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IRIDOID GLYCOSIDES OF *REHMANNIA GLUTINOSA*

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Key Word Index—*Rehmannia glutinosa* var. *purpurea*; *R. glutinosa* var. *hueichingensis*; Scrophulariaceae; iridoid glucosides; galactosyliridoid glucosides; sophorosyliridoid glucoside; ^{13}C NMR spectra.

Abstract—From the roots of *Rehmannia glutinosa* var. *purpurea* and *R. glutinosa* var. *hueichingensis*, besides four known iridoid glucosides, catalpol, leonuride (= ajugol), aucubin and melittoside, four new glycosides of the same class, rehmannioside A, rehmannioside B, rehmannioside C and rehmannioside D, have been isolated and their structures were established as 6′-*O*-α-D-galactosylcatalpol, 6-*O*-α-D-galactosylcatalpol, 8-*O*-α-D-galactosylleonuride and 5-*O*-β-sophorosylmonomelittoside, respectively. Moreover, from the terrestrial parts of both plants catalpol, leonuride, aucubin, dihydrocatalpol and monomelittoside were isolated. Among these glycosides, rehmannioside D showed a weak hypoglycemic action on spontaneously diabetic mice.

INTRODUCTION

Rehmannia glutinosa var. *purpurea* (Japanese name, Akayajio) and *R. glutinosa* var. *hueichingensis* (Japanese name, Kaikeijio) are indigenous to China and have been cultivated as medicinal plants in Japan and China; roots of both plants have been utilized in Chinese medicine as hematopoietics or tonics.

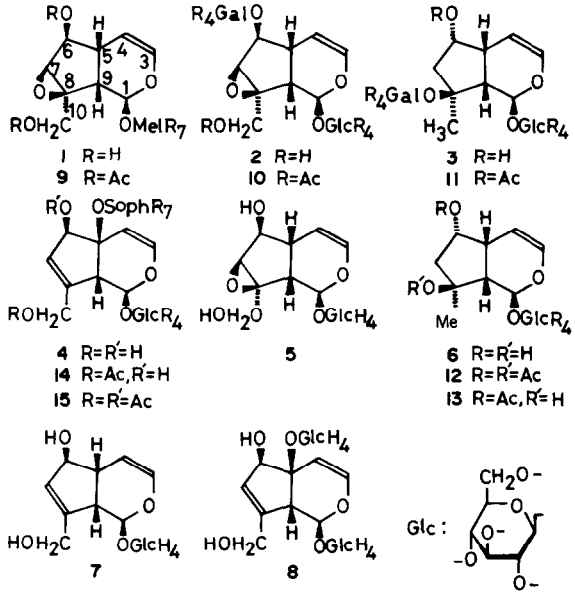
Regarding the iridoid glycosides of these plants, there is only a report by Kitagawa *et al.* [1] on the isolation of catalpol from the roots of *R. glutinosa* var. *hueichingensis*. In the studies on iridoid glycosides of both *R. glutinosa* plants, we isolated from the roots, besides four known glucosides, four new glycosides, and elucidated their structures. Furthermore, we isolated five known glucosides from the terrestrial parts.

RESULTS AND DISCUSSION

From the MeOH extract of the fresh roots of *R. glutinosa* var. *purpurea* or *R. glutinosa* var. *hueichingensis*, four new iridoid glycosides, rehmannioside A (**1**), rehmannioside B (**2**), rehmannioside C (**3**) and rehmannioside D (**4**), were isolated along with four known glucosides, catalpol (**5**) [1], leonuride (= ajugol) (**6**) [2, 3], aucubin (**7**) [4, 5] and melittoside (**8**) [6] according to the procedure described in the Experimental.

Rehmannioside A (**1**) was obtained as the nonaacetate (**9**), which, on alkaline hydrolysis, regenerated **1** as a white powder, $C_{21}H_{32}O_{15}$, $[\alpha]_D^{25}$ −0.1° (MeOH). The ^{13}C NMR spectrum of **1** was similar to that of catalpol (**5**), except for the signals of sugar moieties (Table 1), suggesting **1** to be a catalpol glycoside. On the other hand, **1** and **5** and galactose on hydrolysis with α-galactosidase (prepared from coffee beans), and melibiose (6-*O*-α-D-galactosyl-D-glucose) as the sugar component on hydrolysis with 10% HCO_2H. The structure of **1**, therefore, was elucidated as 6′-*O*-α-D-galactosylcatalpol (= catalpogenin-β-melibioside).

Rehmannioside B (**2**) was obtained as the nonaacetate (**10**), which, on alkaline hydrolysis, regenerated **2** as a white powder, $C_{21}H_{32}O_{15}$, $[\alpha]_D^{25}$ −8.8° (MeOH). The ^{13}C NMR spectrum of **2** indicated that it was also a glycoside of **5** (Table 1). Rehmannioside B (**2**) gave catalpol (**5**) and galactose on hydrolysis with α-galactosidase, but did not afford any hydrolysate on treatment with β-galactosidase (prepared from *E. coli*). On hydrolysis with 10% HCO_2H, **2** gave glucose and galactose, but did not yield any disaccharide. These facts imply that galactose links to the C-6 or C-10 OH group of the catalpol moiety in the glycoside **2**.



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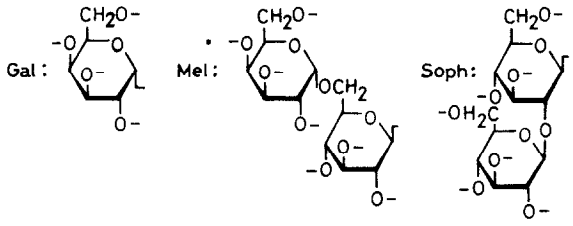


The ^{13}C NMR spectrum (D_2O) of **2** showed signals at δ 36.8 (C-5), 86.5 (C-6) and 62.0 (C-7), whereas the spectrum of **5** showed signals of the corresponding carbons at δ 38.2, 78.4 and 62.9, respectively. The differences in the chemical shifts of the corresponding carbon atoms of both **2** and **5**, [−1.4 (C-5), +8.1 (C-6) and −0.9 (C-7)], clearly showed the glycosidation shift caused by the linkage of galactose to the C-6 OH group of **5** [7, 8]. Furthermore, the appearance of the C-10 carbon signals of both compounds at the same field (δ 60.9) indicated that galactose is not linked to the C-10 OH group of **5**. The chemical shift (δ 100.9) of the anomeric carbon of the galactosyl moiety in rehmannioside B (**2**) was in accordance with the corresponding shift (δ 100.5) of methyl-α-D-galactopyranoside, and agreed with that (δ 99.1) of rehmannioside A (**1**), but disagreed with the value (δ 104.9) of methyl-β-D-galactopyranoside [9]. Thus, the spectral data also demonstrated the α-configuration of the anomeric carbon of galactose moiety in **2**. On the basis of these findings, the structure of rehmannioside B (**2**) was established to be 6-*O*-α-D-galactosylcatalpol.

Rehmannioside C (**3**) was also obtained as the nonaacetate, which gave the original glycoside **3** as a white powder, $C_{21}H_{34}O_{14}$, $[\alpha]_D^{25}$ −11.3° (MeOH) on alkaline hydrolysis. As the signal pattern of the ^{13}C NMR spectrum of **3** was similar to that of leonuride (**6**), except for signals of sugar moieties, it was assumed that **3** is a glycosylleonuride (Table 1). Actually, **3** gave **6** and galactose on treatment with α-galactosidase whereas it was not hydrolysed with β-galactosidase. Furthermore, it gave glucose and galactose on heating with 10% HCO_2H. Compound 3, therefore, should be assigned the structure 6- or 8-*O*-α-D-galactosylleonuride. The absence of the OH group, which resists the usual acetylation with Ac_2O–pyridine at room temperature, however, demonstrated the linkage of galactose to the C-8 OH group. The location of the galactosyl group was also confirmed

Table 1. ^{13}C NMR data of glycosides and some acetates*

C-atom†	1	2	3	4	5	6	8	11	14	18	21
1	95.7	95.5	94.1	96.5	95.5	93.8	96.0	92.2	95.7	95.3	92.4
3	141.4	141.4	139.6	145.4	141.3	139.4	145.5	139.5	143.9	58.2	143.3
4	104.0	104.2	105.8	104.9	104.0	105.8	105.6	103.9	106.6	27.0	104.9
5	38.1	36.8	39.7	82.0	38.2	39.6	81.5	37.6	80.3	85.5	76.6
6	78.5	86.5	77.0[a]	81.0[a]	78.4	77.1	80.7	77.6	82.6	77.0[a]	197.7
7	62.8	62.0	45.8	128.5	62.9	49.0	128.5	44.9	131.2	37.4	128.8
8	66.6	66.4	86.2	144.6	66.6	79.3	144.2	84.8	138.3	32.5	168.0
9	42.6	41.8	49.1	52.1	42.7	50.4	51.5	47.0	53.0	50.9	53.0
10	61.0	60.9	24.1	60.5	60.9	25.1	60.5	23.6	61.7[a]	17.8	61.7[a]
1′	99.5[a]	99.4	98.7	99.0	99.3	98.8	98.9	95.4	96.6	98.2	96.9
2′	73.5	73.6	73.6	73.7	73.7	73.5	73.7[a]	70.6	70.5[b]	73.6[b]	70.0[b]
3′	76.6	77.1	76.9[a]	77.1[b]	77.1	76.7	77.1	72.6	72.6[c]	77.4[a]	72.5[c]
4′	70.4	70.1	70.5	70.2[c]	70.4	70.5	70.2[b]	68.3	68.0[d]	70.3[c]	67.9[d]
5′	75.6	76.5	76.5	76.5[d]	76.5	76.5	76.6[c]	72.0	72.3[c]	76.3[a]	72.3[c]
6′	66.8	61.5	61.6	61.3[e]	61.7	61.6	61.4	61.8[a]	61.5[a]	61.5[d]	61.6[a]
1″	99.1[a]	100.9	94.6	97.2			98.6	91.5	97.5	97.1	97.8
2″	69.2	69.2	69.3	80.8[a]			74.0[a]	66.6	78.1	80.5	78.9
3″	70.4	70.4	70.5	76.8[d]			76.6	68.1	72.9[c]	76.4[a]	73.1[c]
4″	70.0	70.1	70.2	70.2[c]			70.3[b]	67.5	68.8[d]	70.4[c]	68.7[d]
5″	71.9	72.0	71.8	76.4[d]			76.3[c]	68.1	71.1[c]	76.0[a]	72.0[c]
6″	61.9	62.4	62.1	61.7[e]			61.4	61.6[a]	62.0[a]	61.5[d]	61.6[a]
1‴				103.8					101.1	103.3	100.4
2‴				74.8					71.1[b]	74.5[b]	70.7[b]
3‴				77.2[b]					74.5[c]	77.0[a]	73.6[c]
4‴				70.5[c]					68.3[d]	70.5[c]	68.0[d]
5‴				76.4[d]					72.7[c]	76.4[a]	72.9[c]
6‴				61.4[e]					62.0[a]	62.0[a]	61.4[a]

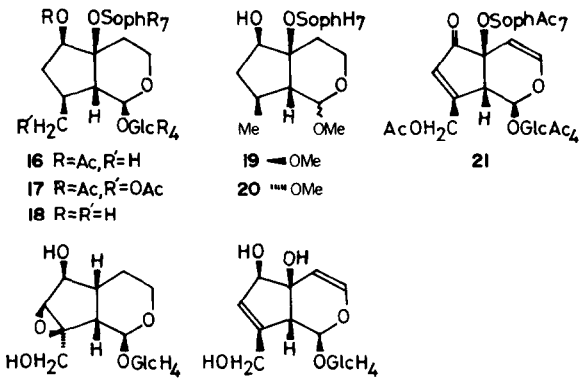
* The spectra of **1**–**6**, **8** and **18** were measured in D_2O and those of the others in $CDCl_3$. δ values of the chemical shifts of OAc C-atoms were not given.

† C-1′–C-6′ are C-atoms of glucose bound to the C-1 OH group. C-1″–C-6″ are C-atoms of galactose moiety of compounds **1**, **2**, **3** and **11** or those of glucose portion bound to C-5 OH group of compounds **4**, **8**, **14**, **18** and **21**. C-1‴–C-6‴ are C-atoms of glucose bound to C-2″ OH group of **4**, **14**, **18** and **21**.

Signals bearing the same alphabetical superscript in any one column can be interchanged.

by the ^{1}H NMR data. The C-1 proton signal of leonuride hexaacetate (**12**) with 8-OAc group appeared at δ 5.81 (*s*) in a lower field, compared with the corresponding signal at δ 5.41 of leonuride pentaacetate (**13**) with the free 8-OH group in accord with the reported regularity [10, 11]. On the other hand, the C-1 proton signal of rehmannioside C nonaacetate (**11**) appeared like that of rehmannioside C (**3**) without the lower field shift at δ 5.42 (*d*, J = 1.8 Hz), suggesting the absence of an OAc group on C-8. Moreover, a significant glycosidation shift was observed in the chemical shift (δ 86.2) of C-8 of rehmannioside C (**3**) in comparison with that (δ 79.3) of leonuride (**6**). The anomeric carbon signal (δ 94.6) of galactose in **3** appeared upfield by 4.5 and 6.3 ppm relative to the corresponding signals (δ 99.1 and 100.9) of the glycosides **1** and **2** possessing an α-galactosyl moiety. On the other hand, the latter two were found in 4.6 and 2.8 ppm upfield from the frequency of the corresponding signal of lactose (δ 103.7) with β-galactosyl moiety, respectively. Since the above-described upfield shift of the anomeric carbon signal of galactose in **3** is ascribed to the linkage of the galactosyl moiety to the *tert*-OH group [8], these spectral data also clearly indicate the α-configuration of the anomeric carbon of galactose in **3**. On the basis of the above findings, the structure of **3** was established to be 8-*O*-α-D-galactosylleonuride.

Rehmannioside D (**4**) was obtained as a white powder, $C_{27}H_{42}O_{20}$, $[\alpha]_D^{25}$ −27.1° (H_2O), with a slightly sweet taste. It gave a dodecaacetate (**14**) as the main product and a tridecaacetate (**15**) as the minor one on acetylation with Ac_2O–pyridine at 1°, but with the same reaction carried out at room temperature yielded **15** as the main product with **14** as the minor one. The ^{13}C NMR spectrum of **4** was similar to that of melittoside (**8**), except for the signals due to the sugar portions, indicating the close structural relationship of both glycosides (Table 1). Actually, **4** slowly gave **8** and glucose on hydrolysis with diastase [12]. Furthermore, it yielded sophorose (2-*O*-β-D-glucosyl-D-glucose) and glucose on heating with 10% HCO_2H. Sophorosylmonomelittoside or 2′-*O*-β-D-glucosylmelittoside, therefore, should be assigned to rehmannioside D (**4**). Rehmannioside D tridecaacetate (**15**) afforded tetrahydrodeoxyrehmannioside D dodecaacetate (**16**) on catalytic hydrogenation over Pd–C, along with a small amount of tetrahydrorehmannioside D tridecaacetate (**17**). In the ^{1}H NMR spectrum of **16**, the Me signal appeared at δ 1.09 as a sharp doublet indicating that only one of the C-8 epimers had been formed.



Likewise, in the ^{13}C NMR spectrum of **18**, prepared by hydrolysis of **16**, only a single Me signal appeared (δ 17.8). It is known that the catalytic hydrogenation (Pd–C) of asperuloside tetraacetate gives rise to 7-deoxyloganin (the 8β-methyl epimer) [13] and in view of the bulky groups present on the β-face of **15**, it seems likely that the C-8 centre of **16** and **18** have the S-configuration as drawn in the figures. By analogy the same configuration is assigned to **17**, although in this case the ^{1}H NMR spectrum could not be analysed in detail. On the other hand, **18** gave a pair of C-1 Me ethers (**19** and **20**), with the same composition, $C_{22}H_{38}O_{14}$, and glucose on treatment with Amberlite IR-120 in dry MeOH. Compound **19** also gave sophorose as a sugar component on treatment with 10% HCO_2H. These findings prove that the glucosyl moiety is linked to C-1 in the original glycoside. The structure of rehmannioside D (**4**), therefore, was concluded to be 5-*O*-β-sophorosyl-monomelittoside.

The stereochemistry on C-1 of both Me ethers (**19** and **20**) was established by comparing the C-1 proton signals of the two compounds which appeared in coupling with C-9 protons at δ 4.98 (*d*, J = 7.0 Hz) and δ 4.54 (*d*, J = 3.0 Hz), respectively. Since the C-9 proton in each compound presumably assumes a pseudoaxial position on the dihydropyran ring, the above coupling constants imply that the C-1 proton of **19** is pseudoaxial and that of **20** is pseudoequatorial position. Consequently, the OMe group of **19** should have the β-configuration and that of 20 the α-configuration. The free OH group of rehmannioside D dodecaacetate was localized on the basis of the structure of its MnO_2 oxidation product (**21**), $C_{51}H_{64}O_{32}$. This showed an absorption at 227 nm (log ε 3.87) due to a conjugated ketone in the UV spectrum, and, in the ^{1}H NMR spectrum, the C-7 proton signal at δ 6.22 as a broad singlet, which appeared at δ 5.80 as a doublet (J = 1.0 Hz) in the spectrum of **14**. Compound **21** further showed in the ^{13}C NMR spectrum a signal at δ 197.7 due to the C-atom of the newly formed 6-keto group and a signal at δ 168.0 due to the C-8 in the allylic position; both in agreement with the proposed structure [14]. Accordingly, it can be concluded that **21** is a 6-keto compound and hence **14** has the free OH group at the C-6 position.

Rehmannioside A (**1**), B (**2**) and C (**3**) are the second examples of iridoid glycosides having a galactose moiety [15], whereas rehmannioside D (**4**) represents the first iridoid glycoside having a sophorose moiety. Moreover, it is noteworthy that **4** shows a weak hypoglycemic action on spontaneously diabetic mice by oral administration. Detailed results on this respect will be reported elsewhere.

Finally, from the terrestrial parts of both *Rehmannia* plants, five known iridoid glucosides, catalpol (**5**), leonuride (**6**), aucubin (**7**), dihydrocatalpol (**22**) [16] and monomelittoside (**23**) [17, 18] were also isolated, of which the last four were obtained as acetates.

EXPERIMENTAL

General procedures. Mps are uncorr. ^{1}H NMR spectra were recorded at 90 MHz using TMS as an int. (in $CDCl_3$) or ext. (in D_2O) standard. ^{13}C NMR spectra were determined at 50 MHz using TMS (in $CDCl_3$) or dioxane (δ 67.4) (in D_2O) as int. standard. Chemical shifts are given in δ (ppm) relative to the standard. Granular charcoal and Si gel 60 (70–230 mesh) were employed for CC. Si gel 60 F_{254} was used for TLC. Iridoid glycosides and sugars were developed with

$CHCl_3$–MeOH–H_2O (6:4:1), and acetates of glycosides with Et_2O. The spots were visualized by spraying with a mixture of anisaldehyde (0.5 ml), conc H_2SO_4 (10 ml) and 50% EtOH (90 ml) followed by heating. GC analyses were carried out after trimethylsilylation of aliquots of samples with *bis*-TMSiacetamide–TMCS in pyridine. Column: 1 m × 3 mm packed with 0.5% OV-1. Carrier gas: N_2 at 70 ml/min. Detector: dual FID. Temps.: programmed (i) 100° → at 3°/min (monosaccharides), (ii) 150° → at 3°/min (disaccharides), (iii) 190° → at 2°/min and 210° → at 4°/min (iridoid glycosides). Injector temp. 270°. Detector temp. 320°.

Plant materials. Roots of *R. glutinosa* Liboschitz var. *purpurea* Makino and *R. glutinosa* var. *hueichingensis* Chao et Schih, cultivated at Fukuchiyama Experimental Farm, Takeda Chemical Industries (Kyoto prefecture), were collected in December 1978 and 1979, while the terrestrial parts of both plants were collected in September 1980. Both plants were identified by G. Murata of Kyoto University and voucher specimens (*R. glutinosa* var. *purpurea*: H. Inouye and H. Oshio No. 1; *R. glutinosa* var. *hueichingensis*: H. Inouye and H. Oshio No. 2) have been deposited in the Herbarium of the Department of Botany, Faculty of Science, Kyoto University.

Isolation of iridoid glucosides from roots of R. glutinosa *var.* purpurea. Fresh roots (2 kg) were sliced and extracted with MeOH (4 l. × 3) under reflux. The MeOH extract was concd *in vacuo* and the resulting viscous soln (*ca* 1.5 l.) was shaken with a mixture of H_2O (1 l.) and EtOAc (2 l.). The aq. layer was concd *in vacuo*, H_2O (3 l.) added to the residue and the insoluble material filtered off. The filtrate was applied to a charcoal (460 g) column and eluted successively with H_2O (60 l.) and MeOH (15 l.). Concn of the first MeOH fraction gave a residue (Fr. 1, 41 g) containing monoglucosides. The 2nd MeOH fraction, on concn, gave a residue (Fr. 2, 15 g) containing other glycosides. Fr. 1 was chromatographed on Si gel (560 g) with $CHCl_3$–MeOH–H_2O (6:4:1) as eluent to yield a fraction of high glucoside content (8.5 g), which gave, on recryst. from EtOH, colourless needles (5.5 g) of catalpol (**5**), mp 206–208°, $[\alpha]_D^{25}$ −103.8° (MeOH, $c = 0.5$). R_f 0.43; R_t 9.0. (Found: C, 49.53; H, 6.10. Calc. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12%.) The mother liquor containing **5** and two additional glucosides, namely leonuride (**6**) (R_f 0.49, R_t 6.7) and aucubin (**7**) (R_f 0.47, R_t 7.4) was concd to dryness and acetylated with Ac_2O–pyridine at 1° overnight. Si gel (180 g) CC of the product with Et_2O as eluent gave three substances, which, on recrystallization from EtOH, yielded the following glucoside acetates. Aucubin hexaacetate, colourless needles (0.086 g), mp 128–129°, $[\alpha]_D^{22}$ −157.0° ($CHCl_3$, $c = 0.5$), R_f 0.85, identical with an authentic sample (TLC, mmp and IR). (Found: C, 54.06; H, 5.71. Calc. for $C_{27}H_{34}O_{15}$: C, 54.18; H, 5.73%.) Catalpol hexaacetate, colourless needles (2.87 g), mp 140–141°, $[\alpha]_D^{22}$ −88.3° ($CHCl_3$, $c = 0.5$), R_f 0.76, identical with an authentic sample (TLC, mmp and IR). (Found: C, 53.08; H, 5.64. Calc. for $C_{27}H_{34}O_{16}$: C, 52.77; H, 5.58%.) Leonuride pentaacetate (**13**), colourless needles (0.207 g), mp 126°, $[\alpha]_D^{22}$ −68.6° ($CHCl_3$, $c = 0.5$), R_f 0.52, identical with an authentic sample (TLC and IR). (Found: C, 53.72; H, 6.19. Calc. for $C_{25}H_{34}O_{14}$: C, 53.76; H, 6.14%.) **13** also gave leonuride hexaacetate (**12**) on acetylation with Ac_2O–pyridine at 50° for 48 hr, which was purified by recrystallization from EtOH to yield colourless needles, mp 171–172°, $[\alpha]_D^{22}$ −147.7° ($CHCl_3$, $c = 0.5$), R_f 0.85. (Found: C, 54.09; H, 6.00. Calc. for $C_{27}H_{36}O_{15}$: C, 54.00; H, 6.04%.) Fr. 2 was further divided into two fractions through Si gel CC (560 g) with $CHCl_3$–MeOH–H_2O (6:4:1) as eluant. From the faster-moving fraction (Fr. 2-1, 0.9 g), the following four glycosides were detected by TLC and GC: rehmannioside A (**1**) (R_f 0.28, R_t 20.6), rehmannioside B (**2**) (R_f 0.24, R_t 22.9), rehmannioside C (**3**) (R_f 0.32, R_t 19.2) and melittoside (**8**) (R_f 0.27, R_t 21.6), while from the slower-moving fraction (Fr. 2-2, 0.4 g) only rehmannioside D (**4**) (R_f 0.18, R_t 28.8) was detected. Fr. 2-1 was acetylated with Ac_2O–pyridine in the usual way and the product chromatographed on Si gel (220 g) with Et_2O as eluent to give the following acetates, which were further purified by recryst. from EtOH. Rehmannioside A nonaacetate (**9**), colourless needles (64 mg) mp 196°, $[\alpha]_D^{22}$ +2.2° ($CHCl_3$, $c = 0.5$); R_f 0.65. IR ν_{max}^{KBr} cm^{-1}: 1740, 1643; ^{1}H NMR ($CDCl_3$): δ 1.95–2.08 (9 × OCOMe), 2.40–2.72 (2 H, *m*, 5-H and 9-H), 5.39 (1 H, *br s*, 1-H), 6.27 (1 H, *q*, J = 6 and 2 Hz, 3-H). (Found: C, 51.95; H, 5.58. $C_{39}H_{50}O_{24}$ requires: C, 51.88; H, 5.58%.) Rehmannioside B nonaacetate (**10**), colourless needles, 65 mg, mp 170–172°, $[\alpha]_D^{22}$ +23.8° ($CHCl_3$, $c = 0.5$), R_f 0.53. IR ν_{max}^{KBr} cm^{-1}: 1752, 1740, 1655; ^{1}H NMR ($CDCl_3$): δ 1.96–2.11 (9 × OCOMe) 2.41–2.77 (2 H, *m*, 5-H and 9-H), 5.44 (1 H, *br s*, 1-H), 6.27 (1 H, *q*, J = 6 and 2 Hz, 3-H). (Found: C, 51.64; H, 5.51. $C_{39}H_{50}O_{24}$ requires: C, 51.88; H, 5.58%.) Rehmannioside C nonaacetate (**11**), colourless plates, 17 mg, mp 156–157°. $[\alpha]_D^{22}$ −10.8° ($CHCl_3$, $c = 0.5$), R_f 0.65. IR ν_{max}^{KBr} cm^{-1}: 1742, 1645; ^{1}H NMR ($CDCl_3$): δ 1.33 (3 H, *s*, 10-H), 1.97–2.12 (9 × OCOMe), 2.69 (2 H, *br s*, 5-H and 9-H), 5.42 (1 H, *d*, J = 1.8 Hz, 1-H), 6.11 (1 H, *q*, J = 6 and 1 Hz, 3-H). (Found: C, 52.94; H, 5.75. $C_{39}H_{52}O_{23}$ requires: C, 52.70; H, 5.90%.) Melittoside decaacetate, colourless needles, 280 mg, mp 149° (lit. [6] 149–149.5°), $[\alpha]_D^{22}$ −39.8° ($CHCl_3$, $c = 0.5$). R_f 0.42. (Found: C, 52.23; H, 5.59. Calc. for $C_{41}H_{52}O_{25}$: C, 52.12; H, 5.55%.) Fr. 2-2 was dissolved in hot EtOH and the insoluble material filtered off. On cooling the filtrate to 1°, rehmannioside D (**4**) precipitated as a white powder (288 mg). $[\alpha]_D^{27}$ −27.1° (H_2O, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 3370, 1645; ^{1}H NMR (D_2O): δ 5.30 (1 H, *d*, J = 6 Hz, 1-H), 5.43 (1 H, *d*, J = 6 Hz, 4-H), 5.90 (1 H, *br s*, 7-H), 6.57 (1 H, *d*, J = 6 Hz, 3-H). (Found: C, 47.02; H, 6.19. $C_{27}H_{42}O_{20}$ requires: C, 47.23, H, 6.17%.)

Isolation of iridoid glycosides from roots of R. glutinosa *var.* hueichingensis. Fresh roots (2.2 kg) were treated in the same way as in the case of *R. glutinosa* var. *purpurea* to yield **4** (0.40 g), **5** (8.57 g), **9** (0.15 g), **10** (0.33 g), **11** (0.07 g), **13** (0.50 g), catalpol hexaacetate (3.68 g), melittoside decaacetate (0.16 g) and aucubin hexaacetate (0.38 g).

Acetylation of rehmannioside D (**4**). **4** (200 mg) was acetylated with Ac_2O–pyridine at room temp. overnight and the product was chromatographed on Si gel (100 g) with Et_2O as eluant to give the tridecaacetate (**15**) (235 mg) and the dodecaacetate (**14**) (33 mg). In contrast to this result, acetylation of **4** (200 mg) at 1° overnight gave **14** (244 mg) as the major product and **15** (21 mg) as the minor one. Dodecaacetate (**14**) was obtained as colourless needles after recrystallization from MeOH, mp 236°, $[\alpha]_D^{22}$ −9.3° ($CHCl_3$, $c = 0.5$); R_f 0.08. IR ν_{max}^{KBr} cm^{-1}: 1738, 1630; ^{1}H NMR ($CDCl_3$): δ 1.97–2.08 (12 × OCOMe), 5.80 (1 H, *d*, J = 1 Hz, 7-H), 6.40 (1 H, *d*, J = 6 Hz, 3-H). (Found: C, 51.49; H, 5.52. $C_{51}H_{66}O_{32}$ requires: C, 51.43; H, 5.59%.) Tridecaacetate (**15**) was obtained as a white powder on cooling a soln of it in EtOH. $[\alpha]_D^{22}$ −45.6° ($CHCl_3$, $c = 0.5$), R_f 0.23. IR ν_{max}^{KBr} cm^{-1}: 1755, 1738, 1650; ^{1}H NMR ($CDCl_3$): δ 1.95–2.14 (13 × OCOMe), 5.80 (1 H, *d*, J = 1 Hz, 7-H), 6.33 (1 H, *d*, J = 6 Hz, 3-H). (Found: C, 51.55; H, 5.62. $C_{53}H_{68}O_{33}$ requires: C, 51.62; H, 5.56%.)

Hydrolysis of glycoside acetates. (i) A soln of rehmannioside A nonaacetate (**9**) (100 mg) and NaOH (1.5 g) in 75% MeOH (50 ml) was stirred at room temp. for 6 hr. After concn of the soln *in vacuo*, the residue was redissolved in H_2O (200 ml) and applied to a charcoal column (20 g). After washing the column with H_2O (2 l.), the glycoside was eluted with MeOH. On concn of the MeOH eluate, the residue was dissolved in hot EtOH and left standing in a freezer to afford a white powder of rehmannioside A (**1**) (30 mg). $[\alpha]_D^{25}$ −0.1° (MeOH, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 3390, 1648 (sh), 1570, 1418; ^{1}H NMR (D_2O): δ 5.11 (1 H, *s*, 1-H), 5.27 (1 H, *q*, J = 6 and 1 Hz, 4-H), 5.56 (1 H, *q*, J = 6 and 1 Hz, 3-H).

(Found: C, 47.93; H, 6.20. $C_{21}H_{32}O_{15}$ requires: C, 48.09; H, 6.15%.) (ii) Rehmannioside B nonaacetate (**10**) (100 mg) was hydrolysed and worked up in the same way as above to afford a white powder of rehmannioside B (**2**) (36 mg). $[\alpha]_D^{25}$ −8.8° (MeOH, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1558, 1416; ^1H NMR (D_2O): δ 6.53 (1 H, *q*, $J = 6$ and 1 Hz, 3-H). (Found: C, 47.95; H, 6.23. $C_{21}H_{32}O_{15}$ requires: C, 48.09; H, 6.15%.) (iii) Rehmannioside C nonaacetate (**11**) (100 mg) afforded, on treatment as above, a white powder of rehmannioside C (**3**) (29 mg), $[\alpha]_D^{25}$ −11.3° (MeOH, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 3390, 1640, 1560, 1410; ^1H NMR (D_2O): δ 1.56 (3 H, *s*, 10-H), 5.71 (1 H, *br s*, 1-H), 6.38 (1 H, *q*, $J = 6$ and 1 Hz, 3-H). (Found: C, 49.21; H, 6.66. $C_{21}H_{34}O_{14}$ requires: C, 49.41; H, 6.71%.) (iv) Melittoside decaacetate (500 mg) afforded, on treatment as above, a white powder of melittoside (**8**) (176 mg), $[\alpha]_D^{27}$ −31.0° (H_2O, $c = 1.0$) (lit. [6] −29° (H_2O, $c = 1.6$)). (Found: C, 47.89; H, 5.99. Calc. for $C_{21}H_{32}O_{15}$: C, 48.09; H, 6.15%.) The above-described glycosides **1**, **2**, **3** and **8** regenerated from the acetates showed the same R_f and R_t values as the respective glycosides detected in the extract, indicating the occurrence of these glycosides in free forms in both plants.

Hydrolysis of glycosides with galactosidase. (i) A soln of rehmannioside A (**1**) (100 mg) and α-galactosidase [prepared from coffee beans (Boehringer Mannheim GmbH, 5 mg/ml)] (0.2 ml) in 0.1 M acetate buffer (pH 5.5) (5 ml) was incubated at 32° for 4 days, neutralized with NH_4OH and then concd *in vacuo*. The residue was chromatographed on Si gel (20 g) with $CHCl_3$–MeOH–H_2O (6:4:1) as eluent to give two products. The more mobile one was recrystallized from EtOH to afford colourless needles, 51 mg, mp 206–208°, which were identified as catalpol (**5**) (TLC, mmp and IR). (Found: C, 49.62; H, 6.30. Calc. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12%.) The less mobile one (35 mg) was identified as galactose (TLC and GC). (ii) Rehmannioside B (**2**) (100 mg) was incubated with α-galactosidase under the same conditions as above for 24 hr. Work-up of the reaction gave catalpol (**5**) (54 mg), mp 206–208°, and galactose (31 mg), which were identical to authentic samples (TLC and GC), respectively. On the contrary, glycoside (**2**) did not afford any hydrolysate on treatment with β-galactosidase prepared from *E. coli* (Boehringer Mannheim GmbH, 5 mg/ml). (iii) Rehmannioside C (**3**) (100 mg) was incubated with α-galactosidase under the same conditions as above for 5 days. The reaction was subjected to Si gel CC in the usual way, giving two products. From a soln of the more mobile substance in EtOH a white powder (53 mg) precipitated on cooling, which was identified as leonuride (**6**) (TLC and GC). Acetylation of **6** with Ac_2O–pyridine at 60° for 48 hr followed by recrystallization of the product from EtOH gave colourless needles, mp 171–172°, which were identical with leonuride hexaacetate (**12**) (mmp and IR). (Found: C, 53.71; H, 6.10. Calc. for $C_{27}H_{36}O_{15}$: C, 54.00; H, 6.04%.) The less mobile substance (36 mg) was identical with galactose (TLC and GC). On treatment with β-galactosidase, **3** also did not afford any hydrolysate.

Hydrolysis of glycosides with HCO_2H. (i) A soln of rehmannioside A (**1**) (100 mg) in 10% HCO_2H (10 ml) was refluxed for 1.5 hr. The resulting dark brown ppt. was filtered off, and the filtrate, after neutralization with Amberlite IR 45 (200 ml), was concd to dryness *in vacuo*. The residue (53 mg) was chromatographed on Si gel (50 g) using $CHCl_3$–MeOH–H_2O (6:4:1) as eluent. The first eluate contained a trace amount of glucose and galactose which were detectable on TLC. The following eluates containing the main product were combined and concd. A soln of the residue in a small amount of H_2O afforded on addition of *ca* 20 vol. EtOH a white powder (26 mg) of melibiose. $[\alpha]_D^{25}$ +132.0° (H_2O, $c = 0.5$, 8 hr after dissolution). R_f 0.13; R_t 23.8 (α-form, *ca* 40%), 24.6 (β-form, *ca* 60%). (Found: C, 39.81; H, 6.62. Calc. for $C_{12}H_{22}O_{11} \cdot H_2O$: C, 39.99; H, 6.73%.) (ii) A soln of rehmannioside B (2) (50 mg) in 10% HCO_2H (10 ml) was refluxed for 3 hr. The sugar portion (21 mg) obtained by work-up in the same way as above was found to be a mixture of glucose (R_f 0.29, R_t 15.0 (α-form) and 19.0 (β-form)) and galactose (R_f 0.29, R_t 14.4). (iii) Rehmannioside C (**3**) (50 mg) was hydrolysed in the same way as above. The resulting sugars (25 mg) were glucose and galactose (TLC and GC). (iv) A soln of rehmnannioside D (**4**) (600 mg) in 10% HCO_2H (100 ml) was refluxed for 8 hr. The reaction was worked up in the same way as above. Si gel CC of the product gave two classes of sugars. One (194 mg) was identical with glucose (TLC and GC). The other (214 mg) gave, on recrystallization from aq. EtOH, colourless needles, mp 196°, $[\alpha]_D^{25}$ −19.8° (H_2O, $c = 0.5$, 7 hr after dissolution), which were identical with sophorose [mmp, TLC and GC; R_f 0.20; R_t 22.6 (α-form, *ca* 1%), 23.8 (β-form, *ca* 99%)]. (Found: C, 39.83: H, 6.59. Calc. for $C_{12}H_{22}O_{11} \cdot H_2O$: C, 39.99; H, 6.73%.) (v) A soln of Me ether (**19**) (12 mg) in 10% HCO_2H (2 ml) was refluxed for 4 hr. The reaction was worked up in the same way as above. The resulting sugar was identical with sophorose (TLC and GC).

Hydrolysis of rehmannioside D (**4**) *with β-glucosidase.* A soln of **4** (200 mg) and β-glucosidase [prepared from diastase (Pharmacopoea Japonica IX) according to ref. [12]] in 0.1 M acetate buffer (pH 5.1, 20 ml) was left standing at 32° for 3 weeks. After the addition of MeOH (30 ml), the resulting ppt was filtered off, and the filtrate concd *in vacuo*. The residue (212 mg) was chromatographed on Si gel (160 g) with $CHCl_3$–MeOH–H_2O (6:4:1) as eluant. The initially eluted substance (70 mg) was glucose (TLC and GC). The next eluted substance (71 mg) coincided with melittoside (**8**) (TLC and GC). The acetate of the latter (colourless needles from EtOH, mp 148.5°) was identical to authentic material (mmp and IR). (Found: C, 51.91; H, 5.45. Calc. for $C_{41}H_{52}O_{25}$: C, 52.12; H, 5.55%.) The last eluted substance (44 mg) was starting material (**4**).

Catalytic hydrogenation of rehmannioside D tridecaacetate (**15**). **15** (3 g) was hydrogenated over 5% Pd–C (1 g) in MeOH (300 ml) until H_2 uptake (3.2 mol) had ceased. After removing the catalyst by filtration, the filtrate was concd and the residue chromatographed on Si gel (200 g) with Et_2O as eluant. From a soln of the more mobile substance in EtOH a white powder (1.46 g) of tetrahydrodeoxyrehmannioside D dodecaacetate (**16**) precipitated on cooling. $[\alpha]_D^{25}$ −26.5° ($CHCl_3$, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 1742; ^1H NMR ($CDCl_3$): δ 1.09 (3 H, *d*, $J = 6$ Hz, 10-H), 1.95–2.13 (12 × OCOMe). (Found: C, 51.76; H, 6.03. $C_{51}H_{70}O_{31}$ requires: C, 51.95; H, 5.98%.) From a soln of the less mobile substance in EtOH a white powder (170 mg) of tetrahydrorehmannioside D tridecaacetate (**17**) precipitated on cooling. $[\alpha]_D^{25}$ −26.2° ($CHCl_3$, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 1745; ^1H NMR ($CDCl_3$): δ 1.95–2.14 (13 × OCOMe). (Found: C, 51.20; H, 5.93. $C_{53}H_{72}O_{33}$ requires: C, 51.45; H, 5.87%.)

Hydrolysis of tetrahydrodeoxyrehmannioside D dodecaacetate (**16**). A soln of **16** (1.8 g) and NaOH (6 g) in 75% MeOH (200 ml) was stirred at room temp. overnight. The successive work-up of the reaction in the usual way gave a white powder (700 mg) of tetrahydrodeoxyrehmannioside D (**18**). $[\alpha]_D^{25}$ −42.0° (H_2O, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 3370, 1625; ^1H NMR (D_2O): δ 1.28 (3 H, *d*, $J = 6$ Hz, C-10), 5.32 (1 H, *s*, 1-H). (Found: C, 47.80; H, 6.61. $C_{27}H_{46}O_{19}$ requires: C, 48.07; H, 6.87%.)

Methanolysis of tetrahydrodeoxyrehmannioside D (**18**). A mixture of **18** (500 mg), Amberlite IR 120B (H^+ form) (600 mg) and dry MeOH (20 ml) was stirred at room temp. for 24 hr. After removal of the resin by filtration, the MeOH soln was concd and the residue chromatographed on Si gel (140 g) with $CHCl_3$–MeOH–H_2O (6:4:1), yielding two fractions. The less mobile fraction (64 mg) was identified as glucose (TLC and GC).

The more mobile fraction (250 mg) was rechromatographed on Si gel (140 g) with *n*-BuOH (satd with H_2O) as eluant to give two substances. From a soln of the more mobile substance in EtOH a white powder of Me ether (**20**) (50 mg) precipitated on cooling. $[\alpha]_D^{25}$ +12.9° (MeOH, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 3400; ^1H NMR (D_2O): δ 1.29 (3 H, *d*, $J = 5.5$ Hz, 10-H), 3.51 (3 H, *s*, OMe), 4.54 (1 H, *d*, $J = 3$ Hz, 1-H). (Found: C, 49.99; H, 7.31. $C_{22}H_{38}O_{14}$ requires: C, 50.18; H, 7.27%.) From a soln of the less mobile substance in EtOH a white powder of Me ether (**19**) (90 mg) precipitated on cooling. $[\alpha]_D^{25}$ −28.7° (MeOH, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: 3400; ^1H NMR (D_2O): δ 1.30 (3 H, *d*, $J = 6$ Hz, 10-H), 3.53 (3 H, *s*, OMe), 4.98 (1 H, *d*, $J = 7$ Hz, 1-H). (Found: C, 50.30; H, 7.11. $C_{22}H_{38}O_{14}$ requires: C, 50.18; H, 7.27%.)

Oxidation of rehmannioside D dodecaacetate (**14**). A soln of **14** (500 mg) in $CHCl_3$ (300 ml) was stirred with active MnO_2 (10 g) at room temp. for 24 hr, and after addition of further oxidant (5 g), the stirring was continued for 24 hr. After removal of MnO_2 by filtration, the filtrate was concd and the residue (280 mg) chromatographed on Si gel (70 g) with Et_2O as eluant. From the main fraction 6-dehydrorehmannioside D dodecaacetate (**21**) (85 mg) was precipitated as a white powder by addition of *n*-hexane. $[\alpha]_D^{22}$ −87.1° ($CHCl_3$, $c = 0.4$); UV λ_{max}^{EtOH} nm (log ε): 227 (3.87); IR ν_{max}^{KBr} cm^{-1}: 1736, 1640; ^1H NMR ($CDCl_3$): δ 1.97–2.14 (12 × OCOMe), 6.22 (1 H, *br s*, 7-H), 6.37 (1 H, *d*, $J = 5.7$ Hz, 3-H). (Found: C, 51.36; H, 5.52. $C_{51}H_{64}O_{32}$ requires: C, 51.52; H, 5.42%.)

Isolation of glucosides from the terrestrial parts of R. glutinosa *var.* purpurea. Fresh plant material (10.5 kg) was extracted with boiling MeOH (20 l. × 2) and the soln was concd *in vacuo* to *ca* 2 l. The resulting soln was shaken with a mixture of H_2O (2 l.) and EtOAc (3 l.). The aq. layer was concd *in vacuo* and the insoluble substance filtered off. The filtrate was subjected to charcoal (800 g) CC, eluting with H_2O (100 l.) and MeOH (50 l.) successively. A fraction containing a mixture of iridoid glucosides (60 g) was further chromatographed on Si gel (2.1 kg) with $CHCl_3$–MeOH–H_2O (6:4:1) to give Fr. 1 containing catalpol (**5**), leonuride (**6**) and acucubin (**7**) (20.7 g) and Fr. 2 containing **5**, dihydrocatalpol (**22**) (R_f 0.36, R_t 9.2) and monomelittoside (**23**) (R_f 0.36, R_t 8.6, identical with authentic sample) (7 g). Fr. 1 was recrystallized from EtOH to give colourless needles of **5** (8.5 g). The residue from the mother liquor was acetylated with Ac_2O–pyridine at 1° overnight and the product chromatographed on Si gel (500 g) with Et_2O as eluant to afford acucubin hexaacetate (0.45 g), catalpol hexaacetate (4.2 g) and leonuride hexaacetate (**13**) (1.6 g). Fr. 2 was also acetylated in the same way as above, and the product was subjected successively to CC on Si gel (2 and 1 kg) with Et_2O as eluant to afford catalpol hexaacetate (3.2 g), monomelittoside hexaacetate (0.07 g) and dihydrocatalpol hexaacetate (1.1 g). Monomelittoside hexaacetate was obtained on recrystallization from EtOH as colourless needles, mp 172–174° (lit. [17] 169–170°). R_f 0.49; $[\alpha]_D^{25}$ −170.0° ($CHCl_3$, $c = 0.5$). (Found: C, 52.81; H, 5.56. Calc. for $C_{27}H_{34}O_{16}$: C, 52.77; H, 5.58%.) It gave, on acetylation with Ac_2O–pyridine at 60° for 3 days, the heptaacetate, which was recrystallized from EtOH to give colourless needles, mp 160° (lit. [17] 158–160°). R_f 0.70; $[\alpha]_D^{24}$ −162.3° ($CHCl_3$, $c = 0.8$). (Found: C, 52.90; H, 5.54. Calc. for $C_{29}H_{36}O_{17}$: C, 53.05; H, 5.53%.) Dihydrocatalpol hexaacetate was obtained, on recrystallization from EtOH, as colourless plates, mp 152–154°. R_f 0.47; $[\alpha]_D^{24}$ −74.4° ($CHCl_3$, $c = 1.0$) identical with authentic sample (mmp and IR). (Found: C, 52.41; H, 5.91. Calc. for $C_{27}H_{36}O_{16}$: C, 52.59; H, 5.89%.)

Isolation of glucosides from the terrestrial parts of R. glutinosa *var.* hueichingensis. Fresh material (10.8 kg) was treated in the same way as above to give **5** 6.90 g, **13** 1.07 g, catalpol hexaacetate 3.82 g, aucubin hexaacetate 0.04 g, dihydrocatalpol hexaacetate 0.08 g and monomelittoside hexaacetate 0.04 g.

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