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ABSOLUTE STEREOSTRUCTURES OF REHMAGLUTINS A, B, AND D THREE NEW IRIDOIDS ISOLATED FROM CHINESE REHMANNIAE RADIX

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Four new iridoids, rehmaglutins A, B, C, and D, were isolated from Chinese Rehmanniae Radix, the dried root of *Rehmannia glutinosa* Libosch. [Kan-jiō (in Japanese) from China]. The absolute stereostructures of rehmaglutins A (2), B (3), and D (4) were elucidated on the basis of chemical and physicochemical evidence which included the application of the benzoate chirality method.

KEYWORDS — *Rehmannia glutinosa*; Scrophulariaceae; iridoid; iridoid chlorinated; rehmaglutin A; rehmaglutin B; rehmaglutin D; dibenzoate chirality method

Rehmanniae Radix (jiō in Japanese) is one of most important crude drugs known as a tonic, an antianemic, and an antipyretic and it is prescribed in many Chinese medicinal preparations. Due to the different processings, Rehmanniae Radix is classified into three types named in Japanese as shō-jiō (fresh root), kan-jiō (dried root), and juku-jiō (steamed root), which are respectively used in different manners in Chinese traditional medicine. In 1971, we isolated catalpol (1) from the fresh root of *Rehmannia glutinosa* Libosch. forma *hueichingensis* (Chao et Schih) Hsiao (kaikei-jiō cultivated in Nara, Japan).[1] Since then, several chemical investigations have been carried out on Japanese Rehmanniae Radix.[2] However, no work has been reported on the chemical constituents of Chinese Rehmanniae Radix, which is now in common use in Japan. As a part of our chemical characterization studies on crude drug processing,[3] we have compared the chemical constituents of differently processed Rehmanniae Radix. This paper deals with the absolute stereostructure elucidation of rehmaglutins A (2), B (3), and D (4) which were isolated together with rehmaglutin C[4] from Chinese Rehmanniae Radix, the dried root of *Rehmannia glutinosa* Libosch. (Scrophulariaceae).[5,6]

The radix was extracted with 50% aq. acetone below 25°C and the aqueous acetone solution was partitioned with ethyl acetate. Repeated chromatography (ordinary silica gel, reversed-phase silica gel, and HPLC) of the organic phase soluble portion furnished acteoside[7] (0.014% from the radix), cerebroside[8] (0.015%), and rehmaglutins A (0.004%), B (0.003%), C (0.001%), and D (0.005%).

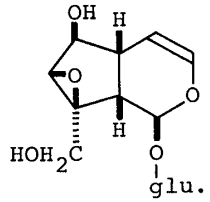
Rehmaglutin A (2), mp 134-136°C, $[\alpha]_D^{19}$ +43.6° (MeOH), $C_9H_{14}O_5$,[9] IR ν (KBr) cm^{-1}: 3450, 2950, 1059, 1035, CI-MS (%): *m/z* 203 [$(M+H)^+$, 98], 185 [$(M+H-H_2O)^+$, 100], had an acetal moiety and two secondary and one tertiary hydroxyl groups as

indicated by the ^{1}H and ^{13}C NMR (Table I) data. Ordinary acetylation of 2 provided the triacetate (2a), mp 128-130°C, $C_{15}H_{20}O_8$, IR ν ($CHCl_3$) cm^{-1}: no OH, 1740, 1240, CI-MS (%): *m/z* 329 [$(M+H)^+$, 1], 269 [$(M+H-AcOH)^+$, 100].

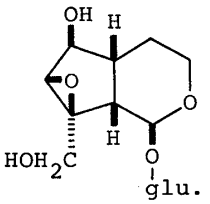
The detailed ^{1}H NMR decoupling experiments (500 MHz, $CDCl_3$) of 2a resulted in the following assignment (J in Hz): δ 5.34 (d, J=5.2; 1β-H), 4.07 (ddd, J=2.4, 11.9, 12.8; 3α-H), 3.63 (dd, J=4.9, 11.9; 3β-H), 1.46 (br d, J=*ca.* 14.6; 4α-H), 1.78 (dddd, J=4.9, 5.2, 12.8, 14.6; 4β-H), 2.64 (ddd, J=5.2, 9.8, 11.0; 5β-H), 5.44 (dd, J=9.5, 11.0; 6α-H), 5.85 (dd, J=1.5, 9.5; 7β-H), 2.74 (dd, J=5.2, 9.8; 9β-H), 4.59 (d, J=10.5; 10α-H), 3.59 (dd, J=1.5, 10.5; 10β-H). Comparison of the ^{13}C NMR data for 2 and 2a (Table I) suggested the presence of the 6-, 7-, and 8-acetoxyl groups and the 1,10-oxide ring in the iridoid structure of 2a. Furthermore, the NOE's observed between the proton pairs of 2a [1β-H & 9β-H (10%),[10] 9β-H & 5β-H (5%), 5β-H & 7β-H (12%), 6α-H & 3α-H (13%), 6α-H & 10α-H (6%)] and the comparison of the ^{1}H-^{1}H coupling constants with those reported for related iridoids,[11] finally led us to formulate the stereostructure of 2a as shown.

The absolute configuration of 2 was determined by the application of the dibenzoate chirality method.[12] Thus, silylation of 2 with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSiCl_2$, 2 mol. eq.)[13] in pyridine (r.t., 5 h) gave 2b, which, after acetylation, was desilylated with *n*-Bu_4NF-tetrahydrofuran (r.t., 2 h) to afford the 8-O-acetate (2c), mp 134-136°C, $C_{11}H_{16}O_6$, IR ν ($CHCl_3$) cm^{-1}: 3470, 1718, 1245. The location of the 8-O-acetyl group was substantiated by the ^{13}C NMR data (Table I). Benzoylation of 2c with benzoyl chloride-pyridine gave the 8-O-acetyl-6,7-di-O-benzoate (2d), colorless oil, $C_{25}H_{24}O_8$, λ_{max} (MeOH): 229 nm (ε 21000), IR ν ($CHCl_3$) cm^{-1}: 1720, 1600, 1278. The CD spectrum of 2d gave the split Cotton curve: $[\theta]_{237}$ +61600 and $[\theta]_{222}$ -24200, thus corroborating the 6*S*, 7*R* configuration of 2d. Thus the absolute configuration of rehmaglutin A (2) was determined. The structure 2 was further confirmed chemically; dihydrocatalpol (1a), prepared by catalytic hydrogenation of catalpol (1)[1] (H_2 3.0 kg/cm^2, 5% Pd-C, 18°C, 30 min), was converted to rehmaglutin A (2) in 15% yield[14] *via* alkaline treatment (10% aq. NaOH, 85°C, 2 h) followed by methanolysis (9% HCl-dry MeOH, r.t., 1 h).

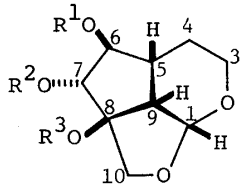
Rehmaglutin B (3), mp 152-153°C, $[\alpha]_D^{19}$ +33.8° (MeOH), $C_9H_{13}O_5Cl$, IR ν (KBr) cm^{-1}: 3280, 2920, 1049, 1031, contained a chlorine atom as shown by the positive Beilstein test and the isotope ion peaks in the CI-MS (%): $(M+H)^+$ at *m/z* 239 (8) and 237 (25), $(M+H-H_2O)^+$ at *m/z* 221 (33) and 219 (100). The ^{1}H and ^{13}C NMR (Table I) data for 3 suggested the presence of two acetal moieties (one with a hydroxyl group) and two secondary hydroxyl groups. Ordinary acetylation of 3 gave the 3,6-di-O-acetate (3a), mp 147-148°C, $C_{13}H_{17}O_7Cl$, IR ν ($CHCl_3$) cm^{-1}: 3300, 1740, 1235, and 3,6,8-tri-O-acetate (3b), colorless oil, $C_{15}H_{19}O_8Cl$, IR ν ($CHCl_3$) cm^{-1}: no OH, 1735, 1230. Silylation of 3 with $TIPDSiCl_2$ (2 mol eq) in pyridine (r.t., 7 h) followed by treatment with MeOH provided 3c. Thus the absence of the α-glycol moiety in 3 was indicated. The ^{1}H NMR data for 3a were assigned as for 2a: δ 5.59 (d, J=4.9; 1β-H), 6.41 (dd, J=6.4, 7.3; 3α-H), 1.63 (ddd, J=4.9, 7.3, 14.7; 4α-H), 2.11 (ddd, J=3.8, 6.4, 14.7; 4β-H), 2.47 (dddd, J=3.8, 4.9, 10.1, 10.5; 5β-H), 5.20 (dd, J=10.1, 10.1; 6α-H), 4.25 (d, J=10.1; 7β-H), 2.71 (dd, J=4.9, 10.5; 9β-H), 4.34 (d, J=11.0; 10α-H), 3.86 (d, J=11.0; 10β-H). In the ^{1}H NMR spectrum of 3b, the proton signals, except those of the 5-, 7-, and 9-H (shifted lower),



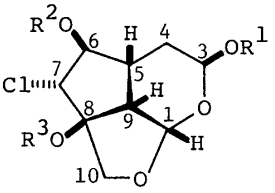
1 (catalpol)



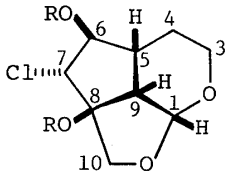
1a (dihydrocatalpol)



2: $R^1=R^2=R^3=H$ (rehmaglutin A)
2a: $R^1=R^2=R^3=Ac$
2b: $R^1, R^2=-Si[CH(CH_3)_2]_2-O-Si[CH(CH_3)_2]_2-$, $R^3=H$
2c: $R^1=R^2=H$, $R^3=Ac$
2d: $R^1=R^2=Bz$, $R^3=Ac$



3: $R^1=R^2=R^3=H$ (rehmaglutin B)
3a: $R^1=R^2=Ac$, $R^3=H$
3b: $R^1=R^2=R^3=Ac$
3c: $R^1=-Si[CH(CH_3)_2]_2-O-Si[CH(CH_3)_2]_2-OCH_3$, $R^2=R^3=H$



4: R=H (rehmaglutin D)
4a: R=Ac

Chart 1

Table I. ^{13}C NMR Data for the Skeletal Carbons of Rehmaglutins A(2), B(3), and D(4) and Their Derivatives[a]

	2	2a	2c	2d	3	3a	3b	4	4a
C-1	101.0(d)[b]	99.1(d)	98.9(d)	99.5(d)	102.1(d)	100.6(d)	100.6(d)	101.3(d)	101.2(d)
3	56.4(t)	55.5(t)	55.8(t)	56.0(t)	85.8(d)	86.2(d)	90.8(d)	56.3(t)	56.3(t)
4	22.4(t)	21.1(t)	21.2(t)	21.6(t)	32.4(t)	26.7(t)	26.5(t)	22.3(t)	21.1(t)
5	34.9(d)	32.5(d)	33.7(d)	33.1(d)	38.9(d)	36.4(d)	37.0(d)	39.0(d)	35.8(d)
6	75.4(d)	73.1(d)	75.2(d)	73.6(d)	74.8(d)	79.0(d)	78.8(d)	73.0(d)	75.6(d)
7	85.0(d)	78.0(d)	82.8(d)	78.3(d)	78.1(d)	70.1(d)	64.9(d)	75.3(d)	67.5(d)
8	85.2(s)	88.6(s)	92.8(s)	88.9(s)	89.9(s)	90.8(s)	92.5(s)	85.5(s)	90.5(s)
9	44.9(d)	41.3(d)	42.6(d)	41.6(d)	48.4(d)	52.8(d)[c]	50.1(d)[c]	46.2(d)	42.7(d)
10	71.0(t)	67.5(t)	67.6(t)	67.8(t)	74.3(t)	76.6(t)[c]	74.8(t)[c]	76.4(t)	69.5(t)

a) The spectra of 2, 3, 3a, 3b, 4, and 4a were taken in d_6-acetone, while those of 2a, 2c, and 2d were in $CDCl_3$, at 22.5 MHz respectively.
b) The characterization of each carbon signal was made by INEPT (Insensitive Nuclei Enhanced by Polarization) and the off-resonance experiments.
c) These signals appeared abnormally shifted lower as compared with those in 3. The reason is yet obscure.

appeared at positions similar to those in the spectrum of 3a.

The detailed comparisons of the ^1H and ^{13}C NMR (Table I) data for 3, 3a, and 3b with those for 2, 2a, and known iridoids led us to assign the iridoid structure 3 to rehmaglutin B, the stereostructure of which was corroborated, as it was for 2a, by examining the NOE's. Finally, the absolute stereostructure of rehmaglutin B (3) was determined by chemical correlation with catalpol (1). Thus, treatment of 1 with 0.6% HCl-dry MeOH (r.t., 20 h) and subsequent hydrolysis with 10% aq. HCl (r.t., 4 h) provided rehmaglutin B (3) in 45% yield.[15]

Rehmaglutin D (4), mp 132-133°C, $[\alpha]_D^{19}$ +60.6° (MeOH), $C_9H_{13}O_4Cl$, IR ν (KBr) cm^{-1}: 3400, 1028, is also a chlorine-containing iridoid as shown from the positive

Beilstein test and $(M+H)^+$ at *m/z* 223 (33) and *m/z* 221 (100) in the CI-MS (%). The ^1H and ^{13}C NMR (Table I) data for 4 indicate the presence of an acetal moiety, one each of secondary and tertiary hydroxyl groups in the iridoid skeleton. The ^1H NMR data for the diacetate (4a), mp 96-97°C, $C_{13}H_{17}O_6Cl$, IR ν ($CHCl_3$) cm^{-1}: no OH, 1733, 1235, were assigned by detailed decoupling experiments: δ 5.46 (d, J=5.2; 1β-H), 4.06 (ddd, J=2.1, 12.0, 12.2; 3α-H), 3.62 (dd, J=5.2, 12.0; 3β-H), 1.47 (br d, J=*ca.* 14.3; 4α-H), 1.77 (dddd, J=4.6, 5.2, 12.2, 14.3; 4β-H), 2.56 (ddd, J=4.6, 9.8, 10.4; 5β-H), 5.39 (dd, J=10.4, 10.4; 6α-H), 4.81 (dd, J=1.5, 10.4; 7β-H), 2.85 (dd, J=5.2, 9.8; 9β-H), 4.61 (d, J=10.7; 10α-H), 3.74 (dd, J=1.5, 10.7; 10β-H).

The ^{13}C NMR data for 4 and 4a (Table I) showed the presence of the 6,8-diacetoxyl and 7-chloro functions and the 1,10-oxide moiety in the iridoid skeleton. The stereostructure of 4a was substantiated by the detailed NOE examinations as carried out for 2a and 3a. Finally, methanolysis of dihydrocatalpol (1a) (3% HCl-dry MeOH, r.t., 3 h) furnished 4 in 53% yield. Thus the absolute stereostructure of rehmaglutin D (4) was determined.

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5) Imported from China (purchased from Tochimoto Tenkaido, Osaka). The botanical identification was kindly undertaken by Dr. Wang Baoqin, National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Temple of Heaven, Beijing, China.
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8) a) The cerebroside was found to have a long-chain base of the same plane structure as occurred in a minor cerebroside (compound B1-b) which was recently isolated from *Tetragonia tetragonoides*[8b]; b) E. Okuyama and M. Yamazaki, Chem. Pharm. Bull., 31, 2209 (1983).
9) The molecular composition of the compound given with the chemical formula was determined either by elemental analysis or by high resolution mass spectrometry.
10) The magnitude of NOE (%) given in the parenthesis was obtained when the underlined proton was irradiated.
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14) The yield was unsatisfactory, presumably due to the partial cleavage of the glucosidic linkage during the alkaline treatment.
15) Previously, we assumed the 6β-OH, 7α-OH, 8β-Cl structure for rehmaglutin B mostly from the ^1H NMR examinations.[6] However, the structure was found to be revised as 3 from this chemical conversion.

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