to give a glycoside mixture (43 g). 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), $[\alpha]_D^{19}$ +43.6° (c=0.28, MeOH). High MS: Found 202.085. Calcd for $C_9H_{14}O_5$ (M^+) 202.084. IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1035. ^1H-NMR (90 MHz, d_6-acetone) δ: 1.48—1.61 (2H, m, 4-H_2), 2.00—2.18 (2H, m, 5, 9-H), 3.22 (1H, dd, J=1, 10 Hz, 10β-H), 3.60—3.82 (2H, m, 3-H_2), 3.79 (1H, dd, J=10, 10 Hz, 6-H), 3.91 (1H, dd, J=1, 10 Hz, 7-H), 4.35 (1H, d, J=10 Hz, 10α-H), 5.19 (1H, d, J=5 Hz, 1-H). ^{13}C-NMR: see Table II. CI-MS m/z (%): 203 [$(M+H)^+$, 98], 185 [$(M+H-H_2O)^+$, 100], 167 (45).

Rehmaglutin B (**4**): mp 152—153 °C (colorless prisms from MeOH), $[\alpha]_D^{19}$ +33.8° (c=0.79, MeOH). High MS: Found 236.035. Calcd for $C_9H_{13}{}^{35}ClO_5$ (M^+) 236.035. IR ν_{max}^{KBr} cm^{-1}: 3280, 2920, 1049, 1031. ^1H-NMR (90 MHz, d_6-acetone) δ: 1.38—1.63 (1H, m, 4α-H), 1.92—2.08 (1H, m, 4β-H), 2.23—2.37 (1H, m, 5-H), 2.43 (1H, dd, J=5, 10 Hz, 9-H), 3.42 (1H, dd, J=1, 10 Hz, 10β-H), 3.81 (1H, dd, J=9, 10 Hz, 6-H), 4.12 (1H, dd, J=1, 10 Hz, 7-H), 4.21 (1H, d, J=10 Hz, 10α-H), 5.25 (1H, dd, J=4, 9 Hz, 3-H), 5.48 (1H, d, J=5 Hz, 1-H). ^{13}C-NMR: see Table II. CI-MS m/z (%): 239 (9), 237 (25) [$(M+H)^+$], 221 (34), 219 (100) [$(M+H-H_2O)^+$].

Rehmaglutin D (**10**): mp 132—133 °C (colorless prisms from Et_2O), $[\alpha]_D^{19}$ +60.6° (c=0.19, MeOH). High MS: Found 220.051. Calcd for $C_9H_{13}{}^{35}ClO_4$ (M^+) 220.050. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1045, 1025. ^1H-NMR (90 MHz, d_6-acetone) δ: 1.62—1.81 (2H, m, 4-H_2), 2.10—2.30 (1H, m, 5-H), 2.35 (1H, dd, J=5, 10 Hz, 9-H), 3.43 (1H, dd, J=2, 10 Hz, 10β-H), 3.60—4.00 (2H, m, 3-H_2), 3.81 (1H, dd, J=9, 10 Hz, 6-H), 4.18 (1H, dd, J=2, 10 Hz, 7-H), 4.36 (1H, d, J=10 Hz, 10α-H), 5.28 (1H, d, J=5 Hz, 1-H). ^{13}C-NMR: see Table II. CI-MS m/z (%): 223 (33), 221 (100) [$(M+H)^+$], 205 (7), 207 (18) [$(M+H-H_2O)^+$], 187 (2), 185 (5).

SIMS *m/z* (%): 223 (20), 221 (66); 205 (10), 203 (31); 169 (10), 167 (30); 55 (100).

Acetylation of Rehmaglutin A (3) A solution of **3** (12 mg) in pyridine (1.0 ml) was treated with Ac_2O (1.0 ml) and the mixture was stirred at room temperature (20 °C) for 8 h, then poured into ice-water. The whole was extracted with AcOEt. The AcOEt extract was washed with diluted aqueous HCl, water, 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 [SiO_2 4 g, *n*-hexane–AcOEt (2:1)] to furnish the triacetate (**3a**, 19 mg).

3a: mp 128—130 °C (colorless needles from Et_2O), $[\alpha]_D^{19}$ +3.6° (*c*=0.35, MeOH). High MS: Found 328.117. Calcd for $C_{15}H_{28}O_8$ (M^+) 328.116. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1240, 1035. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.04, 2.06, 2.10 (3H each, all s, OAc×3), and other signals as given in Table I. NOE (%): as shown in Fig. 1. ^{13}C-NMR δ_C: (d_6-acetone) 21.1 (2C), 21.8, 171.0, 171.3, 171.6; ($CDCl_3$) 20.8 (2C), 21.3, 170.2 (2C), 170.7, and other data as given in Table II. CI-MS *m/z* (%): 329 [$(M+H)^+$, 1], 269 [$(M+H-AcOH)^+$, 100], 209 (49).

Conversion of 3 to 3b A solution of **3** (14 mg) in pyridine (1.5 ml) was treated with $TIPDSiCl_2$ (30 mg) and the mixture was stirred at room temperature (20 °C) under an 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 treated with Ac_2O (1.5 ml) and DMAP (a catalytic amount). The reaction mixture was stirred at room temperature (20 °C) under an 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 *n*-Bu_4NF (104 mg) and the mixture was stirred at room temperature (20 °C) under an 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 [SiO_2 1 g, *n*-hexane–acetone (2:1)] to furnish **3b** (9 mg).

3b: mp 134—136 °C (colorless prisms from MeOH), $[\alpha]_D^{20}$ +12.4° (*c*=0.34, MeOH). High MS: Found 244.095. Calcd for $C_{11}H_{16}O_6$ (M^+) 244.095. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1718, 1245, 1037. ^{1}H-NMR (90 MHz, d_6-acetone) δ: 1.58—1.73 (2H, m, 4-H_2), 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, 3β-H), 3.48 (1H, dd, *J*=2, 10 Hz, 10β-H), 3.72—3.90 (1H, m, 3α-H), 3.78 (1H, dd, *J*=9, 10 Hz, 6-H), 4.16 (1H, dd, *J*=2, 9 Hz, 7-H), 4.51 (1H, d, *J*=10 Hz, 10α-H), 5.35 (1H, d, *J*=6 Hz, 1-H). ^{13}C-NMR ($CDCl_3$) δ_C: 20.9, 173.0, and others as given in Table II. CI-MS *m/z* (%): 245 [$(M+H)^+$, 4], 227 [$(M+H-H_2O)^+$, 3], 185 [$(M+H-AcOH)^+$, 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. The whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the product was purified by column chromatography [SiO_2 1 g, *n*-hexane–AcOEt (4:1)] to furnish **3c** (11 mg).

3c: A colorless oil, $[\alpha]_D^{20}$ +53.2° (*c*=0.59, MeOH). High MS: Found 453.157. Calcd for $C_{25}H_{25}O_8$ $(M+H)^+$ 453.156. UV λ_{max}^{MeOH} nm (ε): 229 (21000). CD (MeOH): $[\theta]_{237nm}$ +61600 (pos. max.), $[\theta]_{222nm}$ −24200 (neg. max.). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1600, 1278, 1091. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.58—1.72 (2H, m, 4-H_2), 2.10 (3H, s, OAc), 2.80—3.00 (2H, m, 5, 9-H), 3.60—3.80 (1H, m, 3β-H), 3.67 (1H, dd, *J*=1, 11 Hz, 10β-H), 4.09—4.41 (1H, m, 3α-H), 4.73 (1H, d, *J*=11 Hz, 10α-H), 5.42 (1H, d, *J*=4 Hz, 1-H), 5.91 (1H, dd, *J*=10, 10 Hz, 6-H), 6.36 (1H, br d, *J*=*ca.* 10 Hz, 7-H), 7.30—7.60 (6H), 7.95—8.09 (4H) (both m, aromatic protons). ^{13}C-NMR ($CDCl_3$) δ_C: 21.5, 128.5 (4C), 128.6 (4C), 130.0 (2C), 133.4 (2C), 165.8 (2C), 170.6, and others as given in Table II. CI-MS *m/z* (%): 453 [$(M+H)^+$, 2] 393 [$(M+H-AcOH)^+$, 100], 271 (74).

Conversion of Catalpol (1) to Rehmaglutin A (3) A solution of catalpol (**1**, 475 mg) in MeOH (15 ml) containing 5% palladium–carbon (252 mg) was stirred at room temperature under a hydrogen atmosphere (3 kg/cm^2) for 40 min. After removal of the catalyst by filtration, the solvent was evaporated off from the filtrate under reduced pressure to yield dihydrocatalpol (**6**, 478 mg) which was identical with an authentic sample[17] as judged from TLC [$CHCl_3$–MeOH–H_2O (65:35:10, lower phase), *n*-BuOH–AcOH–H_2O (4:1:5, upper phase)], IR (KBr), ^{1}H- (pyridine-d_5) and ^{13}C- (pyridine-d_5) NMR comparisons. A solution of **6** (36 mg) in H_2O (1.0 ml) was treated with 20% aqueous NaOH (1.0 ml) and the mixture was stirred at 85 °C under an N_2 atmosphere for 2 h, then neutralized with Dowex 50W×8 (H^+ form). The resin was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the product (crude **7**) was dissolved in 9% HCl–dry MeOH (1.0 ml) and the solution was stirred at room temperature (27 °C) for 30 min, then neutralized with Ag_2CO_3 and filtered. Removal of the solvent from the filtrate gave a product (31 mg), which was purified by column chromatography [SiO_2 10 g, $CHCl_3$–MeOH–H_2O (7:3:1, lower phase)] to furnish **3** (3 mg). **3** thus obtained was shown to be identical with authentic rehmaglutin A, which was isolated previously from Rehmanniae Radix, by TLC [$CHCl_3$–MeOH–H_2O (7:3:1, lower phase), $CHCl_3$–MeOH (10:1), benzene–MeOH (3:1)], IR (KBr), and ^{1}H-NMR (d_6–acetone) comparisons.

Acetylation of Rehmaglutin B (4) Giving 4a and 4b A solution of **4** (12 mg) in pyridine (1.0 ml) was treated with Ac_2O (1.0 ml) and the whole was stirred at room temperature (22 °C) for 4 h. The reaction mixture was poured into ice-water and 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 [SiO_2 1 g, benzene–acetone (5:1)] to furnish **4a** (13 mg) and **4b** (2 mg).

4a: mp 147—148 °C (colorless needles from Et_2O), $[\alpha]_D^{19}$ +61.9° (*c*=0.54, $CHCl_3$). High MS: Found 320.064. Calcd for $C_{13}H_{17}{}^{35}ClO_7$ (M^+) 320.066. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1740, 1235, 1034. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.07, 2.14 (3H, each, both s, OAc×2), and others as given in Table I. NOE (%): as shown in Fig. 1. ^{13}C-NMR δ_C: (d_6-acetone) 21.1, 21.5, 170.2, 170.6; ($CDCl_3$) 20.8, 21.1, 169.5, 170.4, and others as given in Table II. CI-MS *m/z* (%): 323 (0.3), 321 (1) [$(M+H)^+$], 263 (35), 261 (100) [$(M+H-AcOH)^+$].

4b: A colorless oil, $[\alpha]_D^{19}$ +40.6° (*c*=0.72, $CHCl_3$). High MS: Found 362.077. Calcd for $C_{15}H_{19}{}^{35}ClO_8$ (M^+) 362.077. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2928, 1735, 1230, 1036. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.07, 2.12, 2.14 (3H each, all s, OAc×3) and others as given in Table I. ^{13}C-NMR δ_C: (d_6-acetone) 21.1, 21.5, 22.2, 170.2, 171.5, 172.0; ($CDCl_3$) 20.4, 20.9, 21.6, 169.1, 170.1, 170.8, and others as given in Table II. CI-MS *m/z* (%): 365 (0.3), 363 (1) [$(M+H)]^+$], 305 (16), 303 (45) [$(M+H-AcOH)^+$], 245 (20), 243 (54) [$(M+H-2AcOH)^+$], 185 (34), 183 (100) [$(M+H-3AcOH)^+$].

Preparation of 4d from 4 A solution of **4** (7 mg) in pyridine (1.5 ml) was treated with $TIPDSiCl_2$ (22 mg) and the mixture was stirred at room temperature (21 °C) under an N_2 atmosphere for 7 h, then treated with MeOH (2 ml). The reaction mixture was left to stand for 15 min, and poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 2N HCl, aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$. Evaporation of the solvent from the AcOEt extract under reduced pressure gave the product, which was purified by column chromatography [SiO_2 1 g, benzene–acetone (8:1)] to furnish **4c** (9 mg).

4c: A colorless oil, $[\alpha]_D^{20}$ +32.1° (*c*=0.41, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2930, 1037. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.04—1.07 (24H, m, *sec*-CH_3×8), 3.51 (1H, dd, *J*=1, 10 Hz, 10β-H), 3.56 (3H, s, OCH_3), 3.77 (1H, dd, *J*=9, 10 Hz, 6-H), 4.08 (1H, dd, *J*=1, 10 Hz, 7-H), 4.19 (1H, d, *J*=10 Hz, 10α-H), 5.48 (1H, d, *J*=4, 1-H), 5.49 (1H, dd, *J*=4, 8 Hz, 3-H). CI-MS *m/z* (%): 513 (2), 515 (5) [$(M+H)^+$], 222 (35), 220 (100). A solution of **4c** (9 mg) in pyridine (1.0 ml) was treated with benzoyl chloride (0.03 ml) and DMAP (a catalytic amount) and the mixture was stirred at room temperature (21 °C) under an N_2 atmosphere for 14 h, then poured into ice-water. The whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the product was dissolved in THF (1.0 ml). This solution was treated with *n*-Bu_4NF (28 mg), then the mixture was stirred at room temperature (20 °C) under an N_2 atmosphere for 1 h, and 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 [SiO_2 1 g, *n*-hexane–AcOEt (2:1)] to furnish **4d** (6 mg).

4d: A colorless oil, $[\alpha]_D^{20}$ +14.6° (*c*=0.15, MeOH). High MS: Found 445.106. Calcd for $C_{23}H_{22}{}^{35}ClO_7$ $(M+H)^+$ 445.106. UV λ_{max}^{MeOH} nm (ε): 231 (19500). CD (MeOH): $[\theta]_{238nm}$ +50000 (pos. max.), $[\theta]_{225nm}$ −10000 (neg. max.). ^{1}H-NMR (90 MHz, d_6-acetone) δ: 4.19 (1H, d, *J*=10 Hz, 10β-H), 4.56 (1H, d, *J*=10 Hz, 10α-H), 4.98 (1H, dd, *J*=8, 8 Hz, 3-H), 5.35 (1H, d, *J*=10 Hz, 7-H), 5.65 (1H, dd, *J*=10, 10 Hz, 6-H), 5.75 (1H, d, *J*=5 Hz, 1-H), 7.55—7.70 (6H), 8.00—8.18 (4H) (both m, aromatic protons). CI-MS *m/z* (%): 447 (2), 445 (5) [$(M+H)^+$], 325 (16), 323 (49) [$(M+H-BzOH)^+$], 123 (100).

Conversion of Catalpol (1) to Rehmaglutin B (4) A solution of **1** (105 mg) in MeOH (14 ml) was treated with 9% HCl–dry MeOH (1.0 ml) and the

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**), which was dissolved in 3% aqueous HCl (5.0 ml). The solution 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 [SiO_2 10 g, $CHCl_3$–MeOH (10 : 1)] to furnish **4** (31 mg). **4** thus obtained was shown to be identical with authentic rehmaglutin B, which was isolated above from Rehmanniae Radix, by TLC [$CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase), $CHCl_3$–MeOH (10 : 1), benzene–acetone (2 : 1)], IR (KBr), and ^{1}H-NMR (d_6-acetone) comparisons.

Acetylation of Rehmaglutin D (5) A solution of **5** (12 mg) in pyridine (1.0 ml) was treated with Ac_2O (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 [SiO_2 1 g, *n*-hexane–AcOEt (2 : 1)] to furnish **5a** (16 mg).

5a: mp 96—97 °C (colorless prisms from Et_2O), $[\alpha]_D^{13}$ +28.0° (c = 0.48, MeOH). High MS: Found 304.071. Calcd for $C_{13}H_{17}{}^{35}ClO_6$ (M^+) 304.071. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1733, 1235, 1039. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.10, 2.13 (3H each, both s, OAc × 2), and others as given in Table I. NOE (%): as shown in Fig. 1. ^{13}C-NMR δ_C: (d_6-acetone) 22.1, 22.2, 171.3 (2C); ($CDCl_3$) 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) [$(M+H-AcOH)^+$], 187 (32), 185 (100) [$(M+H-2AcOH)^+$].

Conversion of Dihydrocatalpol (6) to Rehmaglutin 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 [SiO_2, 50 g, benzene–acetone (2 : 1)] to furnish **5** (74 mg). **5** thus obtained was shown to be identical with authentic rehmaglutin D, isolated above from Rehmanniae Radix, by mixed melting point determination, and by $[\alpha]_D^{20}$ (+60.0°), TLC [$CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower phase), $CHCl_3$–MeOH (10 : 1), benzene–acetone (2 : 1)], IR (KBr), and ^{1}H-NMR (d_6-acetone) comparisons.

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5) After we reported the chemical elucidation of many new constituents of Chinese Rehmanniae Radix[6-8] and published a review,[9] several additional chemical constituents of Chinese Rehmanniae Radix were reported.[10]
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18) The structure of this product was presumed to be **8**, which is identical with the structure of glutinoside.[7] 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.
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