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MEGASTIGMANE GLYCOSIDES FROM *LONICERA GRACILIPES* VAR. *GLANDULOSA**

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Key Word Index—*Lonicera gracilipes* var. *glandulosa*; Caprifoliaceae; megastigmane glycoside.

Abstract—Two new megastigmane glycosides, (6*R*, 7*E*, 9*R*)-9-hydroxy-4, 7-megastigmadien-3-one 9-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside] and (6*S*, 7*E*, 9*R*)-6,9-dihydroxy-4, 7-megastigmadien-3-one 9-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside], were isolated from the leaves of *Lonicera gracilipes* var. *glandulosa*. Their absolute stereostructures were elucidated on the basis of spectral and chemical evidence.

INTRODUCTION

In previous papers, we reported the structural elucidation of neolignan glycosides [1], from the leaves of *Lonicera gracilipes* var. *glandulosa*. As a continuation of our investigation on this plant, we have isolated two new megastigmane glycosides, of which one has an enantiometric aglycone part of 6*S*,9*S*-pneumonanthoside. This paper deals with the elucidation of their absolute stereostructures.

RESULTS AND DISCUSSION

The water-soluble fraction of a methanol extract of the fresh leaves of *L. gracilipes* var. *glandulosa* was purified by Diaion HP-20, Sephadex LH-20, silica gel column chromatography and preparative HPLC, to yield two compounds.

Compound **1** was obtained as an amorphous powder, $[\alpha]_D$ +202.0°. The ^{1}H NMR and ^{13}C NMR spectra of **1** suggested the presence of a β-glucopyranosyl, an α-arabinopyranosyl and megastigmane for an aglycone moiety with 13 carbon signals, each signal was assigned from ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY spectral data. The D-glucose and L-arabinose, obtained by acidic hydrolysis of **1**, were identified by GC. Thus, the planar structure was established to be 9-hydroxy-4, 7-megastigmadien-3-one 9-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside]. The relative structure was determined as follows. In the ^{1}H-^{1}H COSY spectra, long-range couplings were observed between H-2β and H_3-11, H-2α and H-4. The presence of 'W'-shaped long-range coupling between H-2α and H-6 indicated that the conformation of **1** must be a pseudo-axial side-chain (C-7–C-10) on the half-chair form of the cyclohexenone skeleton. Further, the correlations in the difference NOE experiment as shown in Fig. 1 support these conformational assignments. The absolute configuration at C-6 was determined on the basis of CD spectroscopic evidence. The CD Cotton-effect exhibited a positive maximum at 244 nm (Δε+38.30), caused by the helicity between an enone moiety and 7-ene. Accordingly, C-6 was elucidated to have the *R*-configuration [2, 3]. Pabst *et al.* [3] reported that ^{13}C NMR chemical shifts for the 9*R*- and 9*S*-configuration of 3-oxo-α ionol β-D-glucopyranoside are δ 77.0 and 74.7, respectively. Therefore, the 9*R*-configuration of **1** resulted from the ^{13}C NMR data which showed C-9 at δ 77.1. On the other hand, in pneumonanthoside reported by Chulia *et al.* from *Gentiana pneumonanthe* [4] and the 6*S*, 9*S*-form of pneumonanthoside reported Murai *et al.* [5], the chemical shifts of C-9 were δ 75.1. Consequently, the structure of **1** was determined to be 6*R*,9*R*-configurations of a new stereomer of pneumonanthoside.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D$ +89.9°, whose M_r was confirmed from the $[M+H]^+$ peak at *m/z* 519 in FAB-mass spectrometry. The ^{1}H NMR spectrum of **2** showed differences from that of **1** for olefinic protons, and the absence of H-6. The ^{13}C NMR chemical shifts of the aglycone moiety were identical with those of roseoside isolated by Otsuka *et al.* [5]. As compared with the ^{13}C NMR data of **1**, downfield shifts of C-1, C-2 and C-6 were observed. Highfield shifts of C-11 and C-12 attributed to the γ-gauche effect of the 6-hydroxyl group were

* Part 11 in the series 'Studies on the Constituents of *Lonicera* Species'. For part 10 see ref. [1].

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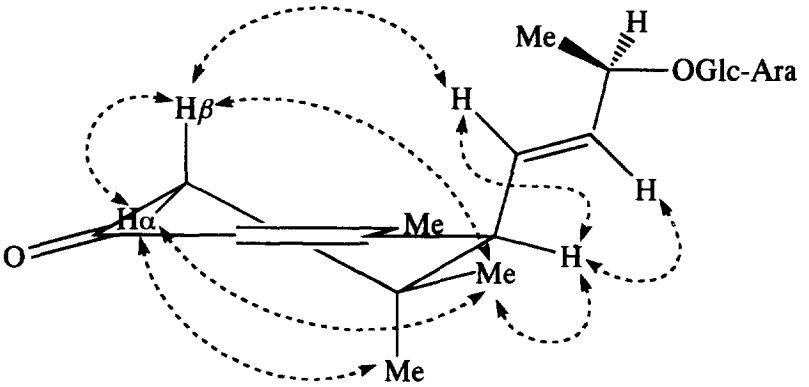


Fig. 1. Stereochemical correlation for **1** obtained from NOE experiments.

also observed, so that the 6-hydroxyl group was deduced to be in the pseudo-equatorial orientation. The ^{1}H-^{1}H COSY spectra showed long-range couplings between H-2β and H$_3$-11, H-2α and H-4. In the difference NOE experiment, a correlation was observed between H-7 and H-2β, H-2β and H$_3$-12, H-2α and H$_3$-11, H-2α and H$_3$-12. The above results indicate that the side-chain (C-7–C-10) is in a pseudo-axial orientation to the cyclohexenone ring had a half-chair conformation, as in **1**. The CD spectrum of **2** showed a positive Cotton effect at 240 nm ($\Delta\varepsilon$ + 17.14), and the ^{13}C NMR data showed C-9 at δ 76.8, indicating the 6*S*- and 9*R*-configurations, respectively. From these results, the absolute structure of **2** was determined to be (6*S*, 7*E*, 9*R*)-6,9-dihydroxy-4,7-megastigmadien-3-one 9-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside].

EXPERIMENTAL

General. ^{1}H and ^{13}C-NMR spectra were recorded at 400 or 270 and 67.8 MHz, respectively, in CD$_3$OD with TMS as int. standard. Prep. HPLC was carried out on Tosoh HPLC system using a ODS-120A column (7.8 mm i.d. × 30 cm) with UV detector. GC was carried out using a 3% SE-52 Chromosorb W(AW) (60–80 mesh, 3 mm i.d. × 2 m) column with FID.

Plant material. The leaves of *L. gracilipes* var. *glandulosa* were collected in October 1990, in Sendai, Japan.

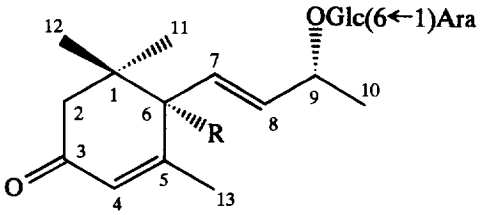
Extraction and isolation. Fresh leaves (0.2 kg) were extracted with MeOH at room temp for 1 month. The MeOH extract was concd under red. press. and the residue suspended in a small excess of H$_2$O. This suspension was successively extracted with CHCl$_3$, Et$_2$O, EtOAc and *n*-BuOH. The H$_2$O-soluble fr. was concd under red. press. to afford a residue (22.2 g). This residue was chromatographed on a Diaion HP-20 column to give a H$_2$O elute and MeOH elute. The MeOH elute was rechromatographed on a Sephadex LH-20 column (MeOH–H$_2$O, 1:1) and the elute was sepd into six frs (frs A–F). Fr. A was subjected to prep. HPLC (MeOH–H$_2$O, 1:2) to give **1** (3 mg) and **2** (5 mg).

(6R, 7E, 9R)-9-*Hydroxy*-4, 7-*megastigmadien*-3-*one* 9-O-[α-L-*Arabinopyranosyl*-(1 → 6)-β-D-*glucopyranoside*] (**1**). Amorphous powder, $[\alpha]_D$ + 202.0° (MeOH, *c* 0.2). UV λ_{max}^{MeOH} nm (log ε): 234.0 (4.47). FAB-MS *m/z*: 503 $[M+H]^+$. ^{1}H NMR (270 MHz, CD$_3$OD): δ 1.00 (3H, *s*, H$_3$-12), 1.03 (3H, *s*, H$_3$-11), 1.29 (3H, *d*, *J* = 6.2 Hz, H$_3$-10), 1.94 (3H, *d*, *J* = 1.1 Hz, H$_3$-13), 2.05 (1H, *d*, *J* = 16.5 Hz, H-2α), 2.43 (1H, *d*, *J* = 16.5 Hz, H-2β), 2.69 (1H, *d*, *J* = 8.8 Hz, H-6), 3.68 (1H, *dd*, *J* = 5.1, 11.2 Hz, H-6′a), 3.84 (1H, *dd*, *J* = 3.0, 12.2 Hz, H-5″b), 4.05 (1H, *dd*, *J* = 2.2, 11.2 Hz, H-6′b), 4.27 (1H, *d*, *J* = 6.6 Hz, H-1″), 4.35 (1H, *d*, *J* = 7.7 Hz, H-1′), 4.41 (1H, *m*, H-9), 5.65 (1H, *dd*, *J* = 15.4, 8.4 Hz, H-7), 5.76 (1H, *dd*, *J* = 15.4, 6.6 Hz, H-8), 5.89 (1H, *br s*, H-4). ^{13}C NMR: see Table 1. CD (MeOH) $\Delta\varepsilon$ + 38.30 (244 nm), − 1.73 (317 nm).

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (in CD$_3$OD)

C	**1**	**2**	(6*S*, 9*R*)-Roseoside*
1	37.2	42.5	42.5
2	48.1	50.8	50.7
3	202.2	201.4	201.2
4	126.2	127.3	127.2
5	166.1	167.3	167.3
6	56.8	80.2	80.0
7	129.0	131.7	131.6
8	138.1	135.0	135.3
9	77.1	76.8	77.3
10	21.1	21.1	21.2
11	27.7	23.5	23.5
12	28.1	24.7	24.7
13	23.9	19.7	19.6
1′	102.6	102.6	102.8
2′	75.2	75.3	75.3
3′	77.9	78.0	78.1
4′	71.5	71.6	71.7
5′	76.8	76.8	78.0
6′	69.5	69.6	62.9
1″	105.2	105.3	
2″	74.3	74.2	
3″	72.4	72.2	
4″	69.4	69.5	
5″	66.8	66.8	

* 100 MHz [5].



R
1 H
2 OH

(6S, 7E, 9R)-6, 9-*Dihydroxy*-4, 7-*megastigmadien*-3-*one* 9-O-[α-L-*arabinopyranosyl*-(1 → 6)-β-D-*glucopyranoside*] (**2**). Amorphous powder, $[\alpha]_D$ +89.9° (MeOH, *c* 0.3). UV λ_{max}^{MeOH} nm (log ε): 233.0 (4.13). FAB-MS *m/z*: 518 $[M+H]^+$. ^1H NMR (270 MHz, CD_3OD): δ 1.02 (3H, *s*, H_3-12), 1.04 (3H, *s*, H_3-11), 1.29 (3H, *d*, *J* = 6.6 Hz, H_3-10), 1.92 (3H, *d*, *J* = 1.5 Hz, H_3-13), 2.15 (1H, *d*, *J* = 16.9 Hz, H-2α), 2.51 (1H, *d*, *J* = 16.9 Hz, H-2β), 3.68 (1H, *dd*, *J* = 5.1, 11.4 Hz, H-6′a), 3.86 (1H, *dd*, *J* = 3.0, 12.2 Hz, H-5″b), 4.06 (1H, *dd*, *J* = 2.2, 11.4 Hz, H-6′b), 4.27 (1H, *d*, *J* = 6.6 Hz, H-1″), 4.35 (1H, *d*, *J* = 7.7 Hz, H-1′), 4.44 (1H, *m*, H-9), 5.84 (1H, *br s*, H-7), 5.85 (1H, *br s*, H-8), 5.89 (1H, *br s*, H-4). ^{13}C NMR: see Table 1. CD (MeOH)Δε+17.14 (240 nm), −1.01 (324 nm).

Acid hydrolysis of **1** *and* **2**. Compounds **1** and **2** were treated with 5% HCl to yield L-arabinose and D-glucose. Two sugars were identified by GC after derivatization of TMS.

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