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Chemical constituents of the Korean endangered species *Rhododendron brachycarpum*

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1. Subject and source

The *Rhododendron* genus containing over 850 species is one of the largest genera in the family Ericaceae. The genus is mostly distributed in Asia and sparsely populated in North America and Europe (Popescu and Kopp, 2013). *Rhododendron brachycarpum* G. Don is an evergreen indeciduous broad-leaved shrub and utilized for alleviation of diabetes, hypertension and rheumatoid arthritis (Jang et al., 2005). Radical climate changes have played a central role in reducing the population, leading its designation as an endangered and rare species (Lee et al., 2002; Lee and Shim, 2011). In our continuing efforts to discover potential value of the Korean endangered species, we herein describe the isolation and structural characterization of a new kaurane-type diterpenoid glycoside, β-sophorosyl *ent*-16-α-hydroxykauran-18-oate (**1**), along with 52 known compounds and discuss the chemotaxonomic significance of these identified compounds. The leaves of *R. brachycarpum* were collected in Gongju, Korea, in 2011. The plant material was authentificated by Prof. MinKyun Na (College of Pharmacy,

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Chungnam National University). A voucher specimen (CNU00195) was deposited at the Pharmacognosy Laboratory of the College of Pharmacy, Chungnam National University, Daejeon, Korea.

2. Previous work

Previous studies regarding the Korean endangered species have identified eight triterpenoids and one steroid (Youn and Cho, 1991; Jang et al., 2005), five flavonoids (Choi et al., 1986; Choi et al., 1987), four phenols (Choi et al., 1987; Jang et al., 2005) and one diterpenoid (Choi et al., 1987). Our recent study on *R. brachycarpum* revealed the presence of a new ursane-type triterpenoid (Choi and Zhou et al., 2012) and flavone glycoside (Zhou et al., 2013), indicating that this endangered species could be a lucrative resource for producing new chemistry and updating its chemotaxonomic profile.

3. Present study

Dried leaves of *R. brachycarpum* (25 kg) were extracted two times with MeOH (250 L × 2) at room temperature for one week and filtered to acquire the MeOH extract. The extract was concentrated to obtain a brownish slurry (6 kg) and half of the extract (3 kg) was suspended in H_2O (10 L) and partitioned with *n*-hexane (10 L × 3), $CHCl_3$ (10 L × 3), EtOAc (10 L × 3) and BuOH (10 L × 3) to acquire four fractions (438, 140, 450, and 320 g, respectively). Each fraction was subjected to various chromatographic techniques to afford 53 secondary metabolites including a new kaurane-type diterpenoid glycoside, β-sophorosyl *ent*-16-α-hydroxykauran-18-oate (**1**), along with 52 known compounds including two grayane-type diterpenoids (**2** and **3**) and their three glycosides (**4**–**6**), fourteen triterpenoids (**7**–**20**), two triterpene saponins (**21** and **22**), four megastigmane glycosides (**23**–**26**), eight flavonoids (**27**–**34**), twelve phenols (**35**–**46**), four glycolipids (**47**–**50**), one monoterpene glycoside (**51**), one lignan (**52**) and one coumarin (**53**) (Fig. 1; for isolation details, see *Extraction and isolation details* in Supplementary data). The known compounds were identified by comparison of experimental and reported physicochemical data (see *Physicochemical properties of compounds* and *References* in Supplementary data). Compound **1** was obtained as a white amorphous powder and the molecular formula was established as $C_{32}H_{52}O_{13}$ based on the HR-ESI-MS data (obsd. $[M+Na]^+$, *m/z* 667.3304; calcd. $[M+Na]^+$, *m/z* 667.3306) (Fig. S1). Analysis of 1D (Table 1), HMBC and COSY spectra indicated that the gross structure of **1** was identical with those of *ent*-16α-hydroxykauran-18-oic acid with the exception of the relatively more shielded resonance of the carbonyl carbon in **1** as compared to that of *ent*-16α-hydroxykauran-18-oic acid (δ_C 178.1 *vs.*181.5 in pyridine-d_5) (Martin et al., 1997) and the presence of a sophorose moiety deduced from 1D NMR spectra (Table 1) [two anomeric protons (δ_H 5.50, 6.91) and carbons (δ_C 105.2, 94.3) and other ^{13}C signals (δ_C 61.8, 63.0, 79.3, 78.1, 70.6, 72.0, 78.4, 78.6, 80.3, 76.1)]. The HMBC signal from the anomeric proton (δ_H 6.91) to the carbonyl carbon (δ_C 178.1) revealed the connectivity between the disaccharide and diterpenoid moieties (Fig. 2). The coupling constants ($J = 8.0$ Hz, 7.8 Hz) of the anomeric proton signals determined β-glucosidic linkages. The NOE correlations between H-5 (δ_H 1.87) and H-9 (δ_H 1.07), H-9 and H-15β (δ_H 1.69), and H-15β and 17-Me (δ_H 1.46) reaffirmed that the relative configuration of **1** is identical with that of *ent*-16α-hydroxykauran-18-oic acid (Martin et al., 1997). When considered in conjunction with the biosynthetic pathway towards *ent*-16-hydroxykaurane and related glycosides (Martin et al., 1997; Qin et al., 2009), the absolute configuration of **1** is presumably identical to that of the known diterpenoid aglycone and its glycosides, permitting the structure to be defined as β-sophorosyl *ent*-16-α-hydroxykauran-18-oate (**1**).

4. Chemotaxonomic significance

This study is the first confirmation of the presence of 21 compounds (**1**, **10**, **12**, **13**, **16**, **20**–**23**, **25**, **26**, **34**, **39**, **41**, **42**, **45** and **47**–**51**) isolated from the family Ericaceae, two of these (**4** and **9**) from the genus *Rhododendron*. Of the 53 compounds reported in this study, 22 compounds (**3**, **5**, **6**, **8**, **11**, **14**, **15**, **24**, **27**–**29**, **31**, **33**, **35**–**37**, **40**, **43**, **44**, **46**, **52** and **53**) were obtained from *R. brachycarpum* for the first time. Highly oxygenated diterpenoids (i.e., grayanane-, leucothane- and kalmane-types) have been reported from genera belonging to the Ericaceae family such as *Kalmia*, *Leucothoe*, *Lyonia*, *Pieris* and *Rhododendron*, whereas *ent*-kaurane diterpenoids, believed to be a biosynthetic precursor of the aforementioned diterpenoids (Chen et al., 2004), have been rarely isolated from this family (Yang and Tian, 2007). The presence of an *ent*-kaurane diterpenoid glycoside (**1**) in *R. brachycarpum* validates that the Ericaceae family can biosynthesize *ent*-kaurane diterpenoid derivatives which have mainly been reported from Compositae (Ohkoshi et al., 2004), Araliaceae (Harinantenaina et al., 2002.) and Rubiaceae (He et al., 2005). Grayanane-type diterpenoids such as compound **4** have previously been reported in *R. molle* (Qiang et al., 2011) and these types of diterpenoids were suggested as chemotaxonomic markers for identification of *R. molle* (Qiang et al., 2011). Various triterpenoids (**7**–**20**) were also identified in this study, demonstrating chemotaxonomic homogeneity in Ericaceae, as these compounds had been previously reported in many other genera in this family (Qiang et al., 2011; Popescu and Kopp, 2013). However, this is the first report from Ericaceae of triterpenoids which possess a secondary methyl group at C-29 and exomethylene group at C-30 (**10**, **12** and **13**). These characteristic triterpenoids have been acquired from Actinidiaceae and Ericaceae, both belonging to the order Ericales, which might imply a chemotaxonomic similarity between the two families. Moreover, our study is the first validation of the presence of triterpenoid saponins (**21** and **22**) from the Ericaceae even though similar triterpenoid saponins were identified in *R. luteum* (Dzuba and Golovko, 2000) and *R. molle* (Ruan, 2008).