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ABSOLUTE STEREOSTRUCTURES OF REHMAGLUTIN 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 Rehmanniae Radix, the dried root of *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 Rehmanniae 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 Rehmanniae 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%), monomelittoside[6] (0.002%), melittoside[4] (0.019%), rehmannioside D[4] (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° (MeOH), CI-MS *m/z* (%): 201 [$(M+H)^+$, 12], 183 [$(M+H-H_2O)^+$, 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 ^{13}C NMR spectra (Table I). Ordinary acetylation (Ac_2O-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° (MeOH), CI-MS *m/z* (%): 327 [$(M+H)^+$, 47], 267 [$(M+H-AcOH)^+$, 100], IR ν ($CHCl_3$) cm^{-1}:

1775, 1739, 1235.

The detailed ^{1}H 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; 4α-H), 3.05 (dd, J=11.3, 18.6; 4β-H), 2.86 (ddd, J=1.8, 5.2, 11.3; 5β-H), 5.35 (br s,[9] 6α-H), 6.07 (dd, J=1.5, 4.0; 7-H), 4.76, 4.80 (AB in ABX, J_{AB}=13.4, J_{AX}=J_{BX}=1.5; 10-H_2). The NOE's were observed between the following pairs of protons[10]: 4α-H & 4β-H (18%), 4α-H & 6α-H (11%), 4β-H & 5β-H (18%), 5β-H & 4β-H (11%), 1-H (at δ 4.25) & 5β-H (7%), 1-H (at δ 4.47) & 4β-H (9%), and 1-H (at δ 4.47) & 5β-H (8%). Based on these spectral data, the stereostructure 4a was assigned to rehmaglutin C triacetate and subsequently the structure 4 to rehmaglutin C.

The absolute configuration of rehmaglutin C (4) was determined by applying the exciton chirality method[11] to the allylic benzoyl derivative of 4. Thus, tritylation of 4 with *p*-anisylchlorodiphenylmethane (MMTrCl) in pyridine (r.t., 48 h) and subsequent benzoylation (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-benzoylrehmaglutin C (4c), colorless oil, $C_{16}H_{16}O_6$, $[\alpha]_D^{21}$ -82.4° (MeOH), UV λ_{max} (MeOH): 231 nm (ε 6700), CI-MS *m/z* (%): 305 [$(M+H)^+$, 22], 183 [$(M+H-C_6H_5COOH)^+$, 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 6*S* configuration of 4c. Thus the absolute stereostructure of rehmaglutin C (4) was determined as shown.

Glutinoside (5), a hygroscopic white powder, $[\alpha]_D^{20}$ -79.2° (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: $(M+H)^+$ at 399 and 401, $(M+Na)^+$ at 421 and 423, and $(M+H+glycerol)^+$ at 491 and 493. The ^{1}H 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, $C_{25}H_{33}O_{15}Cl$, $[\alpha]_D^{20}$ -18.5° ($CHCl_3$), IR ν (CCl_4) cm^{-1}: 3483, 2940, 1755, 1224, 1036, ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.01 (3H), 2.03 (6H), 2.10, 2.11 (3H each)(all s, $OCOCH_3$ x 5), 5.55 (d, J=1.8 Hz; 1α-H), 5.33 (d, J=3.4 Hz; 3β-H), 2.16 (dd, J=3.4, 14.7 Hz; 4α-H),[12] 2.22 (ddd, J=3.1, 9.8, 10.3 Hz; 5β-H), 4.98 (dd, J=3.1, 7.9 Hz; 6α-H), 4.28 (d, J=7.9 Hz; 7β-H), 2.66 (br d, J=*ca.* 9.8 Hz; 9β-H), 3.67 (d, J=12.2 Hz; 10β-H), 4.07 (d, J=12.2 Hz, 10α-H), and the hexaacetate (5b), colorless prisms, mp 150-153°C, $C_{27}H_{35}O_{16}Cl$, $[\alpha]_D^{20}$ -34.4° ($CHCl_3$), IR ν (CCl_4) cm^{-1}: no OH, 2942, 1750, 1222, 1036, ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.01 (3H), 2.03 (6H), 2.08 (3H), 2.11 (6H)(all s, $OCOCH_3$ x 6), 5.55 (d, J=2.2 Hz; 1α-H), 5.34 (d, J=3.1 Hz, 3β-H), 2.17 (dd, J=3.1, 14.0 Hz; 4α-H),[12] 2.26 (ddd, J=2.7, 10.4, 10.4 Hz; 5β-H), 4.91 (dd, J=2.7, 7.9 Hz; 6α-H), 5.31 (dd, J=1.5, 7.9 Hz; 7β-H), 3.51 (br d, J=*ca.* 10.4 Hz; 9β-H), 3.87 (dd, J=1.5, 12.5 Hz; 10β-H), 4.26 (d, J=12.5 Hz; 10α-H). Detailed comparison of the ^{13}C NMR data (Table I) for 5a and 5b with those for rehmaglutins 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

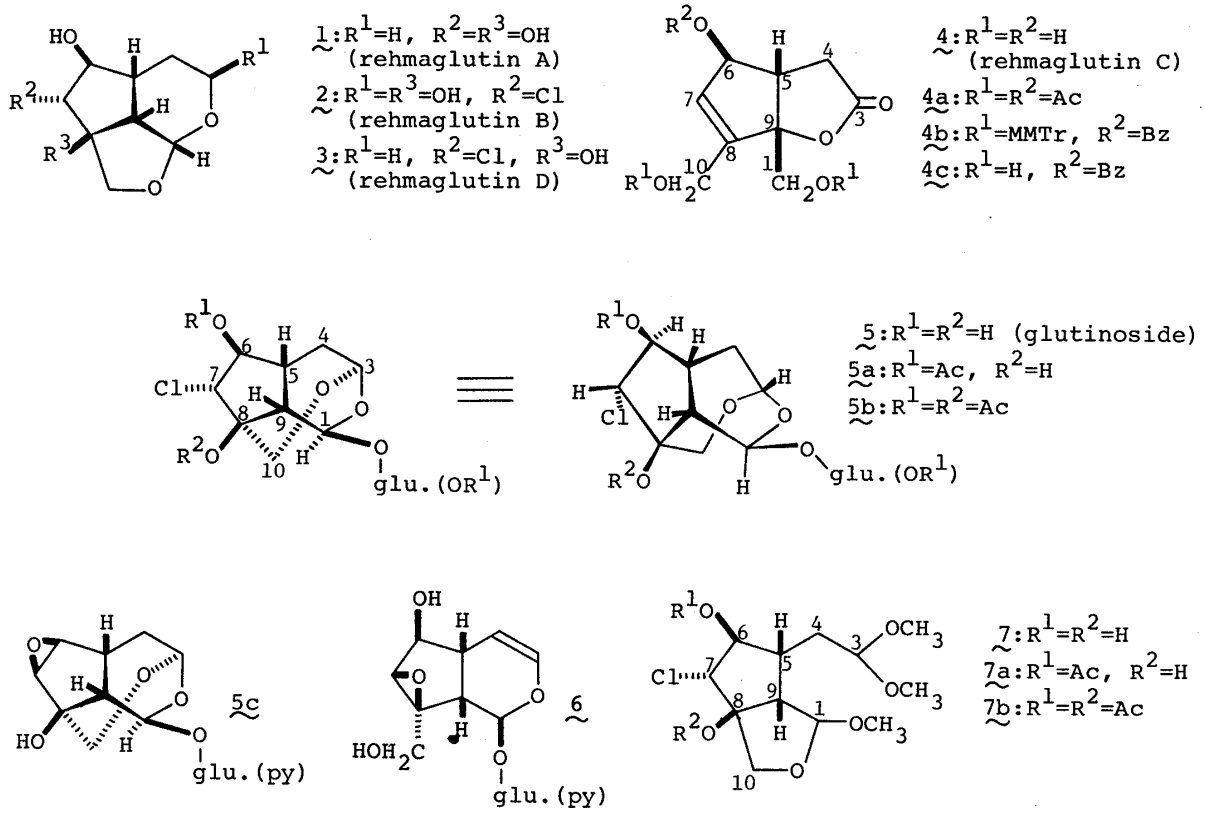

Chart 1

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

	4[a]	4a[b]	7[a]	7a[a]	7b[a]
C-1	59.0	59.3	104.9	104.6	104.7
3	177.0	174.3	107.8	107.5	107.5
4	35.4	34.0	32.8	32.4	32.5
5	50.5	46.6	40.1	38.6	38.4
6	81.0	82.0	75.3	79.3	80.0
7	134.7	131.8	79.9	71.6	67.0
8	147.4	143.6	86.1	85.9	91.0
9	99.6	94.9	57.8	57.7	55.8
10	65.8	65.5	73.9	73.5	71.9

	5[c]	5a[c]	5b[c]	5c[c]
C-1	94.6	94.8	94.8	94.3
3	92.6	93.5	93.1	93.1
4	33.9	33.5	33.8	29.4
5	35.5	33.5	33.8	29.8
6	84.3	86.4	86.2	61.8
7	75.6	70.3	64.5	58.6
8	79.3	79.1	86.0	79.3
9	47.5	47.4	43.1	47.5
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° (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-H_2), 2.38-2.55 (m, 5β-H & 9β-H), 3.29 (6H), 3.31 (3H)(both s, OCH_3 x 3), 3.72 (dd, J=1.5, 9.5 Hz, 10β-H), 4.25 (d, J=9.5 Hz, 10α-H), 4.08 (dd, J=1.5, 9.9 Hz; 7β-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 rehmaglutin 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 6β,7β-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° (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 compositon 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.
11) N. Harada and K. Nakanishi, "Circular Dichroic Spectroscopy - Exciton Coupling in Organic Stereochemistry-," Tokyo Kagaku Dojin, Tokyo, 1982.
12) The signal due to 4β-H overlapped the signal of the acetoxyl methyls.
13) a) As we reported before,[13b] the alkaline treatment (2% KOH/MeOH-H_2O, 45-50°C, 20 min) of linarioside, which had the 6β,8β-dihydroxyl groups and the 7α-chloro residue in the iridoid glucoside structure, gave the 7β,8β-epoxide derivative. However, the present alkaline treatment of 5 yielded the 6β,7β-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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