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ABSOLUTE STEREOSTRUCTURES OF REHMA GLUTIN C AND GLUTINOSIDE
A NEW IRIDOID LACTONE AND A NEW CHLORINATED IRIDOID GLUCOSIDE
FROM CHINESE REHMANNIAE RADIX

Masayuki Yoshikawa, Youichi Fukuda, Toshio Taniyama, and Isao Kitagawa*

Faculty of Pharmaceutical Sciences, Osaka University,
1-6, Yamada-oka, Suita, Osaka 565, Japan

A new iridoid lactone, rehmaglutin C (4), and a new chlorinated iridoid glucoside, glutinoside (5), were isolated from Chinese *Rehmannia glutinosa* Libosch. [Kan-jiō (in Japanese) from China]. Their absolute stereostructures were determined on the basis of chemical and physicochemical evidence and by use of the exciton chirality method for allylic benzoyl derivatives.

KEYWORDS — *Rehmannia glutinosa*; Scrophulariaceae; iridoid lactone; iridoid glucoside chlorinated; rehmaglutin C; glutinoside; exciton chirality method

In the preceding paper,¹⁾ we reported the isolation of four iridoids named rehmaglutins A, B, C, and D from Chinese *Rehmannia Radix*, the dried root of *Rehmannia glutinosa* Libosch. (Scrophulariaceae), which is now in common use in Chinese medicinal preparations in Japan, and described the absolute stereostructures of rehmaglutins A (1), B (2), and D (3). In a continuing study, we isolated a new chlorinated iridoid glucoside named glutinoside from the water-soluble portion of the same *Rehmannia Radix*. This paper deals with the absolute stereostructure elucidation of rehmaglutin C (4) and glutinoside (5).²⁾

The 50% aq. acetone extract of the radix was partitioned with ethyl acetate to furnish the organic phase soluble portion and the aqueous phase soluble portion as described previously.¹⁾ Repeated chromatography of the organic phase afforded acteoside, cerebroside, rehmaglutins A (1), B (2), C (4), and D (3).¹⁾ The aqueous phase, after repeated chromatographic purification with active charcoal, reversed-phase silica gel and ordinary silica gel, furnished catalpol (6)³⁾ (0.004% from the radix), leonuride^{4,5)} (0.003%), monomelittoside⁶⁾ (0.002%), melittoside⁴⁾ (0.019%), rehmannioside D⁴⁾ (0.031%), and glutinoside (5, 0.05%) together with new glucosides named rehmaionosides A, B, and C and rehmapicroside.⁷⁾

Rehmaglutin C (4), colorless oil, $C_9H_{12}O_5$,⁸⁾ $[\alpha]_D^{24} -51.4^\circ$ (MeOH), CI-MS m/z (%): 201 [(M+H)⁺, 12], 183 [(M+H-H₂O)⁺, 100], had one trisubstituted olefin moiety, one secondary hydroxyl group, two primary hydroxyl groups, and one γ -lactone moiety as shown by the IR ν (film) cm^{-1} : 3340, 1758, ¹H NMR and ¹³C NMR spectra (Table I). Ordinary acetylation (Ac₂O-pyridine, r.t., 12 h) of rehmaglutin C (4) afforded the triacetate (4a), colorless oil, $C_{15}H_{18}O_8$, $[\alpha]_D^{20} -42.6^\circ$ (MeOH), CI-MS m/z (%): 327 [(M+H)⁺, 47], 267 [(M+H-AcOH)⁺, 100], IR ν (CHCl₃) cm^{-1} :

1775, 1739, 1235.

The detailed ^1H NMR decoupling experiments (500 MHz, CDCl_3) with **4a** resulted in the following assignment (J in Hz): δ 4.25, 4.47 (both d, $J=11.9$; 1-H_2), 2.65 (dd, $J=5.2, 18.6$; $4\alpha\text{-H}$), 3.05 (dd, $J=11.3, 18.6$; $4\beta\text{-H}$), 2.86 (ddd, $J=1.8, 5.2, 11.3$; $5\beta\text{-H}$), 5.35 (br s,⁹ $6\alpha\text{-H}$), 6.07 (dd, $J=1.5, 4.0$; 7-H), 4.76, 4.80 (AB in ABX, $J_{\text{AB}}=13.4$, $J_{\text{AX}}=J_{\text{BX}}=1.5$; 10-H_2). The NOE's were observed between the following pairs of protons¹⁰: $4\alpha\text{-H}$ & $4\beta\text{-H}$ (18%), $4\alpha\text{-H}$ & $6\alpha\text{-H}$ (11%), $4\beta\text{-H}$ & $5\beta\text{-H}$ (18%), $5\beta\text{-H}$ & $4\beta\text{-H}$ (11%), 1-H (at δ 4.25) & $5\beta\text{-H}$ (7%), 1-H (at δ 4.47) & $4\beta\text{-H}$ (9%), and 1-H (at δ 4.47) & $5\beta\text{-H}$ (8%). Based on these spectral data, the stereostructure **4a** was assigned to rehma-glutin C triacetate and subsequently the structure **4** to rehma-glutin C.

The absolute configuration of rehma-glutin C (**4**) was determined by applying the exciton chirality method¹¹ to the allylic benzoyl derivative of **4**. Thus, tritylation of **4** with *p*-anisylchlorodiphenylmethane (MMTrCl) in pyridine (r.t., 48 h) and subsequent benzylation (benzoyl chloride-pyridine, r.t., 8 h) furnished **4b**, which, by detritylating with *p*-toluenesulfonic acid in MeOH-tetrahydrofuran (r.t., 1 h), was converted to 6-O-benzoylrehma-glutin C (**4c**), colorless oil, $\text{C}_{16}\text{H}_{16}\text{O}_6$, $[\alpha]_{\text{D}}^{21} -82.4^\circ$ (MeOH), UV λ_{max} (MeOH): 231 nm (ϵ 6700), CI-MS m/z (%): 305 $[(\text{M}+\text{H})^+, 22]$, 183 $[(\text{M}+\text{H}-\text{C}_6\text{H}_5\text{COOH})^+, 26]$, IR ν (CHCl_3) cm^{-1} : 3400, 2920, 1769, 1713, 1599. The CD spectrum (MeOH) of **4c** showed a negative first Cotton curve; $[\theta]_{228} -9500$, to substantiate the 6S configuration of **4c**. Thus the absolute stereostructure of rehma-glutin C (**4**) was determined as shown.

Glutinoside (**5**), a hygroscopic white powder, $[\alpha]_{\text{D}}^{20} -79.2^\circ$ (MeOH), IR ν (KBr) cm^{-1} : 3388, 2922, 1047, was positive in the Beilstein test. The secondary ion mass spectrum (SIMS Xe^+ , glycerol matrix, m/z) of **5** had isotope ion peaks due to a chlorine atom: $(\text{M}+\text{H})^+$ at 399 and 401, $(\text{M}+\text{Na})^+$ at 421 and 423, and $(\text{M}+\text{H}+\text{glycerol})^+$ at 491 and 493. The ^1H NMR and ^{13}C NMR (Table I) data for **5** indicated the presence of two acetal moieties, one each of secondary and tertiary hydroxyl groups in the iridoid glucoside skeleton.

Ordinary acetylation (Ac_2O -pyridine, r.t., 3 h) of glutinoside (**5**) provided the pentaacetate (**5a**), colorless prisms, mp 185-186°C, $\text{C}_{25}\text{H}_{33}\text{O}_{15}\text{Cl}$, $[\alpha]_{\text{D}}^{20} -18.5^\circ$ (CHCl_3), IR ν (CCl_4) cm^{-1} : 3483, 2940, 1755, 1224, 1036, ^1H NMR (500 MHz, CDCl_3 , δ): 2.01 (3H), 2.03 (6H), 2.10, 2.11 (3H each) (all s, $\text{OCOCH}_3 \times 5$), 5.55 (d, $J=1.8$ Hz; $1\alpha\text{-H}$), 5.33 (d, $J=3.4$ Hz; $3\beta\text{-H}$), 2.16 (dd, $J=3.4, 14.7$ Hz; $4\alpha\text{-H}$),¹² 2.22 (ddd, $J=3.1, 9.8, 10.3$ Hz; $5\beta\text{-H}$), 4.98 (dd, $J=3.1, 7.9$ Hz; $6\alpha\text{-H}$), 4.28 (d, $J=7.9$ Hz; $7\beta\text{-H}$), 2.66 (br d, $J=ca. 9.8$ Hz; $9\beta\text{-H}$), 3.67 (d, $J=12.2$ Hz; $10\beta\text{-H}$), 4.07 (d, $J=12.2$ Hz, $10\alpha\text{-H}$), and the hexaacetate (**5b**), colorless prisms, mp 150-153°C, $\text{C}_{27}\text{H}_{35}\text{O}_{16}\text{Cl}$, $[\alpha]_{\text{D}}^{20} -34.4^\circ$ (CHCl_3), IR ν (CCl_4) cm^{-1} : no OH, 2942, 1750, 1222, 1036, ^1H NMR (500 MHz, CDCl_3 , δ): 2.01 (3H), 2.03 (6H), 2.08 (3H), 2.11 (6H) (all s, $\text{OCOCH}_3 \times 6$), 5.55 (d, $J=2.2$ Hz; $1\alpha\text{-H}$), 5.34 (d, $J=3.1$ Hz, $3\beta\text{-H}$), 2.17 (dd, $J=3.1, 14.0$ Hz; $4\alpha\text{-H}$),¹² 2.26 (ddd, $J=2.7, 10.4, 10.4$ Hz; $5\beta\text{-H}$), 4.91 (dd, $J=2.7, 7.9$ Hz; $6\alpha\text{-H}$), 5.31 (dd, $J=1.5, 7.9$ Hz; $7\beta\text{-H}$), 3.51 (br d, $J=ca. 10.4$ Hz; $9\beta\text{-H}$), 3.87 (dd, $J=1.5, 12.5$ Hz; $10\beta\text{-H}$), 4.26 (d, $J=12.5$ Hz; $10\alpha\text{-H}$). Detailed comparison of the ^{13}C NMR data (Table I) for **5a** and **5b** with those for rehma-glutins A (**1**),¹ B (**2**),¹ C (**4**), and D (**3**)¹ indicated the presence of 6,8-dihydroxyl groups and a 7-chloro residue in the iridoid skeleton.

Methanolysis of glutinoside (**5**) with 9% HCl-dry MeOH (r.t., 4 h) yielded

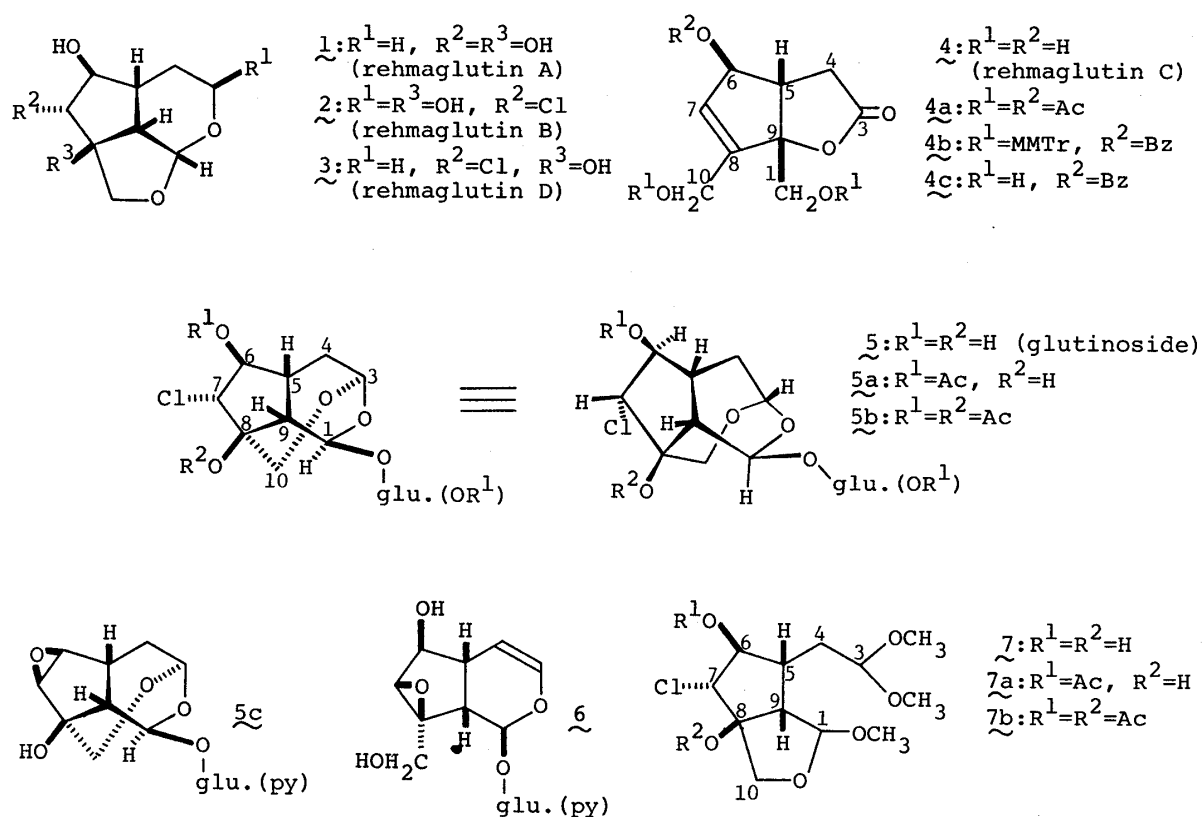


Chart 1

Table I. ^{13}C NMR Data for Rehmaglutin C ($\sim$ 4), the Triacetate ($\sim$ 4a), Glutinoside ($\sim$ 5), and the Derivatives ($\sim$ 5a, $\sim$ 5b, $\sim$ 5c, $\sim$ 7, $\sim$ 7a, $\sim$ 7b)

	$\sim$ 4 ^{a)}	$\sim$ 4a ^{b)}	$\sim$ 7 ^{a)}	$\sim$ 7a ^{a)}	$\sim$ 7b ^{a)}		$\sim$ 5 ^{c)}	$\sim$ 5a ^{c)}	$\sim$ 5b ^{c)}	$\sim$ 5c ^{c)}
C-1	59.0	59.3	104.9	104.6	104.7	C-1	94.6	94.8	94.8	94.3
3	177.0	174.3	107.8	107.5	107.5	3	92.6	93.5	93.1	93.1
4	35.4	34.0	32.8	32.4	32.5	4	33.9	33.5	33.8	29.4
5	50.5	46.6	40.1	38.6	38.4	5	35.5	33.5	33.8	29.8
6	81.0	82.0	75.3	79.3	80.0	6	84.3	86.4	86.2	61.8
7	134.7	131.8	79.9	71.6	67.0	7	75.6	70.3	64.5	58.6
8	147.4	143.6	86.1	85.9	91.0	8	79.3	79.1	86.0	79.3
9	99.6	94.9	57.8	57.7	55.8	9	47.5	47.4	43.1	47.5
10	65.8	65.5	73.9	73.5	71.9	10	61.9	62.1	59.9	64.4
						C-1'	98.6	96.5	96.3	98.5
						2'	74.2	71.5	71.8	74.2
						3'	77.9	73.2	73.3	77.8 ^{d)}
						4'	70.9	68.9	69.0	70.9
						5'	77.9	72.3	72.5	77.9 ^{d)}
						6'	61.9	62.1	62.0	61.5

a)~c) Measured at 22.5 MHz in a) d_6 -acetone,b) $CDCl_3$, or c) d_5 -pyridine.

d) The assignment may be interchanged.

methyl glucoside and the acetal (7), colorless oil, $C_{12}H_{21}O_6Cl$, $[\alpha]_D^{20} +31.8^\circ$ (MeOH), IR ν ($CHCl_3$) cm^{-1} : 3411, 2929, 1100, CI-MS m/z (%): $(M+H)^+$ at 297 (3) and 299 (1); $(M+H-\dot{O}CH_3)^+$ at 265 (100) and 267 (34); $(M+H-\dot{O}CH_3-CH_3OH)^+$ at 233 (98) and 235 (34), 1H NMR (90 MHz, d_6 -acetone, δ): 1.62-2.09 (m, 4-H₂), 2.38-2.55 (m, 5 β -H & 9 β -H), 3.29 (6H), 3.31 (3H) (both s, $OCH_3 \times 3$), 3.72 (dd, $J=1.5, 9.5$ Hz, 10 β -H), 4.25 (d, $J=9.5$ Hz, 10 α -H), 4.08 (dd, $J=1.5, 9.9$ Hz; 7 β -H), 4.48 (t, $J=5.9$ Hz; 3-H), 4.93 (br s, 1-H), ^{13}C NMR (Table I). Acetylation of 7 (Ac_2O -pyridine, r.t., 5 h) provided the monoacetate (7a) and the diacetate (7b). The comparison of the spectral data for 7, 7a, and 7b with those for rehmaglucin D (3)¹⁾ indicated the presence of 6,8-dihydroxyl groups and a 7-chloro residue in the structure. Finally, methanolysis of catalpol (6) under the same reaction conditions described for the methanolysis of glutinoside (5) yielded 7 in 35% yield (major) together with the several minor products. Thus the structure of the methanolysis product of glutinoside (5) was proposed as 7.

Treatment of glutinoside (5) with 10% aq. KOH under reflux for 2.5 h afforded the 6 β ,7 β -epoxide (5c) (in 53% yield),¹³⁾ colorless prisms, mp 139-140°C, $C_{15}H_{22}O_{10} \cdot 2H_2O$, $[\alpha]_D^{20} -46.2^\circ$ (MeOH), IR ν (KBr) cm^{-1} : 3400, 2920, 1073. The ^{13}C NMR data (Table I) for 5c indicated the presence of the 6-hydroxyl group and the 7-chloro residue in the structure of glutinoside (5). On the other hand, treatment of catalpol (6) with 0.6% HCl-dry MeOH at room temperature for 24 h provided glutinoside (5) in 55% yield.

Based on the above evidence, the absolute stereostructure of glutinoside (5) was determined as shown.

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- 8) The molecular composition of the compound given with the chemical formula was determined by elemental analysis or by high resolution mass spectrometry.
- 9) The coupling between the 5-H and the 7-H was shown by the decoupling experiments.
- 10) The magnitude of the NOE (%) shown in the parenthesis was obtained when the underlined proton was irradiated.
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- 12) The signal due to 4 β -H overlapped the signal of the acetoxyl methyls.
- 13) a) As we reported before,^{13b)} the alkaline treatment (2% KOH/MeOH-H₂O, 45-50°C, 20 min) of linarioside, which had the 6 β ,8 β -dihydroxyl groups and the 7 α -chloro residue in the iridoid glucoside structure, gave the 7 β ,8 β -epoxide derivative. However, the present alkaline treatment of 5 yielded the 6 β ,7 β -epoxide (5c), presumably due to stereochemical reason; b) I. Kitagawa, T. Tani, K. Akita, and I. Yosioka, *Chem. Pharm. Bull.*, **21**, 1978 (1973).

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