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ABSOLUTE STRUCTURE OF GIBBOSIDE, AN IRIDOID GLUCOSIDE FROM *PATRINIA GIBBOSA**

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Key Word Index—*Patrinia gibbosa*; Valerianaceae; iridoid glucoside; gibboside; COSY; X-ray crystallographic analysis.

Abstract—A new iridoid glucoside, gibboside, was isolated from the root of *Patrinia gibbosa*, together with three known glucosides, patrinoside, valerosidate and adoxoside. Based on spectroscopic and X-ray crystallographic studies, the absolute structure of the new iridoid glucoside was determined as (4*R*,4a*S*,6*S*,7*R*,7a*S*)-6-β-D-glucopyranosyl-7-hydroxymethyl-4-methyl-1,4,4a,7a-tetrahydrocyclopenta[e]pyran-3-one.

INTRODUCTION

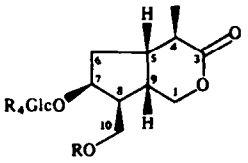
Patrinia gibbosa Maxim. was known to contain patrinoside (**9**) [1] which was first isolated from *Patrinia scabiosaefolia* Fischer [2]. In our recent work, we re-examined the iridoid constituents of this plant and isolated, besides **9**, a new type of iridolactone glucoside named gibboside (**1**) having a β-D-glucopyranosyloxy group at the C-7 position, together with known iridoid glucosides, valerosidate (**5**) [3, 4] and adoxoside (**7**) [5]. This paper deals with the structural elucidation of **1** based on the spectroscopic evidence and X-ray crystallographic analysis.

RESULTS AND DISCUSSION

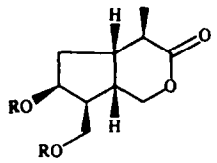
The methanol extract of the dry roots of *Patrinia gibbosa* was fractionated into gibboside (**1**), valerosidate (**5**), adoxoside (**7**) and patrinoside (**9**) by a combination of charcoal and silica gel column chromatography and prep. TLC.

Gibboside (**1**) was obtained as a white amorphous powder, $C_{16}H_{26}O_9$, $[\alpha]_D$ −22.1° (MeOH). Its IR spectrum (KBr) pointed to the presence of hydroxyl groups (3500–3300) and a δ-lactone (1743 and 1240 cm^{-1}). Its ^{13}CNMR spectrum showed 16 signals, six of which appeared at δ62.7, 71.1, 75.4, 77.9, 78.2 and 105.1, thus suggesting the presence of a D-glucopyranosyloxy group.

Enzymatic hydrolysis‡ of **1** with Taka-diastase [6] gave aglucone **2** and D-glucose each in 70% yield. The high resolution mass spectrum of the former showed a molecular ion at *m/z* 200.10435, proving the molecular formula



1* R = H
3 R = Ac



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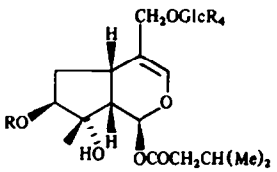

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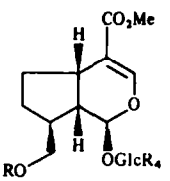
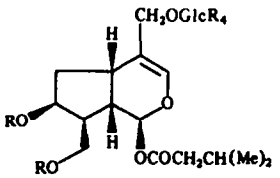
6 R = Ac



7 R = H
8 R = Ac



9 R = H
10 R = Ac

*The numbering in the formulae is arbitrary and hence is not in accordance with the IUPAC recommendation.

*Part 59 in the series 'Studies on Monoterpene Glucosides and Related Natural Products'. For Part 58 see Uesato, S., Miyauchi, M., Itoh, H. and Inouye, H. (1986) *Phytochemistry* **25**, 2515.

‡Treatment of **1** with β-glucosidase (emulsion prepared from almond) gave aglucone **2** in a 50% yield at the maximum. It has been known in our laboratory that the iridoid glucosides possessing an oxygen-bearing group at the 7 or 8 position, such as loganin and plumieride, are apt to resist β-glucosidase-catalysed hydrolysis.

$C_{10}H_{16}O_9$. The 400 MHz ^{1}H NMR spectrum of **2** showed a three-proton doublet at δ1.20 due to a methyl group, as well as a broad triplet at δ4.50 due to an oxygen-bearing methine group. Proton decoupling experiments gave the values of the chemical shifts and proton–proton coupling constants. From these data, the proton–proton connectivity pattern shown in Fig. 1 was constructed and subsequently corroborated by a spin correlated two-dimensional spectrum (COSY). Furthermore, since it was considered that the methylene group at δ3.80 and 3.88 was bound to a hydroxyl group, whereas that at δ4.01 and 4.43 was linked to an acyloxy group, the iridolactone type structure shown in Fig. 1 was therefore presumed for aglucone **2**. In support of this supposition, the ^{13}C NMR spectrum of **2** pointed to the presence of one methyl group, three methylenes, five methines and one carbonyl atom (Table 1).

Subsequently, the relative configuration of **2** was determined in the following way: as shown in Fig. 1, H-9 is coupled to H_a-1 (11.0 Hz) and to H_b-1 (6.0 Hz). Comparing the ^{1}H NMR spectra of 5,9-*cis* or -*trans* irido-3-lactones, Sisido *et al.* reported [7] that, of the 5,9-*cis* irido-3-lactones, iridomyrmecin type lactones have coupling constants of 3.0 Hz between both H-9 and H_a-1 and H-9 and H_b-1, whereas isoiridomyrmecin type lactones have values of 10.1 Hz between H-9 and H_a-1 as well as 5.9 Hz between H-9 and H_b-1 (Fig. 2). On the other hand, 5,9-*trans* irido-3-lactones were shown to have coupling constants of 9.7 Hz between H-9 and H_a-1 and 4.7 Hz between H-9 and H_b-1, regardless of the configuration at C-4. Application of this empirical regularity to compound **2** led us to conclude that **2** has an isoiridomyrmecin type structure. Furthermore, the magnitude of the coupling constant (9.5 Hz) between H-9 and H-8 indicated a *trans* relationship between these protons, which was confirmed by the following NOE experiment: on irradiation at H-8, the H_a-1 signal was enhanced by 5.7%, but the H_b-1 signal was not. This finding suggested that H_a-1 and H-8 are in close proximity, which demands a *trans* disposition of H-9 and H-8. Subsequently, H-5 is coupled to H_a-6 (9.5 Hz) and H_b-6 (7.0 Hz), respectively, whereas H-7 is coupled to H_a-6 (3.5 Hz) and H_b-6 (0.1 Hz), respectively, suggesting H-5 *trans* to H_a-6 and *cis* to H_b-6 as well as H-7 cis to H_a-6 and *trans* to H_b-6. Therefore, H-5 is deduced to be *trans* to H-7.

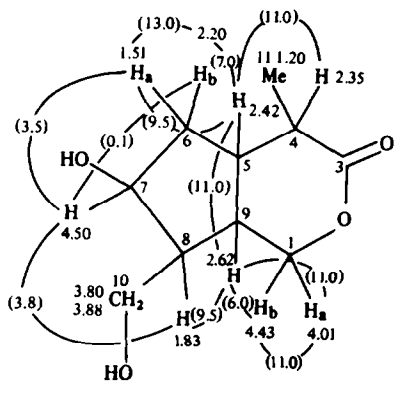


Fig. 1. 400 MHz ^{1}H NMR chemical shifts and the proton–proton coupling constants of compound **2**.

Table 1. ^{13}C NMR* data for gibboside (**1**) and gibboside aglucone (**2**) and their acetates **3** and **4**

	Glucoside		Aglucone	
C	**1**	**3**	**2**	**4**
1	71.0	69.2	69.5	69.1
3	179.2	175.3	176.4	175.3
4	39.9	39.0	37.4	38.8
5	41.2	40.7	41.1	41.0
6	42.3	40.3	41.9	40.0
7	84.7	84.3	75.6	76.5
8	51.4	47.0	49.4	46.0
9	40.5	39.7	39.2	39.2
10	61.6	63.4	61.5	63.0
11	14.4	13.9	13.9	13.8
1′	105.1	101.9		
2′	75.4	71.4		
3′	77.9	71.9		
4′	71.1	68.6		
5′	78.2	72.7		
6′	62.7	62.0		

*The spectra of **1** and **3** were recorded at 50.10 MHz in CD_3OD and $CDCl_3$, respectively, whereas those of **2** and **4** were recorded at 100 MHz in $CDCl_3$.

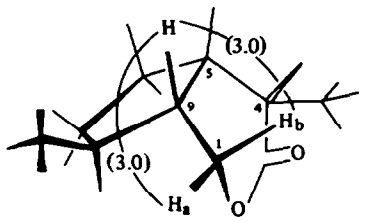
On the basis of the argument outlined above, the relative configuration as shown in **2** was assigned to gibboside aglucone.

The position of the β-glucopyranosyloxy group of **1** was determined by analysing the ^{13}C NMR spectra of **1** and **2**. Each of the carbon signals in the five-membered rings of these compounds was assigned by selective proton decoupling experiments (Table 1). The ^{13}C chemical shifts of the aglucone moiety of **1** were found to be similar to those of aglucone **2** except that the C-7 of **1** resonated 9.1 ppm down-field relative to that of **2**. According to the glycosylation shift rule established for saponins [8, 9], a carbon bound to a glycosyloxy group is shifted down-field by 7–11 ppm relative to the corresponding carbon of its aglycone. Therefore, the D-glucopyranosyloxy group is deduced to be located at the C-7 position of aglucone **2**.

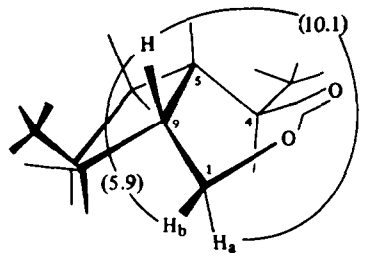
Finally, a single crystal X-ray analysis was carried out in order to determine the absolute configuration of gibboside (**1**). The overall shape of gibboside pentaacetate (**3**) determined from the X-ray is illustrated in Fig. 3, and the bond distances and angles are given in the supplementary material and are normal.* As has been deduced from the NMR studies, the δ-lactone ring and the cyclopentane ring are *cis*-equatorially fused, with torsion angle C(4)–C(5)–C(9)–C(1) $-3.0°$. The torsion angle O(14)–C(7)–C(8)–C(10) is 43.8°, corresponding to the *cis* relationship between the secondary hydroxyl group at C-7 and the hydroxymethyl group at C-8. The methyl group at C-4 could be confirmed to be β-oriented since the torsion angle O(12)–C(3)–C(4)–C(1) is $-3.5°$. The NMR identification given above was therefore fully confirmed, and the X-ray result led us to define the configuration of gibboside (**1**) as illustrated, i.e. 4*R*, 5*S*, 7*S*, 8*R* and 9*S*.

Gibboside (**1**) is the first example of an iridoid glucoside

*Deposited at the Cambridge X-Ray Crystallographic Centre.



Iridomyrmecin



Isoiridomyrmecin

Fig. 2. The conformations of iridomyrmecin and isoiridomyrmecin.

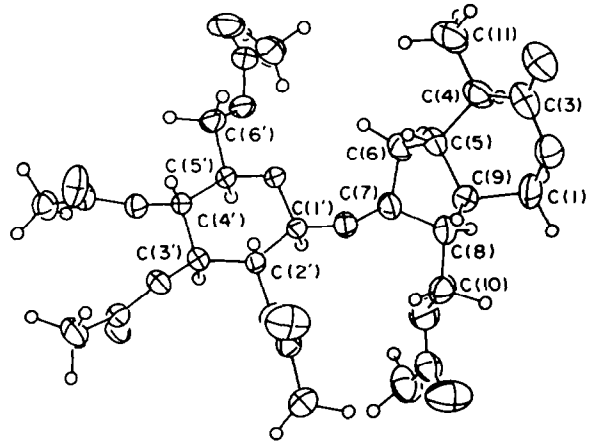


Fig. 3. Stereoscopic drawing of the molecule of gibboside pentacetate (3).

to have a D-glucopyranosyloxy group at the C-7 position of the iridoid. Recently, a similar type of iridoid lactone, villosolside, having a D-glucose moiety at the C-8 position, was isolated along with its aglucone from the same generic plant *Patrinia villosa* [10].

EXPERIMENTAL

General procedures. Mps: uncorr; EI-MS: 75 eV; ^{1}H NMR: 200 and 400 MHz; ^{13}C NMR: 50.10 and 100 MHz. TMS as internal standard. CC: silica gel MN-60 and charcoal; TLC and prep. TLC: silica gel GF_{254} and 60 PF_{254}, respectively. Spots and bands were detected by I_2 vapour or by UV irradiation (254 nm).

Plant material. Patrinia gibbosa was collected in Sado Island (Niigata Prefecture) in September, 1976 and 1983. A voucher specimen (S. Uesato and H. Nishimura No. 1) has been deposited in the Herbarium of the Institute of Botany, Faculty of Science, Kyoto University (KYO), Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto 606, Japan.

Isolation of iridoids. The dry roots of *P. gibbosa* (184 g), which were collected at Sado Island (Niigata Prefecture) in Japan in September 1983, were extracted with MeOH (0.21 × 4) under reflux. The combined extracts were concentrated *in vacuo* to give a residue (25 g), which was taken up in H_2O. The insoluble materials were filtered off, and the filtrate, after washing with EtOAc, was transferred to a charcoal (100 g) column, eluted with MeOH–H_2O of increasing MeOH content. On concn, the 50%, 60–70%, and 80–100% MeOH eluates gave fractions A (1.6 g), B (2.1 g) and C (4.1 g), respectively. Fraction A was chromatographed on a silica gel (100 g) column, eluted with MeOH–$CHCl_3$ of increasing MeOH content. The residue of the combined 15–19% MeOH–$CHCl_3$ eluates was subjected to prep. TLC ($CHCl_3$–MeOH, 7:3) to yield *gibboside* (**1**, 0.920 g) and valerosidate (**5**, 0.015 g) both as a white powder. The latter was acetylated to give valerosidate pentaacetate (**6**, 0.020 g) as a white powder, $[\alpha]_D$ −95.8° (MeOH; *c* 1.00). Fraction B was chromatographed on a silica gel (100 g) column in the same way with MeOH–$CHCl_3$. An aliquot of the residue (0.080 g) of the combined 15–19% MeOH–$CHCl_3$ eluates was acetylated and the product subjected to prep. TLC ($CHCl_3$–MeOH, 97:3) to afford adoxoside pentaacetate (**8**, 0.093 g) as colourless needles, mp 141–142°, $[\alpha]_D$ −60.0 ($CHCl_3$; *c* 1.00). An aliquot (0.100 g) of fraction C was acetylated and the product was subjected to prep. TLC ($CHCl_3$–MeOH, 49:1) to yield patrinoside hexaacetate (**10**, 0.070 g) as colourless needles, mp 131–131.5°, $[\alpha]_D$ −43.2 ($CHCl_3$; *c* 1.00).

Gibboside (**1**). $[\alpha]_D$ −22.1° (MeOH; *c* 1.00); IR ν_{max}^{KBr} cm^{-1}: 3500–3300 (OH), 1743 and 1240 (δ-lactone); ^{1}H NMR (CD_3OD): δ1.14 (*d*, *J* = 6.4 Hz, H_3-11), 4.37 (*t*, *J* = 3.7 Hz, H-7); ^{13}C NMR see Table 1. (Found: C, 52.89; H, 7.41. $C_{16}H_{26}O_9$ requires: C, 53.02; H, 7.24%.)

Acetylation of **1**. **1** (0.100 g) was acetylated followed by recrystallization from EtOH to give gibboside pentaacetate (**3**, 0.040 g) as colourless needles, mp 156.5–157.5°; $[\alpha]_D$ −3.87° ($CHCl_3$; *c* 1.00); IR ν_{max}^{KBr} cm^{-1}: 1740 and 1236 (δ-lactone); ^{1}H NMR ($CDCl_3$) δ1.20 (*d*, *J* = 5.9 Hz, H_3-11), 3.68 (*m*, H-5'), 4.00 (*m*, H-6'), 4.20 (*d*, *J* = 3.9 Hz, H_2-10), 4.54 (*d*, *J* = 8.1 Hz, H-1'), 4.90–5.30 (*m*, H_3-2', 3', 4'); ^{13}C NMR see Table 1. (Found: C, 54.47; H, 6.40. $C_{26}H_{36}O_{14}$ requires: C, 54.53; H, 6.34%.)

Enzymic hydrolysis of **1**. Taka-diastase [6] in H_2O (15 ml) was added to gibboside (**1**) (0.150 g) in H_2O (15 ml), and the mixture was allowed to stand at 36° for 18 hr and then concentrated *in vacuo*. The residue was subjected to prep. TLC ($CHCl_3$–MeOH, 4:1) to yield aglucone **2** (0.058 g) as colourless needles and D-glucose (0.052 g) as powdery crystals. Aglucone **2**: mp 106.5–107°; $[\alpha]_D$ −31.41° (MeOH; *c* 1.00); ^{1}H NMR see Fig. 1; MS: M^+ 200.10435, $C_{10}H_{16}O_4$ requires: 200.10484.

Acetylation of **2**. Aglucone **2** (0.058 g) was acetylated and the product was subjected to prep. TLC ($CHCl_3$–MeOH, 99:1) to yield aglucone diacetate **4** (0.041 g) as a white powder, $[\alpha]_D$ +10.79° ($CHCl_3$; *c* 1.00); ^{1}H NMR ($CDCl_3$): δ1.20 (*d*, *J* = 5.9 Hz, H_3-11), 1.62 (*m*, H_b-6), 2.17 (*m*, H-8), 2.30 (*m*, H_a-6), 2.35 (*m*, H-4), 2.37 (*m*, H-5), 2.51 (*m*, H-9), 4.02 (*dd*, *J* = 7.1, 11.1 Hz, H-10), 4.04 (*t*, *J* = 11.4 Hz, H_b-1), 4.23 (*dd*, *J* = 7.1, 11.1 Hz, H-10), 4.46 (*dd*, *J* = 6.1, 11.5 Hz, H_a-1), 5.36 (*s* (*br*) *t*, *J* = 3.7 Hz, H-7); ^{13}C NMR see Table 1.

X-Ray crystallographic analysis of gibboside pentaacetate (**3**). The crystals of **3** were recrystallized from MeOH at room temperature. The density was measured by the flotation method in the mixture of C_6H_6–hexane–CCl_4 at 296 K. A single crystal

(dimension $0.2 \times 0.1 \times 0.7$ mm) analysis was performed on a Model AFC-5 fourcicle automatic diffractometer. The crystal data were: $C_{26}H_{36}O_{14}$, $M = 572.56$, monoclinic, space group $P2_1$, $a = 5.634(0)$, $b = 12.504(2)$, $c = 21.013(5)$ Å, $D_m = 1.319(1)$ g·cm^{-3}, $Z = 2$, $D_c = 1.316$ g·cm^{-3}, $F(000) = 608$, $\mu = (\text{Cu-K}\alpha) = 9.258$ cm^{-1}. Out of 2600 unique intensities ($\sin\theta/\lambda \leqq 0.558$ Å^{-1}) collected using graphite monochromated Cu-Kα radiation and by the θ-2θ scan mode, 2496 having $Fo > 0$ were treated as observed. No crystal damage was observed in the intensities of four standard reflections measured every 100 reflections. Lorentz and polarization factor corrections were applied, but no absorption correction was made.

The structure was solved by direct method with the MULTAN 76 program. An electron density map using 500 reflections with $E \geqq 1.27$ gave the positions of all non-hydrogen atoms. The atomic coordinates were refined by block-diagonal least-squares method with anisotropic thermal parameters. The positions of all hydrogen atoms were revealed from a difference map. Final R index was 0.076 for 2496 unique reflections. The quantity minimized was $\Sigma w(|Fo| - |Fc|)^2$ where $w = (\sigma^2|Fo| - 0.17626|Fo| + 0.01290|Fo|^2)^{-1}$. All numerical calculations were performed at the Computer Centre of Osaka University using the UNICS program. Atomic positional parameters, equivalent isotropic parameters, bond lengths, bond angles and torsion angles are deposited at the Cambridge Crystallographic Data Centre.

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