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ABSOLUTE CONFIGURATIONS OF REHMAIONOSIDES A, B, AND C AND REHMAPICROSIDE THREE NEW IONONE GLUCOSIDES AND A NEW MONOTERPENE GLUCOSIDE FROM REHMANNIAE RADIX

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Three new ionone glucosides, rehmaionosides A (**1**), B (**2**), and C (**3**), and a new monoterpene glucoside rehmapicroside (**4**), were isolated from Chinese Rehmanniae Radix, the dried root of *Rehmannia glutinosa* Libosch. [kan-jiō (in Japanese) from China]. Their absolute configurations were determined on the basis of chemical and physicochemical evidence which included the application of the exciton chirality method for allylic benzoyl derivatives.

KEYWORDS —— Rehmanniae Radix; *Rehmannia glutinosa*; Scrophulariaceae; rehmaionoside A; rehmaionoside B; rehmaionoside C; rehmapicroside; allylic benzoate exciton chirality method; ionone glucoside; monoterpene glucoside

During the course of chemical characterization studies of crude drug processing,[1)] we have investigated the chemical constituents of Chinese Rehmanniae Radix, the dried root of *Rehmannia glutinosa* Libosch. (Scrophulariaceae) [kan-jiō in Japanese]. We have so far isolated from the Radix various new constituents such as four iridoids (rehmaglutins A, B, C, and D), one iridoid glucoside (glutinoside), three ionone glucosides (rehmaionosides A, B, and C), and one monoterpene glucoside (rehmapicroside), together with eight known glycosides. In our recent papers, we reported the absolute stereostructure of rehmaglutins A,[2)] B,[2)] C,[3)] and D[2)] and glutinoside.[3)] This paper deals with the evidence for the absolute configurations of rehmaionosides A (**1**), B (**2**), and C (**3**) and rehmapicroside (**4**).[4,5)]

Rehmaionoside A (**1**), $C_{19}H_{34}O_8\cdot 2H_2O$,[6)] $[\alpha]_D^{20}$ -49.3° (MeOH), IR (KBr) cm^{-1}: 3400, 2924, 1633, 1075, SIMS (Xe^+, glycerol matrix) *m/z*: 391 $(M+H)^+$, 413 $(M+Na)^+$, 483 $(M+H+glycerol)^+$, rehmaionoside B (**2**), $C_{19}H_{34}O_8\cdot 2H_2O$, $[\alpha]_D^{20}$ -54.2° (MeOH), IR (KBr) cm^{-1}: 3400, 2926, 1631, 1074, SIMS *m/z*: 391 $(M+H)^+$, 413 $(M+Na)^+$, 483 $(M+H+glycerol)^+$, and rehmaionoside C (**3**), mp 217-218°C (prisms), $C_{19}H_{32}O_8$, $[\alpha]_D^{24}$ -59.4° (MeOH), IR (KBr) cm^{-1}: 3270, 1680, 1059, SIMS *m/z*: 389 $(M+H)^+$, 411 $(M+Na)^+$, 481 $(M+H+glycerol)^+$, were indicated as glucosides of ionone derivatives by their ^1H and ^{13}C NMR data (Table I). Oxidation of **1** and **2** with CrO_3-pyridine provided **3** selectively, whereas reduction of **3** with $NaBH_4$ in MeOH yielded **1** and **2** in a 1:1 ratio.

Rehmaionoside C (**3**) had an α,β-unsaturated carbonyl moiety and a tertiary hydroxyl group as indicated by the UV [λmax (MeOH): 232 nm (ε 10700)] and ^1H and

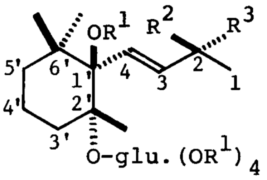
^{13}C NMR (Table I) data. Acetylation of 3 with Ac_2O-pyridine gave the pentaacetate (3a), mp 183-184°C (needles), $C_{29}H_{42}O_{13}$, IR ($CHCl_3$) cm^{-1}: 2938, 1751, 1674, 1241, 1036, UV λmax (MeOH): 228 nm (ε 16600). The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) showed signals assignable to four methyl groups (δ 0.81, 1.08, 1.18, 2.29; 3H each, all s), two olefinic protons (δ 6.19, 7.29; 1H each, both d, J=16.5 Hz), and one β-anomeric proton (δ 4.70, d, J=7.9 Hz). Hydrolysis of 3 with β-glucosidase afforded an ionone derivative (5), mp 115-116°C (prisms), $C_{13}H_{22}O_3$, $[\alpha]_D^{25}$ -53.6° (EtOH), IR ($CHCl_3$) cm^{-1}: 3600, 3450, 1670, 1620. Detailed comparison of the physical data for 5 with those for its optical antipode (5')$^{7)}$ indicated the 1'R, 2'R configurations of 5. Furthermore, the glycosidation shift (between 3 and 5) and the acetylation shift (between 3 and 3a) observed in the ^{13}C NMR data led us to formulate the stereostructure of rehmaionoside C (3) as shown.$^{8)}$

Methanolysis of rehmaionosides A (1) and B (2) under mild conditions (4.5% HCl -dry MeOH, r.t., 8 h) provided 6 (a mixture of C_2-epimers), IR (KBr) cm^{-1}: 3391, 2929, 1598, 1036, ^{1}H NMR (500 MHz, d_5-pyridine) δ: 1.20 (3H d, J=6.1 Hz, 1-H_3), 1.25 (3H s, 2'-CH_3), 1.68, 1.73 [3H each, both s, 6'-$(CH_3)_2$], 3.29 (3H s, OCH_3), 3.72 (1H dq, J=6.1, 8.1 Hz, 2-H), 5.12 (1H d, J=8.6 Hz, 1"-H), 6.00 (1H dd, J=8.1, 15.9 Hz, 3-H), 6.72 (1H d, J=15.9 Hz, 4-H). Comparison of ^{13}C NMR data for 6 and the acetate (6a) and the NOE (14.1%) between 2-OCH_3 and 2-H substantiated the structure 6. Subsequent methanolysis of 6 with 9% HCl-dry MeOH (r.t., 15 h) liberated 7, 8,$^{9)}$ and methyl glucoside.

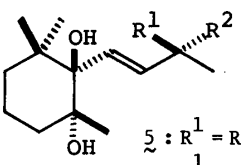
Hydrolysis of rehmaionosides A (1) and B (2) with β-glucosidase afforded 9 (from 1), mp 88-90°C (prisms), $C_{13}H_{24}O_3$, $[\alpha]_D^{28}$ -26.4° (MeOH), IR ($CHCl_3$) cm^{-1}:3610, 3445, 2933, 1600, and 10 (from 2), mp 111-113°C (prisms), $C_{13}H_{24}O_3$, $[\alpha]_D^{28}$ -39.6° (MeOH), IR ($CHCl_3$) cm^{-1}: 3610, 3445, 2933, 1600, respectively. On the other hand, 9 and 10 were obtained in a 1:1 ratio from 5 by $NaBH_4$ reduction in MeOH. Thus, the structures of rehmaionosides A (1) and B (2) were elucidated except the C_2 configuration.

The C_2 absolute configurations of rehmaionosides A (1) and B (2) were determined by the application of the allylic benzoate exciton chirality method.$^{10)}$ $NaBH_4$ reduction of rehmaionoside C pentaacetate (3a) yielded 1a and 2a in 1:1 ratio. Respective benzoylation of 1a and 2a with benzoyl chloride-pyridine afforded 1b (from 1a), colorless oil, $C_{36}H_{48}O_{14}$, UV λmax (MeOH): 228 nm (ε 14100), IR ($CHCl_3$) cm^{-1}: 2935, 1753, 1716, 1600, 1272, 1034, ^{1}H NMR (500 MHz, CD_3OD) δ: 0.78, 0.90 [3H each, both s, 6'-$(CH_3)_2$], 1.16 (3H s, 2'-CH_3), 1.47 (3H d, J=6.4 Hz, 1-H_3), 5.65 (1H dq, J=6.1, 6.4 Hz, 2-H), 5.80 (1H dd, J=6.1, 15.9 Hz, 3-H), 6.34 (1H d, J=15.9 Hz, 4-H), and 2b (from 2a), colorless oil, $C_{36}H_{48}O_{14}$, UV λmax (MeOH): 229 nm (ε 13000), IR ($CHCl_3$) cm^{-1}: 2935, 1753, 1728, 1600, 1272, 1040, ^{1}H NMR (500 MHz, CD_3OD) δ: 0.79, 0.94 [3H each, both s, 6'-$(CH_3)_2$], 1.19 (3H s, 2'-CH_3), 1.47 (3H d, J=6.4 Hz, 1-H_3), 5.65 (1H dq, J=5.8, 6.4 Hz, 2-H), 5.85 (1H dd, J=5.8, 15.9 Hz, 3-H), 6.34 (1H d, J=15.9 Hz, 4-H). Deacylation of 1b and 2b with 1%NaOMe-MeOH regenerated parent 1 and 2. Thus, the structures of 1b and 2b were corroborated. The CD spectrum (MeOH) of 1b gave a positive first Cotton curve: $[\theta]_{226}$ +13100, whereas that of 2b gave a negative first Cotton curve: $[\theta]_{226}$ -15200. Therefore, the 2S configuration in 1b and the 2R configuration in 2b have been substantiated.

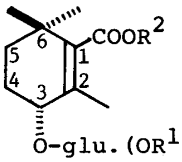
Based on the above evidence, the absolute configurations of rehmaionosides A (1) and B (2) were determined as shown.



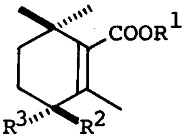
1 : $R^1 = R^3 = H$, $R^2 = OH$ (rehmaionoside A)
1a : $R^1 = Ac$, $R^2 = OH$, $R^3 = H$
1b : $R^1 = Ac$, $R^2 = OBz$, $R^3 = H$
2 : $R^1 = R^2 = H$, $R^3 = OH$ (rehmaionoside B)
2a : $R^1 = Ac$, $R^2 = H$, $R^3 = OH$
2b : $R^1 = Ac$, $R^2 = H$, $R^3 = OBz$
3 : $R^1 = H$, $R^2 = R^3 = O$ (rehmaionoside C)
3a : $R^1 = Ac$, $R^2 = R^3 = O$
6 : $R^1 = H$, R^2, $R^3 = H$ or OCH_3
6a : $R^1 = Ac$, R^2, $R^3 = H$ or OCH_3



5 : $R^1 = R^2 = O$
7, 8 : R^1, $R^2 = H$ or OCH_3
9 : $R^1 = OH$, $R^2 = H$
10 : $R^1 = H$, $R^2 = OH$



4 : $R^1 = R^2 = H$
(rehmapicroside)
4a : $R^1 = Ac$, $R^2 = H$
4b : $R^1 = H$, $R^2 = CH_3$



11 : $R^1 = CH_3$, $R^2 = H$, $R^3 = OH$
11a : $R^1 = CH_3$, $R^2 = H$, $R^3 = OBz$
12 : $R^1 = H$, R^2, $R^3 = H$ or OCH_3
13 : $R^1 = CH_3$, $R^2 = R^3 = O$

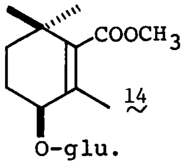


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Table I. ^{13}C NMR Data for 1, 2, 3, 3a, 4, 4a, 4b, 5, 9, 10, and 11 (in d_5-pyridine)

Carbon	1	2	3	3a	5	9	10
1	24.8 (q)[a]	24.7 (q)	25.8 (q)	25.0 (q)	26.9 (q)	24.9 (q)	25.0 (q)
2	68.1 (d)	68.1 (d)	198.3 (s)	198.3 (s)	197.7 (s)	68.3 (d)	68.4 (d)
3	130.8 (d)	130.9 (d)	131.9 (d)	132.0 (d)	131.1 (d)	130.9 (d)	130.9 (d)
4	135.5 (d)	135.1 (d)	151.9 (d)	149.9 (d)	151.4 (d)	135.9 (d)	135.9 (d)
1'	79.4 (s)	79.1 (s)	79.7 (s)	83.9 (s)	79.8 (s)	79.1 (s)	79.1 (s)
2'	82.9 (s)	82.7 (s)	82.6 (s)	79.2 (s)	74.5 (s)	74.8 (s)	74.8 (s)
3'	32.0 (t)	32.0 (t)	32.0 (t)	32.6 (t)	36.5 (t)	37.0 (t)	37.0 (t)
4'	18.3 (t)	18.2 (t)	18.2 (t)	18.1 (t)	18.4 (t)	18.9 (t)	18.9 (t)
5'	36.9 (t)	36.7 (t)	36.6 (t)	36.0 (t)	36.8 (t)	37.2 (t)	37.3 (t)
6'	38.9 (s)	38.7 (s)	39.4 (s)	39.1 (s)	39.0 (s)	38.7 (s)	38.8 (s)
2'-CH_3	22.8 (q)	22.4 (q)	22.8 (q)	21.5 (q)	25.5 (q)	25.5 (q)	25.6 (q)
6'-CH_3	25.6 (q) 27.8 (q)	25.5 (q) 27.6 (q)	27.0 (q) 27.6 (q)	26.4 (q) 27.6 (q)	27.3 (q) 27.6 (q)	27.6 (q) 27.6 (q)	27.6 (q) 27.7 (q)

Carbon	4	4a	4b	11
1	140.5 (s)	142.0 (s)	139.1 (s)	137.4 (s)
2	129.8 (s)	128.9 (s)	132.5 (s)	135.9 (s)
3	73.1 (d)	76.4 (d)	73.1 (d)	68.1 (d)
4	24.0 (t)	25.3 (t)	24.2 (t)	29.4 (t)
5	33.7 (t)	33.8 (t)	34.0 (t)	35.2 (t)
6	32.7 (s)	33.5 (s)	33.3 (s)	33.8 (s)
1-COO-	171.7 (s)	170.2 (s)	170.2 (s)	170.7 (s)
2-CH_3	17.9 (q)	18.5 (q)	18.2 (q)	18.3 (q)
6-CH_3	26.9 (q) 27.9 (q)	27.0 (q) 28.7 (q)	27.1 (q) 28.0 (q)	27.8 (q) 28.3 (q)

a) The characterization of each carbon signal was made by INEPT (Insensitive Nuclei Enhanced by Polarization) and the off-resonance experiments.

Rehmapicroside (4), mp 127-129°C (prisms), $C_{16}H_{26}O_8 \cdot H_2O$, $[\alpha]_D^{20}$ +8.5° (MeOH), SIMS *m/z*: 347 $(M+H)^+$, 369 $(M+Na)^+$, 439 $(M+H+glycerol)^+$, IR (KBr) cm^{-1}: 3405, 1691, 1637, 1073, was shown to be a glucoside having one olefinic methyl group, two tertiary methyl groups, one tetrasubstituted olefin moiety, and one α,β-unsaturated carboxyl group by the ^1H and ^{13}C NMR (Table I) data. Ordinary acetylation of 4 yielded the tetraacetate (4a), mp 146-148°C (prisms), $C_{24}H_{34}O_{12}$, IR ($CHCl_3$) cm^{-1}: 1746, 1698, 1239, 1030, while CH_2N_2 treatment of 4 gave the methyl ester (4b), hygroscopic powder,[11] SIMS *m/z*: 361 $(M+H)^+$, 383 $(M+Na)^+$, 453 $(M+H+glycerol)^+$, IR (KBr) cm^{-1}: 3400, 1722, 1071. Hydrolysis of 4b with β-glucosidase liberated 11, colorless oil, $C_{11}H_{18}O_3$, $[\alpha]_D^{20}$ +53.8° ($CHCl_3$), IR (CCl_4) cm^{-1}: 3420, 1718, 1216, 1059.

Detailed comparison of the ^{13}C NMR data (Table I) for **4**, **4a**, **4b**, and **11** has led us to conclude that rehmapicroside (**4**) is the allylic β-glucoside of **11**-acid. Treatment of **4** with 9% HCl-dry MeOH (reflux for 2 h) liberated **12** (racemic) and methyl glucoside. Oxidation of **11** with CrO_3-pyridine gave the known enone (**13**).[12] Thus, the structure **11** was substantiated.

Finally, the allylic benzoate exciton chirality method was applied to determine the C_3 absolute configuration of rehmapicroside (**4**). Benzoylation of **11** with benzoyl chloride-pyridine furnished **11a**, colorless oil, $C_{18}H_{22}O_4$, UV λmax (EtOH): 229 nm (ε 14600), IR (CCl_4) cm^{-1}: 1717, 1595, 1268, 1101. The CD spectrum of **11a** gave a positive first Cotton curve: $[\theta]_{229}$ +36500. Thus, the 3R configuration of **11a** was determined.

The absolute stereostructure **4** of rehmapicroside was further confirmed by its partial synthesis from α-ionone. The enone methyl ester (**13**), which was prepared from α-ionone,[13] was treated with $NaBH_4$ in MeOH (0°C, 20 min) to afford (±)-**11**. Glycosidation of (±)-**11** with 1-bromo-2,3,4,6-tetra-O-acetylglucose and $Hg(CN)_2$ and subsequent treatment of the reaction product with 0.5% NaOMe-MeOH (r.t., 15 min) followed by HPLC purification provided **4b** (30%) and the diastereoisomer (**14**, 31%). Hydrolysis of **4b** with 10% KOH-aq. MeOH (60°C, 12 h) furnished rehmapicroside (**4**, 69%).

Based on the above evidence, the absolute configuration of rehmapicroside (**4**) was determined as shown.

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