

## Communications to the Editor

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ABSOLUTE STEREOSTRUCTURES OF REHMA GLUTINS 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 *Rehmannia 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

*Rehmannia 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, *Rehmannia 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 (kaikai-jiō cultivated in Nara, Japan).<sup>1)</sup> Since then, several chemical investigations have been carried out on Japanese *Rehmannia Radix*.<sup>2)</sup> However, no work has been reported on the chemical constituents of Chinese *Rehmannia Radix*, which is now in common use in Japan. As a part of our chemical characterization studies on crude drug processing,<sup>3)</sup> we have compared the chemical constituents of differently processed *Rehmannia 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<sup>4)</sup> from Chinese *Rehmannia Radix*, the dried root of *Rehmannia glutinosa* Libosch. (Scrophulariaceae).<sup>5,6)</sup>

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<sup>7)</sup> (0.014% from the radix), cerebroside<sup>8)</sup> (0.015%), and rehmaglutins A (0.004%), B (0.003%), C (0.001%), and D (0.005%).

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

indicated by the  $^1\text{H}$  and  $^{13}\text{C}$  NMR (Table I) data. Ordinary acetylation of 2 provided the triacetate (2a), mp 128–130°C,  $\text{C}_{15}\text{H}_{20}\text{O}_8$ , IR  $\nu$  ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : no OH, 1740, 1240, CI-MS (%):  $m/z$  329 [ $(\text{M}+\text{H})^+$ , 1], 269 [ $(\text{M}+\text{H}-\text{AcOH})^+$ , 100].

The detailed  $^1\text{H}$  NMR decoupling experiments (500 MHz,  $\text{CDCl}_3$ ) of 2a resulted in the following assignment (J in Hz):  $\delta$  5.34 (d,  $J=5.2$ ;  $1\beta\text{-H}$ ), 4.07 (ddd,  $J=2.4$ , 11.9, 12.8;  $3\alpha\text{-H}$ ), 3.63 (dd,  $J=4.9$ , 11.9;  $3\beta\text{-H}$ ), 1.46 (br d,  $J=ca.$  14.6;  $4\alpha\text{-H}$ ), 1.78 (dddd,  $J=4.9$ , 5.2, 12.8, 14.6;  $4\beta\text{-H}$ ), 2.64 (ddd,  $J=5.2$ , 9.8, 11.0;  $5\beta\text{-H}$ ), 5.44 (dd,  $J=9.5$ , 11.0;  $6\alpha\text{-H}$ ), 5.85 (dd,  $J=1.5$ , 9.5;  $7\beta\text{-H}$ ), 2.74 (dd,  $J=5.2$ , 9.8;  $9\beta\text{-H}$ ), 4.59 (d,  $J=10.5$ ;  $10\alpha\text{-H}$ ), 3.59 (dd,  $J=1.5$ , 10.5;  $10\beta\text{-H}$ ). Comparison of the  $^{13}\text{C}$  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 [ $1\beta\text{-H}$  &  $9\beta\text{-H}$  (10%),  $9\beta\text{-H}$  &  $5\beta\text{-H}$  (5%),  $5\beta\text{-H}$  &  $7\beta\text{-H}$  (12%),  $6\alpha\text{-H}$  &  $3\alpha\text{-H}$  (13%),  $6\alpha\text{-H}$  &  $10\alpha\text{-H}$  (6%)] and the comparison of the  $^1\text{H}$ - $^1\text{H}$  coupling constants with those reported for related iridoids,<sup>11)</sup> finally led us to formulate the stereostructure of 2a as shown.

The absolute configuration of 2 was determined by the application of the di-benzoate chirality method.<sup>12)</sup> Thus, silylation of 2 with 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane ( $\text{TIPDSiCl}_2$ , 2 mol. eq.)<sup>13)</sup> in pyridine (r.t., 5 h) gave 2b, which, after acetylation, was desilylated with  $n\text{-Bu}_4\text{NF}$ -tetrahydrofuran (r.t., 2 h) to afford the 8-O-acetate (2c), mp 134–136°C,  $\text{C}_{11}\text{H}_{16}\text{O}_6$ , IR  $\nu$  ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : 3470, 1718, 1245. The location of the 8-O-acetyl group was substantiated by the  $^{13}\text{C}$  NMR data (Table I). Benzoylation of 2c with benzoyl chloride-pyridine gave the 8-O-acetyl-6,7-di-O-benzoate (2d), colorless oil,  $\text{C}_{25}\text{H}_{24}\text{O}_8$ ,  $\lambda_{\text{max}}$  (MeOH): 229 nm ( $\epsilon$  21000), IR  $\nu$  ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : 1720, 1600, 1278. The CD spectrum of 2d gave the split Cotton curve:  $[\theta]_{237} +61600$  and  $[\theta]_{222} -24200$ , thus corroborating the 6S, 7R configuration of 2d. Thus the absolute configuration of rehmaglucin A (2) was determined. The structure 2 was further confirmed chemically; dihydrocatalpol (1a), prepared by catalytic hydrogenation of catalpol (1)<sup>1)</sup> ( $\text{H}_2$  3.0 kg/cm<sup>2</sup>, 5% Pd-C, 18°C, 30 min), was converted to rehmaglucin A (2) in 15% yield<sup>14)</sup> via alkaline treatment (10% aq. NaOH, 85°C, 2 h) followed by methanolysis (9% HCl-dry MeOH, r.t., 1 h).

Rehmaglucin B (3), mp 152–153°C,  $[\alpha]_{\text{D}}^{19} +33.8^\circ$  (MeOH),  $\text{C}_9\text{H}_{13}\text{O}_5\text{Cl}$ , IR  $\nu$  (KBr)  $\text{cm}^{-1}$ : 3280, 2920, 1049, 1031, contained a chlorine atom as shown by the positive Beilstein test and the isotope ion peaks in the CI-MS (%):  $(\text{M}+\text{H})^+$  at  $m/z$  239 (8) and 237 (25),  $(\text{M}+\text{H}-\text{H}_2\text{O})^+$  at  $m/z$  221 (33) and 219 (100). The  $^1\text{H}$  and  $^{13}\text{C}$  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,  $\text{C}_{13}\text{H}_{17}\text{O}_7\text{Cl}$ , IR  $\nu$  ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : 3300, 1740, 1235, and 3,6,8-tri-O-acetate (3b), colorless oil,  $\text{C}_{15}\text{H}_{19}\text{O}_8\text{Cl}$ , IR  $\nu$  ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : no OH, 1735, 1230. Silylation of 3 with  $\text{TIPDSiCl}_2$  (2 mol eq) in pyridine (r.t., 7 h) followed by treatment with MeOH provided 3c. Thus the absence of the  $\alpha$ -glycol moiety in 3 was indicated. The  $^1\text{H}$  NMR data for 3a were assigned as for 2a:  $\delta$  5.59 (d,  $J=4.9$ ;  $1\beta\text{-H}$ ), 6.41 (dd,  $J=6.4$ , 7.3;  $3\alpha\text{-H}$ ), 1.63 (ddd,  $J=4.9$ , 7.3, 14.7;  $4\alpha\text{-H}$ ), 2.11 (ddd,  $J=3.8$ , 6.4, 14.7;  $4\beta\text{-H}$ ), 2.47 (dddd,  $J=3.8$ , 4.9, 10.1, 10.5;  $5\beta\text{-H}$ ), 5.20 (dd,  $J=10.1$ , 10.1;  $6\alpha\text{-H}$ ), 4.25 (d,  $J=10.1$ ;  $7\beta\text{-H}$ ), 2.71 (dd,  $J=4.9$ , 10.5;  $9\beta\text{-H}$ ), 4.34 (d,  $J=11.0$ ;  $10\alpha\text{-H}$ ), 3.86 (d,  $J=11.0$ ;  $10\beta\text{-H}$ ). In the  $^1\text{H}$  NMR spectrum of 3b, the proton signals, except those of the 5-, 7-, and 9-H (shifted lower),

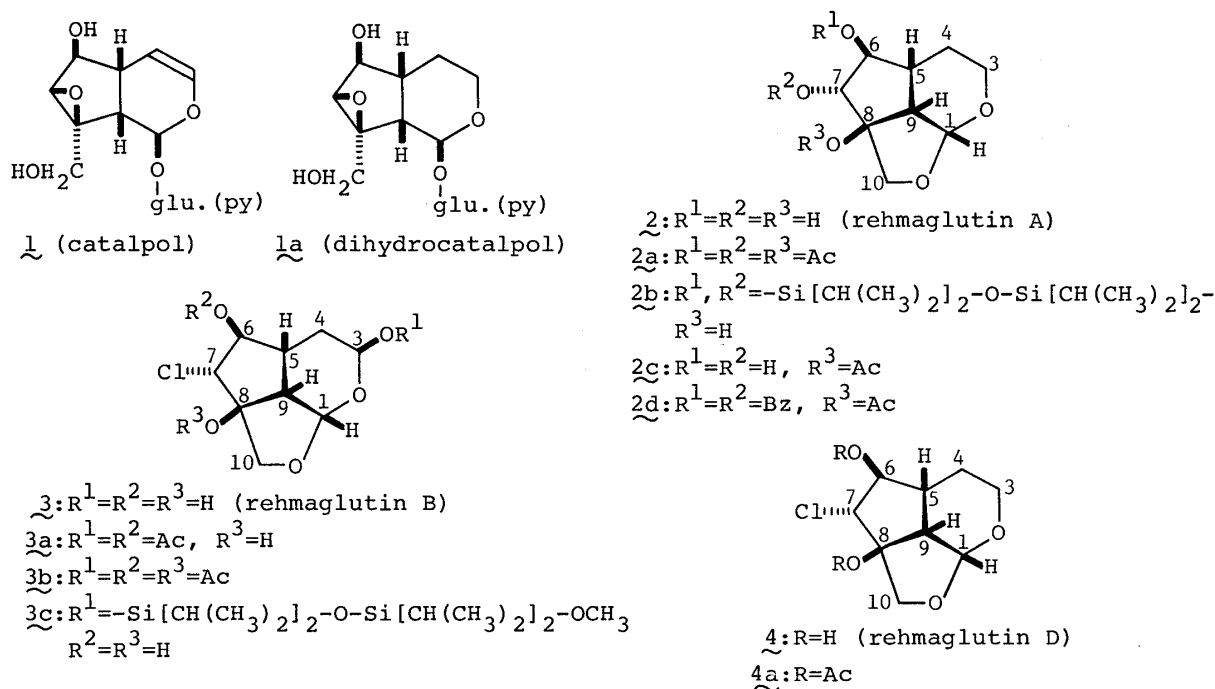


Chart 1

Table I.  $^{13}C$  NMR Data for the Skeletal Carbons of Rehmaglutinins A( $\underline{2}$ ), B( $\underline{3}$ ), and D( $\underline{4}$ ) and Their Derivatives<sup>a)</sup>

|     | $\underline{2}$        | $\underline{2a}$ | $\underline{2c}$ | $\underline{2d}$ | $\underline{3}$ | $\underline{3a}$      | $\underline{3b}$      | $\underline{4}$ | $\underline{4a}$ |
|-----|------------------------|------------------|------------------|------------------|-----------------|-----------------------|-----------------------|-----------------|------------------|
| C-1 | 101.0(d) <sup>b)</sup> | 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) <sup>c)</sup> | 50.1(d) <sup>c)</sup> | 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) <sup>c)</sup> | 74.8(t) <sup>c)</sup> | 76.4(t)         | 69.5(t)          |

a) The spectra of  $\underline{2}$ ,  $\underline{3}$ ,  $\underline{3a}$ ,  $\underline{3b}$ ,  $\underline{4}$ , and  $\underline{4a}$  were taken in  $d_6$ -acetone, while those of  $\underline{2a}$ ,  $\underline{2c}$ , and  $\underline{2d}$  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  $\underline{3}$ . The reason is yet obscure.

appeared at positions similar to those in the spectrum of  $\underline{3a}$ .

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

Rehmaglutin D ( $\underline{4}$ ), mp 132-133°C,  $[\alpha]_D^{19} +60.6^\circ$  (MeOH),  $C_9H_{13}O_4Cl$ , IR  $\nu$  (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  $\nu$  ( $CHCl_3$ )  $cm^{-1}$ : no OH, 1733, 1235, were assigned by detailed decoupling experiments:  $\delta$  5.46 (d,  $J=5.2$ ;  $1\beta-H$ ), 4.06 (ddd,  $J=2.1, 12.0, 12.2$ ;  $3\alpha-H$ ), 3.62 (dd,  $J=5.2, 12.0$ ;  $3\beta-H$ ), 1.47 (br d,  $J=ca. 14.3$ ;  $4\alpha-H$ ), 1.77 (dddd,  $J=4.6, 5.2, 12.2, 14.3$ ;  $4\beta-H$ ), 2.56 (ddd,  $J=4.6, 9.8, 10.4$ ;  $5\beta-H$ ), 5.39 (dd,  $J=10.4, 10.4$ ;  $6\alpha-H$ ), 4.81 (dd,  $J=1.5, 10.4$ ;  $7\beta-H$ ), 2.85 (dd,  $J=5.2, 9.8$ ;  $9\beta-H$ ), 4.61 (d,  $J=10.7$ ;  $10\alpha-H$ ), 3.74 (dd,  $J=1.5, 10.7$ ;  $10\beta-H$ ).

The  $^{13}C$  NMR data for 4 and 4a (Table I) showed the presence of the 6,8-diacetoxy 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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