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FIVE CYCLOPENTANOID MONOTERPENES FROM *REHMANNIA GLUTINOSA**

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Key Word Index—*Rehmannia glutinosa* var. *hueichingensis*; Scrophulariaceae; cyclopentanoid monoterpenes; iridoids; jioglutins; jioglutolide; jiofuran; rehmaglutins; glutinoside.

Abstract—Five new cyclopentanoid monoterpenes named jioglutins A, B, C, jioglutolide and jiofuran, along with other known compounds, have been isolated from the steamed roots of *Rehmannia glutinosa* var. *hueichingensis* and their structures elucidated on the basis of chemical and spectroscopic methods.

INTRODUCTION

In previous papers [1, 2], we reported the isolation of iridoid glycosides (jioglutosides) from the fresh roots of *Rehmannia glutinosa* Libosch. var. *hueichingensis* (Chao et Schih) Hsiao and 6-*O*-acylated ajugols from the dried roots of *R. glutinosa* Libosch. var. *purpurea* Makino. In continuing our studies on the chemical components of Rehmanniae radix, we have isolated five new cyclopentanoid monoterpenes named jioglutins A–C, jioglutolide and jiofuran from the steamed roots of *R. glutinosa* var. *hueichingensis*, together with three known iridoids [rehmaglutins A, D and glutinoside] [3, 4] and five common constituents (uracil, etc.) of the plant. This paper describes the structural elucidation of the five new compounds.

RESULTS AND DISCUSSION

Fractions D and El [5] afforded 13 compounds: four new non-glycosidic iridoids [jioglutins A–C (**1**–**3**) and jioglutolide (**4**)], one new cyclopentanoid monoterpene [jiofuran (**5**)], two known non-glycosidic iridoids [rehmaglutins A (**6**) and D (**7**)], one known iridoid glucoside [glutinoside (**8**)] and five common compounds (uracil, uridine, 5-oxoproline Na salt, 5-hydroxymethylfuroic acid and succinic acid). Compounds **6**, **7** and **8** were identified by comparison of their spectral data with those of authentic samples [3, 4].

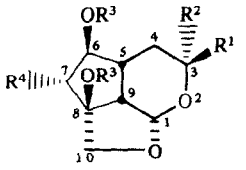
Jioglutin A (**1**) was obtained as a white amorphous powder, $[\alpha]_D$ +63.3° (MeOH), FDMS *m/z*: 250 $[M]^+$. Acetylation of **1** afforded the diacetate **1a** as a colourless oil, $C_{14}H_{19}O_7Cl$. The ^1H and ^{13}C NMR spectra of **1** were closely correlated with those of rehmaglutins A (**6**) and D (**7**), which were also isolated from the same plant by Kitagawa *et al.* and characterized as tricyclic non-glycosidic C_9-iridoids [3]. In particular, rehmaglutin D was established as a rare iridoid having a chlorine atom at C-7. The ^1H NMR spectra of tricyclic iridoids such as **6** and **7** show a long-range coupling ($J = ca$ 1.0 Hz) between H-7 and H-10β, and in the ^{13}C NMR spectra the C-7 chlorinated carbon (δ74.1) in **7** appears at higher field (*ca* 10 ppm) than the hydroxylated one (δ84.6) in **6**. In the ^1H and ^{13}C NMR spectra of **1**, the H-7 and H-10β protons gave rise to long-range coupled signals at δ3.67 (*dd*, J = 10.4, 0.8 Hz) and 3.96 (*dd*, J = 9.8, 0.8 Hz), respectively, and the C-7 methine carbon appeared at δ73.3 (*d*) (Tables 1 and 2). Hence **1** was a tricyclic C_9-iridoid carrying a chlorine atom at C-7. This was further verified by the observation of characteristic isotope ion peaks in the EI and CIMS of **1a**: e.g. CIMS *m/z* (rel. int.): 337 (1), 335 $[M+H]^+$ (3), 305 (33), 303 $[M-OMe]^+$ (100), 277 (14), 275 $[M-OAc]^+$ (43), etc.

The ^1H NMR spectrum of **1** showed OMe signals at δ3.40 (3H, *s*) and two acetalic proton signals at δ4.87 (*dd*, J = 7.6, 5.4 Hz, H-3) and 5.46 (*d*, J = 5.3 Hz, H-1). These assignments were confirmed by a 2D ^1H–^1H COSY experiment. Moreover, a NOESY experiment showed a strong cross peak between the OMe signal and the H-3 signal, indicating that the OMe group was located at C-3.

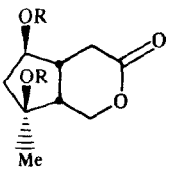
To determine the absolute configuration of the asymmetric centres of **1**, chemical correlation with rehmaglutin B permethylether (**1b**) via rehmaglutin B (**9**), which was prepared from catalpol (**10**), was performed. Rehmaglutin B is a C-7 chlorinated tricyclic iridoid having the β-hydroxyl group at C-3 and its absolute structure has been established by Kitagawa *et al.* Treatment of **10** with 1% HCl–MeOH gave **9** which was identified as rehmaglutin B by comparing its spectral data with those of an authentic sample [3]. Methylation of **1** with MeI–Ag_2O in dimethylformamide provided the dimethylether **1b** as a colourless oil, $[\alpha]_D$ +39.2° ($CHCl_3$). Compound **1b** was identical in all respects with the permethylether of **9** prepared by the same method. On the basis of the above findings, jioglutin A (**1**) was elucidated as 3-*O*-methylrehmaglutin B.

Jioglutin B (**2**) was isolated as a white amorphous powder, $[\alpha]_D$ −63.2° (MeOH). It showed an $[M+Na]^+$ ion peak at *m/z* 273 in the FDMS, and gave the diacetate **2a** as a colourless oil whose EI and CIMS exhibited the same fragmentation pattern as that of jioglutin A diacetate (**1a**). The molecular formula of **2a**, $C_{14}H_{19}O_7Cl$, was confirmed by high resolution EIMS and was coinci-

*Part 5 in the series 'Chemical and Biological Studies on Rehmanniae Radix', For Part 4 see ref. [1].



	R^1	R^2	R^3	R^4
1	OMe	H	H	Cl
1a	OMe	H	Ac	Cl
1b	OMe	H	Me	Cl
2	H	OMe	H	Cl
2a	H	OMe	Ac	Cl
3	OMe	H	H	OH
3a	OMe	H	Ac	OAc
6	H	H	H	OH
7	H	H	H	Cl
9	OH	H	H	Cl



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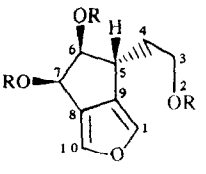
4a R = Ac



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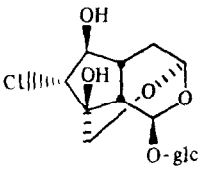
5a R = Ac



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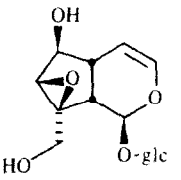

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dent with that of **1a**. The close resemblance of the ^1H and ^{13}C NMR spectra of **2** to those of **1**, except for the signals due to a C-3 methine and a C-4 methylene led us to the assumption that **2** might be a C-3 epimer of **1**. The NOESY spectrum of **2** showed cross peaks between the OMe signal [δ3.39 (*s*)] and the H-10α signal [δ4.10 (*d*, J = 10.1 Hz)] as well as between the OMe signal and the H-3 signal [δ4.72 (*dd*, J = 4.4, 2.6 Hz)] (Fig. 1). This indicates that the OMe group at C-3 must be in the α orientation. Thus, **2** was identified as 3-epijioglutin A.

Jioglutin C (**3**) was obtained as a white amorphous powder, $[\alpha]_D$ +58.1° (MeOH), FDMS *m/z*: 233 $[M+H]^+$. Acetylation of **3** afforded the triacetate **3a** as a colourless oil whose molecular formula, $C_{16}H_{22}O_9$, was confirmed by high resolution EIMS. The ^1H and ^{13}C NMR spectra of **3** were very similar to those of **1**, indicating that **3** is a tricyclic C_9-iridoid having a β-OMe group at C-3. A significant difference between **3** and **1** was present in the ^{13}C NMR spectral data. Thus the C-7 methine carbon in **3** appeared at lower field [δ84.9 (*d*)] than that in **1** [δ73.3 (*d*)], cf. rehmaglutins A (**6**) and D (**7**). This indicates that the chlorine atom at C-7 in **1** was replaced by a hydroxyl group in **3**. Accordingly, jioglutin C (**3**) was determined to be 3β-methoxyrehmaglutin A.

The possibility that jioglutins A–C (**1–3**) are derived from rehmaglutin B (**9**) by recyclization of a tetrahydropyran ring during isolation can be ruled out, because **1–3** were not formed upon treatment of **9** by the procedures as used in the isolation process.

Jioglutolide (**4**) was isolated as colourless needles, mp 141–142°, $[\alpha]_D$ −8.4° (MeOH), $C_9H_{14}O_4$. It also seemed to be a non-glycosidic C_9-iridoid from analysis of its ^1H and ^{13}C NMR spectral data, but their features were different from those of jioglutins A–C (**1–3**). In the ^{13}C NMR spectrum of **4**, an ester carbonyl carbon signal was observed at δ175.8 (*s*). The IR spectrum of **4** showed an absorption band at 1736 cm^{-1}. These data indicate the presence of the δ-lactone group [6]. The ^1H NMR spectrum of **4** showed a singlet methyl signal at δ1.26 (3H, *s*) and a hydroxylated methine signal at δ3.82 (1H, *ddd*, J = 5.4, 5.3, 4.0 Hz). This observation, as well as the formation of the diacetate **4a**, reminded us of the cyclopentane ring of ajugol, which has two hydroxyl groups at C-6 and C-8 and a tertiary methyl at C-8 [2]. In addition, the ^{13}C chemical shifts for the cyclopentane ring carbons of **4** were in good agreement with those of ajugol.

Regarding the δ-lactone moiety, the ^1H NMR spectrum of **4** exhibited three pairs of *gem*-coupled methylene signals: (A) δ1.85 and 1.89 (*gem* J = 13.3 Hz), (B) δ2.47 and 2.71 (*gem* J = 14.5 Hz) and (C) δ4.22 and 4.29 (*gem* J = 11.9 Hz). The first one, (A), was readily ascribed to the C-7 methylene, because a *peri*-effect due to acetylation of the C-6 and C-8 hydroxyl groups was observed in **4a** (δ2.06 and 2.57, H-7). The last two signals, (B) and (C), could be allocated to the methylenes of the δ-lactone group and were assigned to C-4 and C-1 methylenes, respectively, by analysis of ^1H–^1H COSY spectrum of **4**. Thus, the oxo group of the δ-lactone was placed at C-3 in **4**. The structure of jioglutolide was thus formulated as **4**.

In order to complete the structural elucidation of **4**, an X-ray crystallographic analysis was performed. The stereoscopic view of the molecule is shown in Fig. 2 and is depicted on the assumption that the configurations of H-5 and H-9 protons in **4** are in the β-form as those in usual iridoids. The δ-lactone ring of **4** has a boat conformation ($_1B_4$) with a V_7 conformation of the cyclopentane ring in the crystal state.

Jiofuran (**5**),* a white amorphous powder, $[\alpha]_D$ −30.4° (MeOH), yielded the triacetate **5a** as a colourless oil, $C_{15}H_{18}O_7$, upon acetylation. The ^{13}C NMR spectrum of **5** exhibited four olefinic and five aliphatic carbon signals. But no acetalic carbon signals were observed among the signals. Therefore, the linkage between C-1 and O-2 found in common iridoids seemed to be absent [7] and hence **5** was assumed to be a cyclopentanoid monoterpene such as eucommiol [8, 9]. Compound **5** exhibited a reddish-violet coloration with Erlich reagent and showed a UV maximum at 216 nm. These facts and the observation of the olefinic proton and carbon signals [δ_H 7.23 and 7.40 (each 1H, *s*); δ_c 131.1, 132.3 (each *s*), 135.6 and 137.4 (each *d*)] suggested the existence of 3,4-disubstituted furan ring.

With regard to the aliphatic moiety, the ^1H NMR spectrum of **5a** showed three acetylation-shifted signals:

*The numbering system of **5** was applied as the iridoid for convenience of explanation.

Table 1. ^{1}H NMR spectral data for iridoids **1–4**, **6**, **7**, **9** and **1b** (500 MHz, CD_3OD)*

H	1	1b†	2	3	4	6‡	7	9
1	5.46 *d* (5.3)	4.48 *d* (5.3)	5.48 *d* (6.6)	5.43 *d* (5.3)	4.22 *dd* (11.9, 6.8) 4.29 *dd* (11.9, 5.5)	5.27 *d* (5.3)	5.31 *d* (5.5)	5.51 *d* (5.3)
3β	—	—	4.72 *dd* (4.4, 2.6)	—	—	3.52 *ddd* (12.3, 5.3, 2.5)	3.54 *dddd* (11.7, 5.3, 2.0, 0.8)	—
3α	4.87 *dd* (7.6, 5.4)	4.94 *dd* (7.1, 6.3)	—	4.87 *dd* (8.1, 4.7)	—	3.89 *ddd* (12.3, 10.5, 4.0)	3.85 *ddd* (12.8, 11.7, 2.9)	5.23 *dd* (9.1, 3.6)
4	1.51 *ddd* (14.5, 7.6, 5.7) 2.07 *ddd* (14.5, 5.4, 2.6)	1.53 *ddd* (14.8, 7.1, 5.3) 2.16 *ddd* (14.8, 6.3, 2.7)	1.77 *ddd* (14.7, 7.2, 4.4) 1.87 *ddd* (14.7, 2.6, 1.8)	1.46 *ddd* (14.1, 8.1, 5.7) 2.01 *ddd* (14.1, 4.7, 2.6)	2.47 *dd* (14.5, 6.2) 2.71 *dd* (14.5, 7.2)	1.70 *m* (2H)	1.66 *dddd* (14.4, 2.9, 2.0, 2.0) 1.78 *dddd* (14.4, 12.8, 5.5, 5.3)	1.51 *ddd* (14.3, 9.1, 5.7) 2.01 *ddd* (14.3, 3.6, 3.0)
5	2.21 *dddd* (10.3, 10.1, 5.7, 2.6)	2.24 *dddd* (10.3, 10.3, 5.3, 2.7)	2.11 *dddd* (11.5, 10.0, 7.2, 1.8)	2.15 *dddd* (10.4, 10.3, 5.7, 2.6)	2.66 *m*	2.05 *m*	2.15 *ddddd* (10.3, 10.3, 5.5, 2.0, 0.8)	2.26 *m*
6	3.75 *dd* (10.1, 9.8)	3.64 *dd* (10.3, 9.3)	4.23 *dd* (10.0, 9.6)	3.61 *dd* (10.4, 8.9)	3.82 *ddd* (5.4, 5.3, 4.0)	3.70 *t* (9.5)	3.81 *dd* (10.3, 10.1)	3.75 *dd* (10.0, 10.0)
7	3.96 *dd* (9.8, 0.8)	4.17 *dd* (9.3, 0.8)	3.92 *dd* (9.6, 0.8)	3.81 *dd* (8.9, 1.1)	1.85 *dd* (13.3, 5.3) 1.89 *dd* (13.3, 5.4)	3.86 *dd* (9.5, 1.5)	4.06 *dd* (10.1, 1.6)	4.01 *dd* (10.0, 1.2)
9	2.45 *dd* (10.3, 5.3)	2.61 *dd* (10.3, 5.3)	2.51 *dd* (11.5, 6.6)	2.29 *dd* (10.3, 5.3)	2.55 *m*	2.16 *dd* (10.2, 5.3)	2.29 *dd* (10.3, 5.5)	2.37 *dd* (10.3, 5.3)
10β	3.67 *dd* (10.4, 0.8)	4.04 *dd* (11.0, 0.8)	3.63 *dd* (10.1, 0.8)	3.52 *dd* (10.1, 1.1)	1.26 *s* (3H)	3.30 *dd* (10.5, 1.5)	3.43 *dd* (10.3, 1.6)	3.53 *dd* (10.4, 1.2)
10α	4.16 *d* (10.4)	4.16 *d* (11.0)	4.10 *d* (10.1)	4.21 *d* (10.1)		4.40 *d* (10.5)	4.39 *d* (10.3)	4.26 *d* (10.4)
OMe	3.40 *s*	3.35, 3.46, 3.62 *s*	3.39 *s*	3.41 *s*	—	—	—	—

*Coupling constants (Hz) are given in parentheses. Assignments are based on ^{1}H–^{1}H COSY and in part on NOESY experiments.
†In $CDCl_3$.
‡Measured at 200 MHz.

Table 2. ^{13}C NMR spectral data for iridoids **1**, **1b**, **2**–**7** and **9** and some acetates (**1a**–**5a**) (50 MHz)*

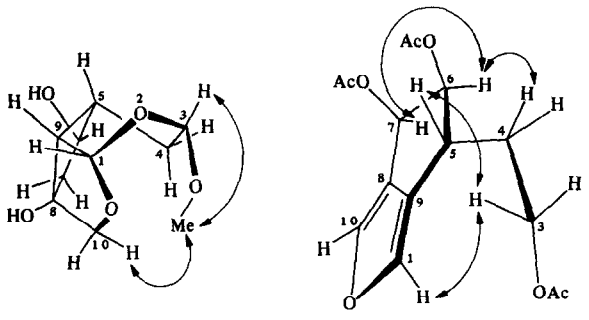
C	1	1a	1b	2	2a†	3	3a†	4	4a	5	5a	6	7	9
1	100.6 *d*	98.2	98.0[a]	101.3 *d*	99.6	101.2 *d*	98.2	68.1 *t*	66.4	135.6[a] *d*	134.6‡	101.2 *d*	101.4 *d*	102.3 *d*
3	97.9 *d*	97.3	97.2[a]	98.5 *d*	96.6	97.6 *d*	97.0	175.8 *s*	171.8	62.1 *t*	62.7	56.6 *t*	56.5 *t*	89.3 *d*
4	27.4 *t*	26.8	27.0	26.8 *t*	25.6	27.7 *t*	26.7	33.1 *t*	32.4	36.5 *t*	30.9	22.2 *t*	22.0 *t*	29.1 *t*
5	38.1 *d*	36.0	35.8	35.4 *d*	32.4	36.5 *d*	34.3	45.1 *d*	41.4	41.4 *d*	37.8	34.9 *d*	36.7 *d*	38.7 *d*
6	78.8 *d*	78.0	87.6	80.4 *d*	78.8	77.6 *d*	77.8	78.7 *d*	79.2	68.1 *d*	67.5	75.2 *d*	76.3 *d*	73.7 *d*
7	73.3 *d*	63.1	67.2	72.5 *d*	63.2	84.9 *d*	75.8	48.4 *t*	43.0	85.6 *d*	81.8	84.6 *d*	74.1 *d*	77.9 *d*
8	86.1 *s*	91.8	90.8	87.8 *s*	92.8	86.0 *s*	90.7	80.3 *s*	88.5	131.1[b] *s*	126.1§	85.1 *s*	85.3 *s*	85.6 *s*
9	50.7 *d*	49.0	47.4	46.7 *d*	41.5	‡*d*	48.2	49.1 *d*	45.6	132.3[b] *s*	129.1§	44.8 *d*	45.9 *d*	48.0 *d*
10	75.7 *t*	74.0	71.7	75.4 *t*	73.4	73.6 *t*	71.7	24.2 *q*	22.3	137.4[a] *d*	138.1‡	70.9 *t*	72.8 *t*	74.1 *t*
OMe (*q*)	55.9	55.8	55.2	55.7	55.7	56.0	55.9	—	—	—	—	—	—	—
			55.6											
			60.1											
Me (*q*)		20.8			20.9		20.8		20.6		20.7			
|		21.9			21.9		20.9		21.0		21.0 (2C)			
|							21.6							
CO (*s*)		170.6			169.9		170.3		170.5 (2C)		170.1			
		171.0			170.9		170.9				170.3			
							171.0				171.0			

*The spectra of iridoids were measured in CD_3OD and those of the acetates in $CDCl_3$. Assignments are based on ^{13}C–^{1}H COSY experiments. Multiplicity was confirmed by off-resonance or DEPT spectra.

†Measured at 125 MHz.

‡The signal was concealed behind the solvent signal.

[a,b]May be reversed in each column.



2 5a

Fig. 1. Possible conformation of jioglutin B (2) and jiofuran triacetate (5a). Arrows refer to the observed NOEs.

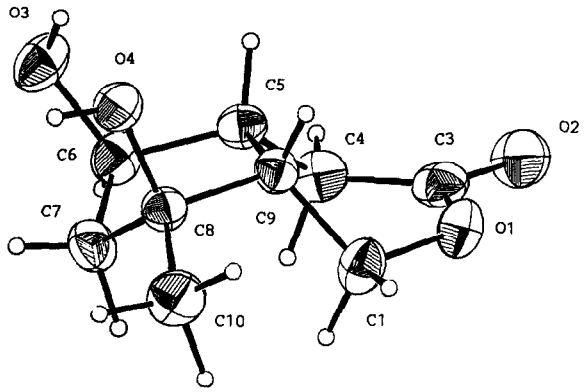
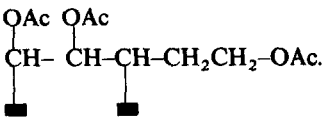


Fig. 2. X-Ray stereoscopic view of jioglutolide (4).

one methylene [δ4.24 (2H, *t*, J=6.6 Hz)] and two methines [δ5.17 (1H, *dd*, J=8.4, 5.3 Hz) and 5.94 (1H, *d*, J=5.3 Hz)]. Besides two more non-hydroxylated methylene [δ1.92 (1H, *ddt*, J=14.1, 8.6, 6.6 Hz) and 2.08 (1H, *m*)] and methine [δ3.32 (1H, *ddd*, J=8.6, 8.4, 1.8 Hz)] signals were observed. Successive ^{1}H spin decoupling and ^{1}H–^{1}H COSY experiments allowed the assignment of all the aliphatic proton signals and revealed the connectivity of these protons as follows:



In view of the degrees of unsaturation and the mode of biosynthesis of cyclopentanoid monoterpenes [10], it is likely that a cyclopenta[*c*]furan ring has been formed in **5**.

A NOESY experiment on **5a** showed cross peaks between the signals of H-1 and H-3, H-3 and H-5, H-4 and H-6, H-6 and H-7. No cross peaks, however, were observed between H-5 and H-6. This reveals the relative disposition of protons from H-5 to H-7 as shown in Fig. 1, assuming the usual stereochemistry at C-5 for iridoids. Thus, jiofuran was characterized as a cyclopentanoid-triol having a condensed furan ring and its structure was proposed as **5**.

The isolation of various iridoid glycosides such as catalpol, ajugol, etc. from the fresh or dried roots of *R. glutinosa* has been reported [1, 2, 11, 12]. However, we have hardly obtained these iridoid glycosides except for glutinoside (**8**). Instead we isolated non-glycosides, jioglutins, rehmaglutins, etc. from the steamed roots of this plant. This result suggests that the iridoid glycosides in the fresh or dried plant might have been converted into non-glycosidic compounds during the processing of the crude drug. Preliminary biological tests showed that jioglutolide (**4**) has a weak testosterone 5α-reductase inhibiting activity.

EXPERIMENTAL

Mps: uncorr; ^{1}H and ^{13}C NMR: 500 (in part 200) and 50 (in part 125) MHz, respectively, with TMS as int. standard; 2D NMR: 500 MHz in common conditions; EI and CIMS: 70 eV; prep. HPLC: prepacked CIG Si-10 column (silica gel, 15 mm i.d. × 30 cm); CC: silica gel 60 (70–230 mesh). Acetylation was conducted with Ac_2O, pyridine and a catalytic amount of 4-dimethylaminopyridine. Plant material was purchased from Yamamoto Yakuhin Kogyo Co., Ltd., Tokyo.

Isolation procedure. Steamed roots of *R. glutinosa* var. *hueichingensis* (100 kg) were extracted with EtOH (500 l, twice) under reflux. The EtOH extract was concentrated to a brown mass (6.94 kg), which was dissolved in H_2O and successively extracted with Et_2O, EtOAc (fr. D, 85 g) and *n*-BuOH (fr. E, 654 g). Fr. E was applied to a Diaion HP-20 CC (2.5 kg), eluted with H_2O (fr. E1, 443 g), 50% MeOH–H_2O (fr. E2, 161 g) and MeOH (fr. E3, 36 g) [5].

Fr. D was passed through a charcoal column (200 g) with H_2O and the Me_2CO as an eluent. The Me_2CO eluate (17 g) was subjected to silica gel CC (400 g) using increasing amount of MeOH in $CHCl_3$ (0:1→1:5), and was divided into 4 fractions, D1 (1.1 g), D2 (1.9 g), D3 (0.9 g) and D4 (1.0 g). Fr. D1 was repeatedly subjected to prep. HPLC, developed with $CHCl_3$–MeOH (19:1) or $CHCl_3$–MeCN (4:1), to give **1** (19 mg), **2** (10 mg), **5** (30 mg) and **7** (15 mg).

Fr. E1 was passed through a charcoal column (800 g) for removal of sugars by elution with H_2O. The MeOH eluate (56 g) was subjected to silica gel CC (1 kg), developed with an increasing amout of MeOH in $CHCl_3$ (0:1→1:2), and was divided into 5 fractions, E1-1 (26.9 g), E1-2 (13.2 g), E1-3 (6.6 g), E1-4 (4.4 g) and E1-5 (4.9 g). Fr. E1-1 was further chromatographed on a silica gel column (500 g) with an increasing amount of MeOH in $CHCl_3$ (0:1→1:4), and was subjected to prep. HPLC, eluted with $CHCl_3$–MeCN (3:2), to give **4** (21 mg). Fr. E1-2 was repeatedly subjected to prep. HPLC, eluted with a mixture of MeOH in $CHCl_3$, e.g. (1:9) etc. to yield **3** (27 mg), **6** (105 mg) and succinic acid (60 mg). Fr. E1-3 was chromatographed on a Lobar LiChroprep RP-8 column (25 mm i.d. × 30 cm) with H_2O to furnish **8** (231 mg). Fr. E1-4 was allowed to stand at room temp. in MeOH and gave a ppt., which was identified as 5-oxoproline Na salt (225 mg). Fr. E1-5 was subjected to silica gel CC (100 g), developed with $CHCl_3$–MeOH (5:1), to give uracil (75 mg), uridine (60 mg) and 5-hydroxymethylfuroic acid (345 mg), which were identified with authentic samples by direct comparison.

Jioglutin A (**1**). A white amorphous powder, $[\alpha]_D^{20}$ +63.3° (MeOH; *c* 1.00). ^{1}H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2. FDMS *m/z*: 250 $[M]^+$.

Jioglutin A diacetate (**1a**). A colourless oil, $[\alpha]_D^{27}$ +61.6° ($CHCl_3$; *c* 0.98). ^{1}H NMR ($CDCl_3$): δ1.42 (1H, *ddd*, J=14.6, 7.7 4.2 Hz, H-4α), 2.00 (1H, *ddd*, J=14.6, 6.1, 2.5 Hz, H-4β), 2.10, 2.1 (each 3H, *s*, OAc × 2), 2.69 (1H, *dddd*, J=10.0, 10.0, 4.2, 2.5 Hz H-5), 3.05 (1H, *dd*, J=10.0, 5.0 Hz, H-9), 3.45 (3H, *s*, OMe), 4.0

(1H, *dd*, J = 11.0, 0.6 Hz, H-10β), 4.36 (1H, *d*, J = 11.0 Hz, H-10α), 4.92 (1H, *dd*, J = 10.0, 0.6 Hz, H-7), 5.06 (1H, *dd*, J = 7.7, 6.1 Hz, H-3), 5.26 (1H, *dd*, J = 10.0, 10.0 Hz, H-6), 5.50 (1H, *d*, J = 5.0 Hz, H-1). ^{13}C NMR ($CDCl_3$): see Table 2. EIMS *m/z* (rel. int.): 336 (0.7), 334.0828 $[M]^+$ (calc. for $C_{14}H_{19}O_7Cl$: 334.0818) (2), 305 (3), 303 (7), 245 (1), 243 (3), 183 (50), 119 (7), 85 (100). CIMS (*iso*-butane), *m/z* (rel. int.): 337 (1), 335.0925 $[M+H]^+$ (calc. for $C_{14}H_{20}O_7Cl$: 335.0898) (3), 305 (33), 303 (100), 277 (14), 275 (43), 245 (17), 243 (48), 217 (5), 215 (17), 185 (4), 183 (14).

Conversion of catalpol (**10**) *into rehmaglutin B* (**9**). A soln of **10** (500 mg) in 1% HCl–MeOH (5 ml) was stirred at room temp. overnight. After concn, the reaction mixture was further treated with 10% HCl aq. (5 ml) at room temp. for 4 hr and then extracted with EtOAc. The EtOAc layer was evapd and subjected to prep. HPLC with $CHCl_3$–MeOH (9:1) as an eluent to afford **9** (240 mg).

Rehmaglutin B (**9**). Colourless needles (EtOAc–EtOH), mp 149–150°. $[\alpha]_D^{23}$ +34.4° (MeOH; *c* 1.19). IR ν_{max}^{KBr} cm^{-1}: 3292 (OH), 2936, 2889. ^{1}H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2. FDMS *m/z*: 239 $[M+H]^+$. (Found: C, 45.78; H, 5.59. Calc. for $C_9H_{13}O_5Cl$: C, 45.68; H, 5.54%). Compound **9** was identified as rehmaglutin B by direct comparison of ^{1}H, ^{13}C NMR and IR spectral data with those of an authentic sample [3].

Methylation of **1** *and* **9**. A soln of **1** (10 mg) or **9** (20 mg), with MeI (1.5 ml) and Ag_2O (500 mg) in DMF (2 ml) was stirred at room temp. overnight [13]. The reaction mixture was poured into EtOAc (15 ml) and washed with H_2O. After concn, the EtOAc layer was subjected to prep. HPLC [*n*-hexane–EtOAc (17:3)] to give **1b** (11 mg from **1**; 19 mg from **9**).

Jioglutin A dimethylether (**1b**). A colourless oil, $[\alpha]_D^{26}$ +39.2° ($CHCl_3$; *c* 1.00). ^{1}H NMR ($CDCl_3$): see Table 1. ^{13}C NMR ($CDCl_3$): see Table 2. EIMS *m/z* (rel. int.): 280 (1), 278 $[M]^+$ (4), 250 (2), 248 (6), 221 (2), 219 (7), 164 (5), 162 (14), 100 (100).

Jioglutin B (**2**). A white amorphous powder, $[\alpha]_D^{22}$ −63.2° (MeOH; *c* 0.94). ^{1}H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2. FDMS *m/z*: 273 $[M+Na]^+$.

Jioglutin B diacetate (**2a**). A colourless oil, $[\alpha]_D^{24}$ −64.0° ($CHCl_3$; *c* 0.18). ^{1}H NMR ($CDCl_3$): δ 1.74 (1H, *ddd*, J = 14.7, 6.7, 4.2 Hz, H-4), 1.84 (1H, *ddd*, J = 14.7, 2.3, 1.7 Hz, H-4), 2.11 (6H, *s*, OAc × 2), 2.58 (1H, *dddd*, J = 11.5, 9.8, 6.7, 1.7 Hz, H-5), 3.17 (1H, *dd*, J = 11.5, 6.5 Hz, H-9), 3.46 (3H, *s*, OMe), 3.92 (1H, *dd*, J = 10.8, 0.7 Hz, H-10β), 4.38 (1H, *d*, J = 10.8 Hz, H-10α), 4.75 (1H, *dd*, J = 4.2, 2.3 Hz, H-3), 4.86 (1H, *dd*, J = 9.8, 0.7 Hz, H-7), 5.53 (1H, *d*, J = 6.5 Hz, H-1), 5.85 (1H, *t*, J = 9.8 Hz, H-6). ^{13}C NMR ($CDCl_3$): see Table 2. EIMS *m/z* (rel. int.): 336 (0.7), 334.0809 $[M]^+$ (calc. for $C_{14}H_{19}O_7Cl$: 334.0818) (2), 305 (3), 303 (9), 245 (5), 243 (15), 183 (47), 119 (59), 85 (100). CIMS (*iso*-butane), *m/z* (rel. int.): 337 (0.7), 335 $[M+H]^+$ (2), 305 (32), 303 (100), 277 (3), 275 (10), 245 (17), 243 (48), 217 (15), 215 (10), 185 (14), 183 (36).

Jioglutin C (**3**). A white amorphous powder, $[\alpha]_D^{20}$ +58.1° (MeOH; *c* 0.89). ^{1}H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2. EIMS *m/z* (rel. int.): 202 $[M-OMe]^+$ (8), 182 (6), 156 (6), 145 (6), 97 (21), 85 (99), 58 (100).

Jioglutin C triacetate (**3a**). A colourless oil, $[\alpha]_D^{25}$ +32.5° ($CHCl_3$; *c* 0.45). ^{1}H NMR ($CDCl_3$): δ 1.45 (1H, *ddd*, J = 14.7, 7.9, 4.8 Hz, H-4), 1.97 (1H, *ddd*, J = 14.7, 5.7, 2.7 Hz, H-4), 2.05 (3H, *s*, OAc), 2.07 (6H, *s*, OAc × 2), 2.73 (1H, *dddd*, J = 10.7, 10.0, 4.8, 2.7 Hz, H-5), 2.96 (1H, *dd*, J = 10.0, 5.2 Hz, H-9), 3.47 (3H, *s*, OMe), 3.97 (1H, *br d*, J = 11.0 Hz, H-10β), 4.40 (1H, *d*, J = 11.0 Hz, H-10α), 5.05 (1H, *dd*, J = 7.9, 5.7 Hz, H-3), 5.31 (1H, *dd*, J = 10.7, 9.1 Hz, H-6), 5.49 (1H, *d*, J = 5.2 Hz, H-1), 5.90 (1H, *d*, J = 9.1 Hz, H-7). ^{13}C NMR ($CDCl_3$): see Table 2. EIMS *m/z* (rel. int.): 358.1266 $[M]^+$ (calc. for $C_{16}H_{22}O_9$: 358.1264) (5), 328 (5), 300 (16), 241 (95), 150 (100).

Jioglutolide (**4**). Colourless needles (Me_2CO), mp 141–142°. $[\alpha]_D^{20}$ −8.4° (MeOH; *c* 1.19). IR ν_{max}^{KBr} cm^{-1}: 3504, 3344 (OH), 1736 (C=O). ^{1}H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2. FDMS *m/z*: 187 $[M+H]^+$. (Found: C, 58.05; H, 7.58. $C_9H_{14}O_4$ requires: C, 58.16; H, 7.46%).

Jioglutolide diacetate (**4a**). Colourless needles (EtOH), mp 91–92°. $[\alpha]_D^{23}$ +4.0° ($CHCl_3$; *c* 0.50). ^{1}H NMR ($CDCl_3$): δ 1.58 (3H, *s*, H-10), 1.99, 2.02 (each 3H, *s*, OAc × 2), 2.06 (1H, *dd J* = 14.7, 6.3 Hz, H-7α), 2.54 (1H, *dd*, J = 15.2, 5.9 Hz, H-4), 2.57 (1H, *dddd*, J = 14.7, 4.2, 1.2, 1.2 Hz, H-7β), 2.74 (1H, *dd*, J = 15.2, 6.8 Hz, H-4), 2.78 (1H, *m*, H-9), 2.84 (1H, *m*, H-5), 4.28 (1H, *dd*, *J* = 12.1, 6.1 Hz, H-1), 4.32 (1H, *dd*, J = 12.1, 5.3 Hz, H-1), 4.78 (1H, *ddd*, J = 6.3, 4.2, 4.1 Hz, H-6). ^{13}C NMR ($CDCl_3$): see Table 2. CIMS (*iso*-butane), *m/z* (rel. int.): 271 $[M+H]^+$ (99), 211 (29), 151 (100).

X-Ray crystallographic analysis of **4**. The crystal size of **4** was 0.3 × 0.3 × 0.2 mm. Unit cell dimension was obtained by least-squares refinement using 21 centred reflections for which 20° < 2θ < 28° (graphite monochromatized MoKα, λ = 0.71073 Å). Intensity data were collected at $\omega/2\theta$ scans on an Enraf-Nonius CAD-4 with three check reflection at intervals of 200 reflections. Other crystal data were: $C_9H_{14}O_4$, orthorhombic, space group $P2_12_12_1$, Z = 4, a = 8.258 (3) Å, b = 16.525 (6) Å, c = 6.571 (2) Å, V = 896.8 (8) Å^3, D_{calc} = 1.38 g cm^{-3} and (MoKα) = 1.0 cm^{-1}. Intensities were measured for 959 reflections in the range 2° ≦ 2θ ≦ 50° with 866 considered as observed by the criterions $I > 3\sigma$ (*I*). The data were corrected for Lorents and polarization effects. No absorption correction was applied. The structure was solved by the direct-methods program Multan [14] and was refined by full-matrix least-squares, using the Enraf-Nonius SDP programs [15]. All the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located from difference maps. The last difference Fourier map was essentially featureless with no peaks greater than 0.17 eÅ^{-3}. The final discrepancy index was R = 0.038. Full crystal data are deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Jiofuran (**5**). A white amorphous powder, $[\alpha]_D^{24}$ −30.4° (MeOH; *c* 0.19). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH). ^{1}H NMR (200 MHz, CD_3OD): δ 1.76, 2.02 (each 1H, *m*, H-4), 2.97 (1H, *m*, H-5), 3.80 (2H, *m*, H-3), 3.90 (1H, *dd*, J = 7.6, 4.8 Hz, H-6), 4.76 (1H, *d*, *J* = 4.8 Hz, H-7), 7.23, 7.40 (each 1H, *s*, H-1, 10). ^{13}C NMR (CD_3OD): see Table 2. FDMS *m/z*: 184 $[M]^+$.

Jiofuran triacetate (**5a**). A colourless oil, $[\alpha]_D^{24}$ −154.9° ($CHCl_3$; *c* 0.26). UV λ_{max}^{EtOH} nm (log ε): 215.6 (3.69). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.92 (1H, *ddt*, J = 14.1, 8.6, 6.6 Hz, H-4), 2.08 (1H, *m*, H-4, overlapped with OAc signals), 2.05, 2.09, 2.12 (each 3H, *s*, OAc × 3), 3.32 (1H, *ddd*, J = 8.6, 8.4, 1.8 Hz, H-5), 4.24 (2H, *t*, J = 6.6 Hz, H-3), 5.17 (1H, *dd*, J = 8.4, 5.3 Hz, H-6), 5.94 (1H, *d*, J = 5.3 Hz, H-7), 7.22 (1H, *dd*, J = 1.8, 1.2 Hz, H-10), 7.40 (1H, *d*, J = 1.2 Hz, H-1). ^{13}C NMR ($CDCl_3$): see Table 2. EIMS *m/z* (rel. int.): 310.1049 $[M]^+$ (calc. for $C_{15}H_{18}O_7$: 310.1052) (3), 250 (32), 208 (53), 190 (24), 165 (41), 148 (100).

Rehmaglutin A (**6**). Colourless needles (Me_2CO–EtOAc), mp 132–134°. $[\alpha]_D^{20}$ +52.1° (MeOH; *c* 0.26). IR ν_{max}^{KBr} cm^{-1}: 3452, 3300 (OH). ^{1}H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2. EIMS *m/z* (rel. int.): 202 $[M]^+$ (3), 184 (38), 172 (69), 166 (47), 154 (48), 138 (36), 125 (100). Compound **6** was identified as rehmaglutin A by direct comparison of ^{1}H, ^{13}C NMR and IR spectral data with those of an authentic sample [3].

Rehmaglutin D (**7**). Colourless needles (Me_2CO–EtOAc), mp 129–130°. $[\alpha]_D^{22}$ +53.5° (MeOH; *c* 0.30). IR ν_{max}^{KBr} cm^{-1}: 3416 (OH). ^{1}H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see

Table 2. FDMS *m/z*: 221 $[M+H]^+$. Compound **7** was identified as rehmaglutin D by direct comparison of ^1H, ^{13}C NMR and IR spectral data with those of an authentic sample [3].

Glutinoside (**8**). A white amorphous powder, $[\alpha]_D^{20}$ −50.8° (MeOH; *c* 0.52); IR ν_{max}^{KBr} cm^{-1}: 3388 (OH); ^1H NMR (200 MHz, CD_3OD): δ1.65 (1H, *dd*, *J* = 13.4, 3.2 Hz, H-4), 2.2–2.6 (3H, *m*, H-4, 5, 9), 3.1–4.1 (10 H, *m*, H-6, 7, 10, 2′, 3′, 4′, 5′, 6′), 4.69 (1H, *d*, *J* = 7.8 Hz, H-1′), 5.25 (1H, *d*, *J* = 3.2 Hz, H-3), 5.62 (1H, *d*, *J* = 2.2 Hz, H-1); ^{13}C NMR (50 MHz, pyridine-d_5): δ34.0 (*t*, C-4), 36.2 (*d*, C-5), 48.1 (*d*, C-9), 62.3, 62.5 (each *t*, C-10, 6′), 71.3 (*d*, C-4′), 74.9 (*d*, C-2′), 76.4 (*d*, C-7), 78.7 (2C, *d*, C-3′, 5′), 79.8 (*s*, C-8), 84.7 (*d*, C-6), 93.1 (*d*, C-3), 95.1 (*d*, C-1), 99.8 (*d*, C-1′). FDMS *m/z*: 421 $[M+Na]^+$, 437 $[M+K]^+$. Compound **8** was identified as glutinoside by direct comparison of ^1H, ^{13}C NMR and IR spectral data with those of an authentic sample [4].

Exposure of rehmaglutin B (**9**) *to the conditions used for isolating jioglutins A–C* (**1–3**). A soln of **9** (12.0 mg) in MeOH (1 ml) was adsorbed on to a silica gel column (20 mm i.d. × 25 cm) and allowed to stand at room temp. for 3 days. Elution with $CHCl_3$–MeOH (3:1) (200 ml) and concn gave unchanged **9** (11.6 mg) which was taken up in MeOH (0.5 ml) and subjected to prep. HPLC, eluted with $CHCl_3$–MeOH (5:1) (150 ml). The unchanged **9** (10.8 mg) from HPLC was taken up in MeOH (1 ml) and applied to a charcoal CC (15 mm i.d. × 25 cm) eluted with H_2O (150 ml) and then MeOH (150 ml). The recovery of **9** was 10.5 mg. No formation of **1–3** from **9** was detectable by TLC ($CHCl_3$–MeOH, 5:1) in any of the steps just described.

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