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IRIDOIDS IN *ROTHMANNIA GLOBOSA*

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Key Word Index—*Rothmannia globosa*; Rubiaceae; iridoids; α-gardiol; β-gardiol; genipin; geniposide; mussaenoside; scandoside methylester; gardenoside; artifacts.

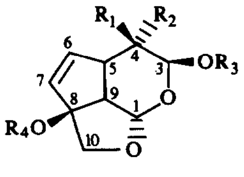
Abstract—The fruits of *Rothmanina globosa* contained the known iridoid glucosides geniposide, mussaenoside, scandoside methyl ester and gardenoside. In addition, they contained genipin and two novel non-glucosidic iridoids named α- and β-gardiol. It has been demonstrated that the two gardiols are epimeric at C-4 and that they can be formed from gardenoside by enzymatic hydrolysis. It is suggested that genipin and the gardiols are artifacts formed during work-up.

INTRODUCTION

The genus *Rothmannia* has only been sparsely investigated for chemical constituents. Kooiman has examined the seeds of *R. hispida* and *R. longiflora*, but did not detect iridoids in either [1]. Here the results with *R. globosa.* (Hochst.) Keay are reported.

RESULTS AND DISCUSSION

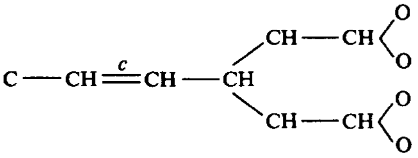
From fruits of *R. globosa*, the known [2] iridoid glucosides geniposide (**8**), mussaenoside (**9**), scandoside methyl ester (**10**) and gardenoside (**11**) were isolated. In addition, the non-glucosidic iridoid genipin (**7**) and two novel iridoids, which have been named α- and β-gardiol, were isolated. Both of the latter compounds can be produced from gardenoside (**11**) by enzymatic hydrolysis (see below). The gardiols differ from **11** by not having an absorption maximum at 235 nm due to a conjugated ester group. Thus, despite the fact that both gardiols analyse for $C_{11}H_{14}O_6$, as gardenoside aglucone would do, a rearranged skeleton must be present in these compounds.



1 $R_1 = COOMe$, $R_2 = R_3 = R_4 = H$
2 $R_1 = COOMe$, $R_2 = R_4 = H$, $R_3 = Bz$
3 $R_1 = COOMe$, $R_2 = H$, $R_3 = R_4 = Bz$
4 $R_1 = R_3 = R_4 = H$, $R_2 = COOMe$
5 $R_1 = R_4 = H$, $R_2 = COOMe$, $R_3 = Bz$
6 $R_1 = H$, $R_2 = COOMe$, $R_3 = R_4 = Bz$

Bz = Benzoyl

The ^1H NMR spectrum (Table 1) of β-gardiol (**1**) resembles that of the aglucone moiety of **11**, except that the absorption from H-3 found at δ7.5 in the latter, is lacking in **1**. On the other hand, the presence of two signals at *ca* δ5.5 indicates that **1** contains two acetalic centres. Decoupling experiments demonstrated the partial structure A, a modified gardenoside aglucone moiety. The ^{13}C NMR spectrum (Table 2) showed the presence of two acetalic centres (δ101.5 and 89.2) and also that only one double bond was present in **1**, consistent with the partial structure above. However, two formulae are compatible with this partial structure and the NMR data, namely **12** and **1**, disregarding stereochemistry. Structure **12** could have been formed by a Michael-addition of the C-10 oxygen to the 3,4-double bond, whereas the alternative **1** could have arisen by a ring-closure between the C-10 oxygen and C-1, followed by the addition of water to the double bond. The latter ring-closure has some precedent since iridoid aglucones with a CH_2–OH group in the 8α-position are known to react in this way [3, 4]; however, in such cases the 3,4-double bond is kept intact.



A

Benzoylation of β-gardiol (**1**) under mild conditions afforded a monobenzoate (**2**). In the ^1H NMR spectrum of this compound, the low field position (δ6.74) of the signal from one of the acetalic protons, showed that this centre had been acylated. The ^{13}C NMR spectrum of **2**

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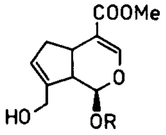
*This work was carried out during a stay at the Department of Organic Chemistry, University of Melbourne, Victoria, Australia.

Table 1. ^1H NMR data for compounds **1–6** (100 MHz, $CDCl_3$, TMS as int. standard)

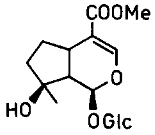
H No.	**1**	**2**	**3**	**4**	**5**	**6**
1	5.62 *d* (6.3)	5.68 *d* (5.5)	5.71 *d* (5.3)	5.61 *d* (5.4)	5.63 *d* (5.0)	5.70 *d* (4.7)
3	5.37 *dd* (2.1; 7.4*)	6.74 *d* (2.5)	6.75 *d* (2.8)	5.34 *d* (8.4)	6.53 *d* (8.5)	6.63 *d* (8.4)
4	2.82 *dd* (2.0; 6.4)	3.09 *dd* (2.5; 10.5)	3.20 *dd* (2.8; 10.0)	2.97 *dd* (5.4; 8.4)	3.07 *dd* (4.8; 8.5)	3.15 *dd* (4.7; 8.5)
5	3.7 *m*	3.8 *m*	3.8 *m*	3.48 *m*	3.7 *m*	3.7 *m*
6	5.97 *dd* (1.8; 5.1)	6.15 *dd* (2.1; 5.6)	6.24 *dd* (1.7; 5.4)	5.95 *s*	5.93 *m*	6.05 *dd* (1.7; 5.7)
7	5.87 *dd* (2.1; 5.4)	5.94 *dd* (1.7; 5.6)	6.38 *dd* (1.9; 5.4)			6.33 *dd* (2.6; 5.7)
9	2.70 *dd* (6.3; 8.9)	2.92 *dd* (5.4; 9.2)	3.44 *dd* (5.1; 9.1)	2.70 *dd* (5.4; 8.4)	2.93 *dd* (4.9; 8.2)	3.40 *dd* (4.5; 8.1)
10	3.67 3.92 AB (9.5)	3.72 4.01 AB (9.7)	3.98 4.43 AB(10.5)	3.96 4.05 AB (9.6)	4.10 4.17 AB(10.5)	4.47 *brs*
OMe	3.82	3.76	3.72	3.76	3.73	3.74

The values given are in δ-values; multiplicity and coupling constants (Hz) in parentheses.

*The additional coupling to OH at C-3 (δ3.93).



7 R = H
8 R = Glc



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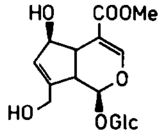

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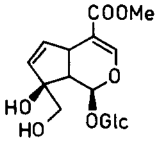

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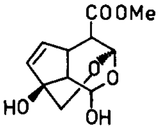

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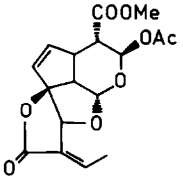

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Table 2. ^{13}C NMR data for compounds **1–8** and **11** (25.1 MHz)

C. No.	**1***	**2†**	**3†**	**4***	**5†**	**6†**	**11***	**7†**	**8***
1	101.5	99.5	98.8	101.2	101.1	98.8	94.1	96.1	98.1
	(180)	(175)	(176)		(172)		(174)		(174)
3	89.2	89.3	89.3	90.3	89.0	91.2	151.6	152.4	153.4
	(166)	(182)	(182)		(179)		(192)		(194)
4	46.9	46.1	45.7	47.3	44.1	44.0	110.9	110.4	112.4
	(135)	(130)	(133)		(132)		*s*		*s*
5	41.7	39.0	38.7	43.4	41.7	41.9	37.9	36.5	35.1
	(137)	(138)	(140)		(139)		(136)		(138)
6	138.5	137.3	139.0	135.7	134.2	136.4	134.2	38.8	38.9
	(166)	(166)	(167)		(168)		(167)		(133)
7	134.1	134.7	131.6	135.7	136.5	133.3	135.8	130.1	129.8
	(166)	(168)	(171)		(170)		(167)		(163)
8	93.4	93.4	99.2	93.0	93.5	99.4	86.0	142.0	142.3
	s	*s*	*s*		*s*		*s*		*s*
9	46.9	49.3	47.3	51.1	54.5	52.8	51.4	47.6	46.6
	(135)	(138)	(142)		(137)		(132)		(139)
10	73.0	74.4	74.0	75.8	78.3	77.4	66.0	60.9	60.6
	(147)	(149)	(149)		(150)		(142)		(144)
11	173.8	170.3	170.2	174.4	171.0	170.7	169.8	167.4	170.8
	s	*s*	*s*		*s*		*s*		*s*
OMe	53.4	52.5	52.5	53.6	52.6	52.8	52.7	51.3	52.7

*D_2O (dioxane).
†$CDCl_3$ (TMS).

showed only minor changes from that of **1**, but due to the change of solvent, no certain conclusions could be drawn from this fact.

Further benzyolation, under more drastic conditions, gave rise to the dibenzoate, **3**. The two structural possibilities could be distinguished by comparison of the ^{1}H NMR spectra of **2** and **3**. The second benzoyl group must have been introduced at the tertiary centre C-8, as medium downfield acylation shifts expected for the signals of the protons at C-7, C-9 and C-10 were observed. The values found were δ0.44 for H-7, *ca* 0.34 for the C-10 protons and 0.52 for a third proton which must, therefore, be H-9. The remaining signals showed only negligible shifts. In the spectrum of **3**, the signal thus assigned to H-9 (δ3.44) showed a coupling to the signal at δ5.71, assigned to the proton on C-1. In conclusion, the low field signal (δ6.75) must be assigned to the proton at C-3; hence, this centre carried the benzoyl group, consistent only with the structure **3**, disregarding the stereochemistry. α-Gardiol (**4**) gave rise to the mono- and di-benzoates **5** and **6**, respectively, and arguments similar to those based on the NMR spectra demonstrated that **1** and **4** had the same gross structure. As both compounds can be formed from gardenoside (**11**) under very mild conditions, the stereochemistry could be considered proven in all centres, except at C-3 and C-4, and furthermore, the difference between **1** and **4** had to reside within these centres. The compound allamandin isolated from *Allamanda cathartica* [5] has been assigned the structure **13**, which is structurally similar to **1** and **4**. Comparison of the available ^{1}H NMR data for 3-*O*-acetylallamandin with those of **6**, showed a complete coincidence of the coupling constants and a reasonable agreement between the shift-values for H-1–H-9. Following the arguments given in ref. [5], the stereochemistry for α-gardiol must be that depicted for **4** with the 3-hydroxyl group in the β-position and the 4-methoxycarbonyl group in the α-position. The configuration at C-3 and C-4 in **1** could be deduced from the ^{1}H NMR spectrum of **3**. The coupling constant $J_{4,5} = 10$ Hz indicates a *trans*-diaxial arrangement of H-4 and H-5 and examination of a Dreiding model showed that if the tetrahydropyran ring assumed a boat-like conformation $_1B_4$ with the 3-*O*-substituent and the methoxycarbonyl group both in the β-positions, the expected coupling constants between the ring protons are in accord with those measured for **3**. In order to verify this, a series of NOE experiments were carried out. In **3** the relatively large NOE effects between H-3 and H-4 (6.5 and 7 %; see Table 3) together with the smaller effects between H-4 and H-5 (2 and 3 %) confirmed the *cis*-disposition of the former pair, as well as the *trans*-disposition of the latter pair. Furthermore, notable effects were found between H-1 and H-10β (8.5 and 3.5 %), showing that the tetrahydrofuran ring assumes an E_0-confirmation with a 1,3-diaxial proximity between these protons. Re-examination of the model demonstrated that such a conformation of the tetrahydrofuran ring was in good agreement with the $_1B_4$ conformation of the six-membered ring. In conclusion, β-gardiol has the structure depicted as **1** and, thus, the two gardiols are epimeric at C-4.

In addition to the iridoid glucosides mentioned above, ethyl glucoside could be identified as a constituent of the isolated mixture. However, when the isolation was repeated using methanol as the solvent instead of ethanol, methyl glucoside replaced ethyl glucoside as a constituent of the crude mixture. This indicated that the fruits of *R. globosa* contained large amounts of an enzyme(s) capable of converting glucose present in the fruits into the glucoside of the alcohol used as the solvent for work-up. When half-dry leaves of the plant were examined after work-up with ethanol, only geniposide (**8**) and gardenoside (**11**) were detected by TLC. Furthermore, when gardenoside was treated with β-glucosidase in aqueous solution and the reaction followed by TLC it could be demonstrated (see Experimental) that the aglucone initially formed was quickly converted to a *ca* 1:1 mixture of the gardiols, and that **1** and **4** could be prepared in this way. Therefore, it must be suspected that genipin as well as the gardiols are artifacts formed during work-up of the fruits. Unfortunately, no more plant material was available to test this suggestion.

Table 3. ^{1}H NMR NOEs obtained for **3** (%)

Irradiated proton	Observed proton 1	3	4	5	6	9	10α	10β
1	—	1	—	—	—	9	—	3.5
3	0.5	—	6.5	—	—	—	—	—
4	2	7	—	3	3	—	—	—
5	1.5	—	2	—	4	6	—	—
10α	—	—	—	—	—	—	—	30
10β	8.5	—	—	—	—	—	30	—

The values given are estimated to be correct within ±10% relative, they were obtained at 270 MHz ($CDCl_3$ without degassing) at 310 K in the difference mode.

EXPERIMENTAL

MPs are uncorr. and were determined in capillary tubes in a heated bath; Prep. TLC: 1 mm Si gel PF_{254} bands detected with UV light; microanalyses were performed by the Australian Microanalytical Service, Melbourne. Plant vouchers were deposited at the National Herbarium in Melbourne (SRJ 150) and in Sydney (R.G.C. 10189), respectively.

Isolation of iridoids. Fresh unripe fruits of *Rothmannia globosa* were homogenized with EtOH and filtered. The ethanolic extract was evaporated to near dryness, then partitioned between H_2O and Et_2O. The organic phase was again evaporated to dryness and partitioned between H_2O–MeOH (2:1) and hexane, and the two aq. phases combined. Evaporation onto Si gel was followed by elution with Me_2CO. Fruits collected in Melbourne (June 1978; 167 g) gave 3.3 g Me_2CO eluate, while those collected in Sydney (368 g) gave 3.9 g eluate. The two extracts seemed identical in composition (TLC, NMR) and were combined and chromatographed on Si gel (500 g) with $CHCl_3$–MeOH (20:1–2:1) as eluant. This gave fractions A (1.5 g) and B (3.8 g). An aliquot of fraction A (315 mg) was rechromatographed by TLC (Et_2O–MeOH, 19:1) to give, as the fastest moving band, genipin (7, 18 mg); ^{1}H NMR (60 MHz, $CDCl_3$): δ7.53 (*br s*, H-3), 5.85 (*m*, H-7), 4.79 (*d*, $J_{1,9} = 9$ Hz; H-1), 4.28 (*m*, CH_2-10), 3.72 (*s*, OMe). It was identical (TLC, ^{1}H NMR) to a sample prepared by hydrolysis (β-glucosidase) of geniposide (**8**). The next band (not detected by UV) consisted of β-gardiol (**1**, 124 mg), crystals from EtOAc, mp 122–124°; $[\alpha]^{21}_{D}$ +211° (MeOH; *c* 0.3); NMR data in Tables 1–2. (Found: C, 54.25; H, 5.76. $C_{11}H_{14}O_6$ requires: C, 54.54; H, 5.83 %.) The next band contained α-gardiol (**4**, 118 mg), a syrup; $[\alpha]^{21}_{D}$ +152° (MeOH; *c* 0.8); NMR data in Tables 1–2. (Found: C, 54.49; H, 5.94. $C_{11}H_{14}O_6$ requires, as above.) Fraction B contained geniposide (**8**, *ca* 1 g, 0.2 %), mussaenoside (**9**, 150 mg, 0.03 %), scandoside methyl ester (**10**, 175 mg, 0.03 %) and gardenoside (**11**, *ca* 1 g, 0.2 %). These compounds were identified by comparison (TLC, ^{1}H NMR) with authentic samples. Also, ethyl glucoside (*ca* 300 mg) was isolated. It was solely characterized by ^{1}H NMR (60 MHz, D_2O): δ4.42 (*d*, $J_{1,2} = 7$ Hz, H-1′), 3.75 (*q*, O–CH_2), 1.19 (*t*, Me). This compound was shown to be an artifact formed during work-up of the plant material in EtOH, as it was not present when MeOH was used. In the latter case, methyl glucoside was formed as seen in the ^{1}H NMR spectrum: δ3.55 (*s*, OMe).

3-O-Benzoyl β-gardiol (**2**). Benzoylation (pyridine, C_6H_5COCl, 0°, 15 min) gave **2**. Crystallization from Et_2O–hexane, mp 124°; $[\alpha]^{21}_{D}$ +158° ($CHCl_3$; *c* 0.3). NMR data in Tables 1–2. (Found: C, 62.68; H, 5.35. $C_{18}H_{18}O_7$ requires: C, 62.42; H, 5.24 %.)

3,8-*Di-O-benzoyl β-gardiol* (**3**). Benzoylation (1hr, 25°) gave **3** as a syrup. $[\alpha]_D^{21}$ + 146° ($CHCl_3$; *c* 1.5). NMR data in Tables 1–3. (Found: C, 66.81; H, 4.93. $C_{25}H_{22}O_8$ requires: C, 66.66; H, 4.92%.)

3-O-*Benzoyl α-gardiol* (**5**). Prepared as **2**. Mp 160–161°; $[\alpha]_D^{21}$ + 104° ($CHCl_3$; *c* 0.4). NMR data in Tables 1–2. (Found: C, 62.13; H, 5.15. $C_{18}H_{18}O_7$ requires: C, 62.42; H, 5.24%.)

3,8-*Di-O-benzoyl α-gardiol* (**6**). Prepared as **3**, mp 166–167°; $[\alpha]_D^{21}$ + 150° ($CHCl_3$; *c* 0.6). NMR data in Tables 1–2. (Found: C, 66.79; H, 5.03. $C_{25}H_{22}O_8$ requires: C, 66.66; H, 4.92%.)

Enzymatic hydrolysis of gardenoside (**11**). β-Glucosidase (4 mg) and **11** (20 mg) were dissolved in H_2O (2.5 ml) and stirred. The reaction was followed by TLC (EtOAc–C_6H_6–EtOH; 4:1:1); detection by spraying with dilute H_2SO_4 and heating.

After a few min a UV-active spot (presumably gardenoside aglucone) could be seen at R_f 0.58 (blue) in addition to **11** at R_f 0.15 (blue). After 10 min a spot (brown-black) corresponding to the gardiols could be seen at R_f 0.63. Gardenoside had been completely hydrolysed after 40 min, and after 80 min only the gardiols could be detected. In another expt, **11** (244 mg) in H_2O (5 ml) was treated as above. After satn with $NaSO_4$, extraction with several portions of Et_2O gave 134 mg (90%) of a *ca* 1:1 mixture of **1** and **4**.

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