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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 *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 — *Rehmannia 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 *Rehmannia 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 D²⁾ 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⁾ $[\alpha]_D^{20} -49.3^\circ$ (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), $C_{19}H_{34}O_8 \cdot 2H_2O$, $[\alpha]_D^{20} -54.2^\circ$ (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), $C_{19}H_{32}O_8$, $[\alpha]_D^{24} -59.4^\circ$ (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 [λ_{max} (MeOH): 232 nm (ϵ 10700)] and 1H and

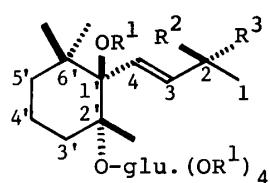
^{13}C NMR (Table I) data. Acetylation of **3** with Ac_2O -pyridine gave the pentaacetate (**3a**), mp 183–184°C (needles), $\text{C}_{29}\text{H}_{42}\text{O}_{13}$, IR (CHCl_3) cm^{-1} : 2938, 1751, 1674, 1241, 1036, UV λ_{max} (MeOH): 228 nm (ϵ 16600). The ^1H NMR spectrum (500 MHz, CDCl_3) showed 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), $\text{C}_{13}\text{H}_{22}\text{O}_3$, $[\alpha]_{\text{D}}^{25}$ -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, ^1H NMR (500 MHz, d_5 -pyridine) δ : 1.20 (3H d, $J=6.1$ Hz, 1-H₃), 1.25 (3H s, 2'-CH₃), 1.68, 1.73 [3H each, both s, 6'-(CH₃)₂], 3.29 (3H s, OCH₃), 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-OCH₃ 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), $\text{C}_{13}\text{H}_{24}\text{O}_3$, $[\alpha]_{\text{D}}^{28}$ -26.4° (MeOH), IR (CHCl_3) cm^{-1} : 3610, 3445, 2933, 1600, and **10** (from **2**), mp 111–113°C (prisms), $\text{C}_{13}\text{H}_{24}\text{O}_3$, $[\alpha]_{\text{D}}^{28}$ -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 benzylation of **1a** and **2a** with benzoyl chloride-pyridine afforded **1b** (from **1a**), colorless oil, $\text{C}_{36}\text{H}_{48}\text{O}_{14}$, UV λ_{max} (MeOH): 228 nm (ϵ 14100), IR (CHCl_3) cm^{-1} : 2935, 1753, 1716, 1600, 1272, 1034, ^1H NMR (500 MHz, CD_3OD) δ : 0.78, 0.90 [3H each, both s, 6'-(CH₃)₂], 1.16 (3H s, 2'-CH₃), 1.47 (3H d, $J=6.4$ Hz, 1-H₃), 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, $\text{C}_{36}\text{H}_{48}\text{O}_{14}$, UV λ_{max} (MeOH): 229 nm (ϵ 13000), IR (CHCl_3) cm^{-1} : 2935, 1753, 1728, 1600, 1272, 1040, ^1H NMR (500 MHz, CD_3OD) δ : 0.79, 0.94 [3H each, both s, 6'-(CH₃)₂], 1.19 (3H s, 2'-CH₃), 1.47 (3H d, $J=6.4$ Hz, 1-H₃), 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: $R^1 = Ac$, $R^2 = H$, $R^3 = OH$

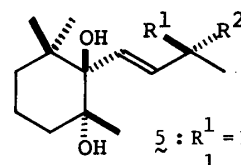
2b: $R^1 = Ac$, $R^2 = H$, $R^3 = OBz$

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

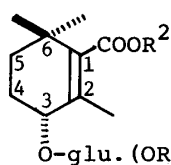


5: $R^1 = R^2 = O$

7, 8: $R^1, R^2 = H$ or OCH_3

9: $R^1 = OH$, $R^2 = H$

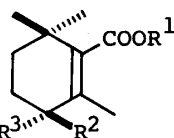
10: $R^1 = H$, $R^2 = OH$



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: $R^1 = CH_3$, $R^2 = R^3 = O$

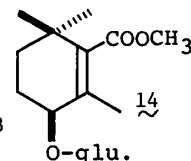


Table I. ^{13}C NMR Data for 1, 2, 3, 3a, 4, 4a, 4b, 5, 9, 10, and 11 (in d_5 -pyridine)

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)	25.5 (q)	27.0 (q)	26.4 (q)	27.3 (q)	27.6 (q)	27.6 (q)
	27.8 (q)	27.6 (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)	27.0 (q)	27.1 (q)	27.8 (q)
	27.9 (q)	28.7 (q)	28.0 (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), $C_{16}H_{26}O_8 \cdot H_2O$, $[\alpha]_D^{20} +8.5^\circ$ (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, $C_{11}H_{18}O_3$, $[\alpha]_D^{20} +53.8^\circ$ ($CHCl_3$), 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 λ_{max} (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 ($0^\circ C$, 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 ($60^\circ C$, 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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