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Epi-α-bisabolol 6-deoxy-β-D-gulopyranoside from the glandular trichome exudate of *Brillantaisia owariensis*

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ABSTRACT

A new sesquiterpene glycoside, (−)-epi-α-bisabolol 6-deoxy-β-D-gulopyranodide (**1**), has been isolated from the glandular trichome exudate of *Brillantaisia owariensis* (Acanthaceae). The structure of compound **1** was determined by spectroscopic analysis as well as acidic hydrolysis of 1 leading to (−)-epi-α-bisabolol (**2**) and 6-deoxy-D-gulose (**3**). This is the first study to analyze secondary metabolites from glandular trichome exudates of plants belonging to the Acanthaceae family. 6-Deoxygulopyranoside is the first example of an epi-α-bisabolol glycoside of plant origin.

1. Introduction

Glandular trichomes produce secondary metabolites of diverse classes such as terpenoids, phenylpropanoids, polyketides and fatty acid derivatives (Schilmiller et al., 2008). We have been analyzing non-volatile secondary metabolites from glandular trichome exudates of plants belonging to various families, in particular poorly exploited families, to seek for possible relationships between their chemical structures and families or genera (Spring et al., 1994). The popular occurrence of sucrose esters in the family of several genera of the Solanaceae family is a well-known example (King et al., 1993). We recently demonstrated that the glandular trichome exudates from two plants belonging to different genera of the Martyniaceae family have essentially identical glycerides (Asai et al., 2010). More recently the glandular trichome exudates of three plants of the *Geranium* genera (Geraniaceae) were found to contain *n*-alkyl disaccharides (Asai et al., 2011). A series of unique cyclic glycolipids have been isolated from the glandular trichome exudates of two different genera (*Silene* and *Cerastium*) of the Caryophyllaceae family (Asai and Fujimoto, 2010; Asai et al., unpublished results). These findings suggest that secondary metabolites contained in glandular trichome exudates could be much more diverse in their structures than previously expected and phytochemical analysis of secondary metabolites in glandular trichome exudates are worthy of further investigation.

Literature survey revealed that there is no previous report on secondary metabolites from glandular trichome exudates of Acanthaceae plants, which prompted us to investigate this family. As a continuation of our study, we chose *Brillantaisia owariensis* P. Beauv. as the first target plant of the Acanthaceae family. The genus *Brillantaisia*, distributed in tropical Africa and Madagascar, is comprised of 17 species (The Plant List, 2010). *B. owariensis* (synonym *Brillantaisia patula* T. Anderson) is a perennial herb common in damp sites and massive forest zones and originates from equatorial Africa. The plant is cultivated and traditionally prescribed for soothing effect, antiplasmoidal, vermifugal, antifungal properties, and antiseptic effects on skin (Olufunke et al., 2009). There is only one paper for the phytochemical study of *B. owariensis* in which volatile essential oil was analyzed (Olufunke et al., 2009). *Brillantaisia nitens* of this genus is also traditionally used for the management of many diseases including cardiovascular disorders (Dimo et al., 2007) and stem extract of *Brillantaisia palisatii* is reported to show peripheral, supra-spinal analgesic activities (Matheus et al., 2005). *B. owariensis* is rich in glandular trichomes in pedicels and calyxes that exude a large amount of an oily material. In this article, we describe the isolation and structure elucidation of a sesquiterpene glycoside from the glandular trichome exudate of *B. owariensis*.

2. Results and discussion

The glandular trichome exudate was obtained by wiping the calyx and pedicels of the flowering plant with cotton, which was

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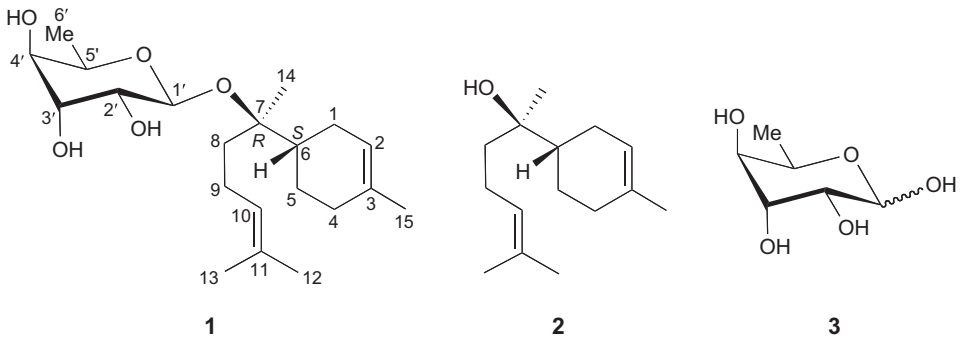

Fig. 1. Structures of compound **1**, (−)-epi-α-bisabolol **2** and 6-deoxy-D-gulose **3**.

washed with ether. The concentrated oily residue was fractionated by silica gel column chromatography to give compound **1** (Fig. 1) as a main constituent. Compound **1** was obtained as colorless oil and its molecular formula, $C_{21}H_{36}O_5$, was determined on the basis of a molecular ion at *m*/*z* 368.2575 ($C_{21}H_{36}O_5$ requires 368.2563) in EIMS. The IR spectrum showed absorptions for hydroxy groups at 3580 cm^{-1}. The ^1H NMR spectrum showed signals of a doublet methyl at δ 1.21, an anomeric proton at δ 4.74 and four oxymethine protons, assignable to a 6-deoxyhexose, and three singlet methyls at δ 1.68, 1.65 and 1.61 and two olefinic protons at δ 5.37 and 5.11, presumably due to a monocyclic sesquiterpene unit (Table 1). The deoxy sugar moiety was assigned as 6-deoxygulopyranose, on the basis of ^1H–^1H COSY correlations and interpretation of the coupling constants for $J_{H\text{-}2,H\text{-}3}$ (4.0 Hz), $J_{H\text{-}3,H\text{-}4}$ (4.0 Hz) and $J_{H\text{-}4,H\text{-}5}$ (nearly 0 Hz). A large coupling constant for $J_{H\text{-}1,H\text{-}2}$ (8.0 Hz) indicated a β-glycosidic linkage. Axial orientation of H-5′ was ascertained by an NOE correlation between H-5′ and the anomeric proton. The ^{13}C NMR spectrum exhibited 21 signals, among which six were unambiguously assigned to the 6-deoxygulose moiety based on HMBC correlations (Table 1). The HMBC data also permitted us to determine the sesquiterpene moiety as α-bisabolol or epi-α-bisabolol, and the remaining 15 signals, including a tertially oxymethine, two sp^2 methine and two sp^2 quaternary carbons, were assigned as listed in Table 1. Comparison of the ^1H and ^{13}C NMR data of **1** with those of known compounds could not permit us to differentiate the two bisabolol diastereomers due to the lack of the data for epi-α-bisabolol glycosides. Furthermore, we thought that it was necessary to investigate the absolute configuration of the aglycon moiety.

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data for compound **1**.[a,b]

Position	δ_C[c]	δ_H[d]	HMBC (H → C)
1	26.8	2.00 (*m*), 1.80 (*m*)	C-2, C-3, C-6, C-7
2	120.7	5.37 (*brs*)	C-1, C-4, C-6, C-15
3	134.3	–	
4	31.0	1.98 (*m*, 2H)	C-2, C-3
5	23.5	1.58 (*m*), 1.28 (*m*)	C-1, C-3, C-7
6	42.1	1.75 (*m*)	C-1, C-2, C-4, C-8
7	81.7	–	
8	35.7	1.57 (*m*), 1.49 (*m*)	C-6, C-7, C-9, C-10
9	22.4	2.02 (*m*), 1.92 (*m*)	C-7, C-8, C-10, C-11
10	124.8	5.11 (*t*, 7.0)	C-8, C-9, C-12, C-13
11	131.3	–	
12	25.7	1.65 (*brs*, 3H)	C-10, C-11, C-13
13	17.7	1.61 (*brs*, 3H)	C-10, C-11, C-12
14	21.8	1.21 (*s*, 3H)	C-6, C-7, C-8
15	23.4	1.68 (*s*, 3H)	C-2, C-3, C-4
1′	94.6	4.74 (*d*, 8.0)	C-2′, C-3′, C-5′
2′	71.9	3.55 (*dd*, 8.0, 4.0)	C-1′, C-3′, C-4′
3′	71.4	4.07 (*t*, 4.0)	C-1′, C-2′, C-4′, C-5′
4′	69.1	3.51 (*brd*, 4.0)	C-2′, C-3′, C-5′
5′	68.9	4.02 (*brq*, 6.6)	C-1′, C-3′, C-6′
6′	16.0	1.21 (*d*, 6.6)	C-4′, C-5′

[a] Assignments were based on COSY, HSQC and HMBC experiments.
[b] Multiplicity and coupling constants (*J* in Hz) are in parentheses.
[c] Recorded in $CDCl_3$.
[d] Recorded in $CDCl_3$–CD_3OD (9:1).

Thus, compound **1** was hydrolyzed under an acidic condition to yield a sesquiterpene alcohol (**2**) and 6-deoxygulose (**3**). The ^1H and ^{13}C NMR data of **2** were in good agreement with those of epi-α-bisabolol, but not with those of α-bisabolol (for example, δ 22.3, 22.3 and 26.9 for C-1 signal of **2**, epi-α-bisabolol and α-bisabolol, respectively) (Babin et al., 1981; Carman and Duffield, 1989; Miyazawa et al., 1995), thus indicating that the structure of the sesquiterpene moiety of **1** is epi-α-bisabolol. Furthermore, the aglycon showed a negative sign of optical rotation $[\alpha]_D^{25}$ −65.6 (*c*, 0.09, MeOH) [lit. −67.5 for 6*S*,7*R*-form (Günther et al., 1993)]. The deoxy sugar (**3**) was identified as 6-deoxy-D-gulose by direct comparison with authentic (−)-6-deoxy-D-gulose that was synthesized according to the published method (Lerner, 1975) (co-TLC, GLC as TMS ether, $[\alpha]_D^{25}$ −32.9 [lit. −39.3 (Lerner, 1975)]). Hence, compound **1** was determined to be (−)-epi-α-bisabolol 6-deoxy-β-D-gulopyranoside.

In summary, we have isolated a new sesquiterpene glycoside from the glandular trichome exudate of *B. owariensis* and elucidated its structure as (−)-epi-α-bisabolol 6-deoxy-β-D-gulopyranoside. This is the first report of the chemical analysis of the glandular trichome exudates from Acanthaceae plants. (+)-Epi-α-bisabolol was previously isolated from the glandular trichome of *Rosa rugosa* (Rosaceae) (Hashidoko et al., 2000). α-Bisabolol fucopyranoside was isolated as a cytotoxic compound from the aerial part of a few plants, such as *Carthamus lanatus* and *Carthamus turkistanikus* (Rustaiyan et al., 1981; Feliciano et al., 1990; Mikhova et al., 2004). However, epi-α-bisabolol glycosides have not been reported from plants, but isolated from a fermentation product of the microorganism, *Stachybotrys elegans* (Yoshida et al., 1993). This is therefore the first report of the isolation of epi-α-bisabolol glycosides from plants. A guaianolide glycoside is the only one sesquiterpene glycoside reported so far (Spring et al., 1994). In view of the very rare occurrence of α-bisabolol, epi-α-bisabolol and their glycosides in glandular trichome exudates, it is of significance that compound **1** represents 86% of non-volatile constituents of the exudate of *B. owariensis*. Several α-bisabolol glycosides have been chemically synthesized to evaluate their cytotoxic activity (Piochon et al., 2009).

3. Experimental

3.1. General experimental procedures

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX500 (500 MHz for ^1H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$, $CDCl_3$–CD_3OD (9:1) or CD_3OD solution. Tetramethylsilane (δ 0.00) signal was used as an internal standard for ^1H shifts, and the $CDCl_3$

(δ 77.00) signal was used as a reference for ^{13}C shifts. For the spectra taken in CD_3OD, the residual nondeuterated solvent signal at δ 3.30 and the solvent signal at δ 49.00 were referenced for ^{1}H and ^{13}C shifts, respectively. EIMS (70 eV) and HREIMS spectra were obtained on a JEOL JMS-700 spectrometer. IR spectra were recorded on a JASCO-FT/IR-5300 spectrometer. Optical rotations were measured on a JASCO P-2200 polarimeter. Silica gel 60 N (spherical neutral, 40–100 μm, Kanto Chemical, Japan) was used for column chromatography (CC). GLC was carried out on a Shimadzu GC-14B apparatus equipped with a J&W Scientific DB-5 capillary column (15 m × 0.25 mm, 0.25 μm film thickness) and split (40:1) injection with FID detection.

3.2. Plant material

B. owariensis (Acanthaceae) was cultivated in a green house of Fukuoka botanical garden, Fukuoka, Japan, from which the sample was collected in October 2008. The plant was identified by Mr. Patrik Chalo Mutiso, the University of Nairobi Herbarium. A voucher specimen (CMS20-01) was deposited in the Department of Chemistry and Materials Science, Tokyo Institute of Technology.

3.3. Extraction and isolation

Glandular trichome exudate was collected by sweeping pedicel and calyx of flowering shoots with cotton. The cotton was washed with ether and removal of the solvent in vacuo gave a yellowish oily residue (29 mg). This was subjected to silica gel (4 g) CC. The column was eluted with a discontinuous gradient of $CHCl_3$ (20 mL), $CHCl_3$–MeOH (40:1 → 20:1 → 10:1, 20 mL each). The fraction eluted with $CHCl_3$–MeOH 20:1 gave compound **1** (25 mg).

3.4. (−)-Epi-α-bisabolol 6-deoxy-β-D-gulopyranoside (1)

Colorless oil. $[\alpha]_D^{25}$ −64.8 (c, 1.8, MeOH), IR ($CHCl_3$) ν_{max} 3580, 3005, 2965, 2925 cm^{-1}. HREIMS *m*/*z* 368.2575 ($C_{21}H_{36}O_5$) requires 368.2563. EIMS *m*/*z*: 368 (3), 350 (1), 285 (1), 273 (2), 256 (2), 233 (3), 205 (51), 204 (61), 149 (27), 147 (25), 121 (20), 119 (62), 109 (64), 94 (51), 93 (38), 81 (47), 73 (32), 69 (100), 57 (32), 55 (34). ^{1}H ($CDCl_3$–CD_3OD (9:1)) and ^{13}C ($CDCl_3$) NMR spectroscopic data, see Table 1. The HSQC and HMBC spectra were recorded in $CDCl_3$. ^{1}H NMR ($CDCl_3$) δ: 5.37 (*brs*, H-2), 5.12 (*t*, *J* = 7.0 Hz, H-10), 4.74 (*d*, *J* = 8.0 Hz, H-1′), 4.16 (*t*, *J* = 4.0 Hz, H-3′), 4.04 (*brq*, *J* = 7.0 Hz, H-5′), 3.58 (*m*, H-2′), 3.57 (*m*, H-4′), 2.01 (*m*, Ha-9), 2.00 (*m*, Ha-1), 1.97 (*m*, H-4), 1.95 (*m*, Hb-9), 1.80 (*m*, Hb-1), 1.75 (*m*, H-6), 1.68 (*s*, H_3-15), 1.65 (*brs*, H_3-12), 1.62 (*m*, Ha-5), 1.61 (*brs*, H_3-13), 1.60 (*m*, Ha-8), 1.50 (*m*, Hb-8), 1.28 (*m*, Hb-5), 1.21 (*s*, H_3-14), 1.21 (*d*, *J* = 7.0 Hz, H_3-6′).

3.5. Acidic hydrolysis of compound 1

A mixture of **1** (12.6 mg), THF (0.5 mL) and 2 N HCl (0.75 mL) was stirred at room temperature overnight. The mixture was neutralized by addition of $NaHCO_3$ (powder, 137 mg) and partitioned with ether and water. The ether layer was concentrated and the residue was chromatographed over silica gel with hexane–EtOAc (15:1) as an eluent to give (−)-epi-α-bisabolol (1.1 mg) as colorless oil. $[\alpha]_D^{25}$ −65.6 (*c*, 0.09, MeOH) (lit. −67.5) (Günther et al., 1993). ^{1}H NMR ($CDCl_3$) δ: 5.40 (*m*, 1H) 5.13 (*t*, *J* = 1.4 Hz, 1H), 1.69 (*s*, 3H), 1.62 (*s*, 3H), 1.65 (*s*, 3H), 1.14 (*s*, 3H). ^{13}C NMR ($CDCl_3$) δ: 133.9, 131.8, 124.6, 120.8, 74.4, 43.3, 39.3, 31.1, 26.1, 25.7, 24.0, 24.0, 23.3, 22.3, 17.7. The water layer was concentrated and the residue was chromatographed over silica gel with $CHCl_3$–MeOH (3:1) as an eluent to give (−)-6-deoxy-D-gulose (4.2 mg) as colorless oil. $[\alpha]_D^{25}$ −32.9 (*c*, 0.42, water). ^{1}H NMR (CD_3OD) δ: 4.77 (*d*, *J* = 8.2 Hz, H-1), 4.02 (*qd*, *J* = 6.7, 1.2 Hz, H-5), 3.95 (*t*, *J* = 3.5 Hz, H-3), 3. 50 (*dd*, *J* = 8.2, 3.5 Hz, H-2), 3.46 (*dd*, *J* = 3.5, 1.2 Hz, H-4), 1.20 (*d*, *J* = 6.7 Hz, H_3-6), ^{13}C NMR (CD_3OD) δ: 95.7 (C-1), 73.8 (C-4), 73.4 (C-3), 70.8 (C-2), 70.1 (C-5), 16.4 (C-6). The sugar was identified by direct comparison with authentic sample. TLC:Rf 0.30 ($CHCl_3$–MeOH, 4:1). GLC (as TMS ether): retention times 4.28 and 4.85 min for the α- and β-anomers under the GLC conditions: injection temperature 270 °C, column temperature 150 °C, detection temperature 270 °C, He carrier gas flow rate of P1, 50 kPa and P2, 120 kPa, H_2 flow rate 55 kPa, air flow rate 45 kPa.

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