Biochemical Systematics and Ecology 45 (2012) 179–182

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Chemical constituents from the leaves of *Craibiodendron yunnanense*

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ARTICLE INFO

Article history:
Received 28 March 2012
Accepted 1 July 2012
Available online 25 August 2012

Keywords:
Ericaceae
Craibiodendron yannanense
Grayanane diterpenoids
Chemotaxonomic significance

ABSTRACT

Phytochemical investigation on the leaves of *Craibiodendron yunnanense* resulted in the isolation of twenty-five compounds, including nine grayanane diterpenoids, two grayanane diterpenoid glycosides, four triterpenoids, four flavonoid and flavonoid glycosides, and six other types of compounds. The chemotaxonomic significance of grayanane diterpenoids of the family Ericaceae based on our chemical investigation on the toxic and non-toxic plants of this family and literatures were discussed.

1. Subject and source

Craibiodendron W.W. Smith belongs to Ericaceae Subfam. Andromededoideae (Drude) E. Busch. This genus contains six species and is limited to southeastern Asia with a center of distribution in south Yunnan, China (Hsu, 1993). These species are *Craibiodendron yunnanense* W.W. Smith, *Craibiodendron scleranthum* (Dop) Judd, *Craibiodendron kwangtungense* S. Y. Hu, *S. stellatus* W. W. Smith, *Craibiodendron vietnamense* Judd and *Craibiodendron henryi* W.W Smith. Based on a comparison of morphological and anatomical characters, the genus *Craibiodendron* is related closely to *Lyonis* Nutt. and secondarily to *Pieris* D. Don within Andromededoideae (Kron et al., 1999).

C. yunnanense W.W. Smith, a well-known toxic plant, is an evergreen tree or shrub and distributed in hilly and valley regions of China. It has been reported that eating seven pieces of leaves of this plant would put people in a coma state for more than one day (Chen and Zheng, 1987). By cladistic analyses and method of plant morphology-plant geography, Hsu T.Z. studied the evolutionary trend of species in *Craibiodendron* and recognized *C. yunnanense* is the most original species of this genus. In addition, southeastern Yunnan may be the origin of this genus (Hsu, 1993).

The leaves of *C. yunnanense* were obtained from Weihe pharmaceutical Co., LTD, Yunnan Province, China, in September 2008, and were identified by Dr. Hai-Zhou Li. A voucher specimen (KMUST, 20080908) was deposited at the Laboratory of Phytochemistry, the Faculty of Life Science and Technology, Kunming University of Science and Technology.

2. Previous work

Previous phytochemical investigations on the genus *Craibiodendron* were mainly concentrated on *C. henryi* and *C. yunnanense*, which have revealed the occurrence of triterpenoids (Huang et al., 2007a, b; Ma et al., 2008), diterpenoids (Zhang et al., 2005), diterpenoid glycosides (Zhang et al., 2005), flavonoids (Song et al., 2009a, b), flavonoid glycosides (Li et al., 2005),

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http://dx.doi.org/10.1016/j.bse.2012.07.004

iridoid glycosides (Huang et al., 2005), and phenolic compounds (Li et al., 2005). No research has been reported on other species in the genus.

3. Present study

Air-dried and powered leaves of *C. yunnanense* (3.6 kg) were extracted with 75% Me_2CO/H_2O (3 × 36 L, 24 h, each) at room temperature. After the removal of Me_2CO under vacuum, the residue was suspended in water (10 L) and partitioned sequentially with petroleum ether, EtOAC and *n*-BuOH, respectively.

The EtOAc extract (110 g) was separated into five fractions Fr.1 ~ Fr.5 by using Sephadex LH-20 column ($MeOH/H_2O$, v/v, gradient 0:1, 3:7, 6:4, 1:0). Fr.2 (3 g, 30% $MeOH/H_2O$) was subjected to MCI gel column ($MeOH/H_2O$ 3:7, 6:4, 9:1, 1:0), followed by repeated MPLC over on silica gel (Merck, $CHCl_3$/MeOH 100:1, 50:1, $CHCl_3/Me_2CO$ 5:1, and $CHCl_3$/isopropyl alcohol 20:1) to give grayanotoxin III (**1**, 9 mg) (Ohnishi et al., 1973), rhodomollein (**2**, 8 mg) (Chen et al., 2004), asebotoxin (**3**, 7 mg) (Hikino et al., 1970), asebotoxin (**4**, 4 mg) (Harada, 1983), arichannatoxin I (**5**, 7 mg) (Nishida et al., 1990), grayanotoxin (**6**, 12 mg) (Huang et al., 2007a, b), pierisformosin (**7**, 7.5 mg) (Wang et al., 1998), grayanotoxin (**8**, 4.2 mg) (Thomas and Lorraine, 1980), and 4-hydroxybenzeneethanol (**20**, 3.9 mg) (Wang et al., 1982). Taken the same method as those of Fr.2, Fr.3 (11 g, 60% MeOH) was further subjected to repeated silica gel CC (petroleum ether/Me_2CO 10:1, and petroleum ether/EtOAc 6:1) to yield (Z)-p-hydroxy cinamic acid (**21**, 30 mg)(Susanne et al., 1996), (E)-p-hydroxy cinamic acid (**22**, 5 mg) (Wang et al., 2010a,b,c), β-sitoserol (**24**, 7 mg) (Li et al., 2005), and daucosterol (**25**, 8.5 mg) (Fu et al., 2010). Fr.4-1 ~ Fr.4-7 were obtained from Fr.4 (25 g, 90% MeOH) by MCI gel column ($MeOH/H_2O$ 3:7, 6:4, 9:1, 1:0). Through repeated MPLC on ODS CC and Sephadex LH-20 (MeOH), Fr.4-4 gave quercetin 3-O-β-D-glucopyrsnodide (**16**, 5 mg) (Tsukasa and Shunji, 1990), craibiodendronin A (**17**, 4.6 mg) (Wang et al., 1997) and (−)-epicatechin (**19**, 3.1 mg) (Ulla et al., 2002), and Fr.4-3 gave luteolin-7-O-α-L-rhamnoside (**18**, 3.2 mg)(Awouafack et al., 2011). Fr.4-7 was chromatographed over repeated silica gel CC to afford 2α-hydroxyursolic acid (**12**, 7 mg) (Cheng et al., 2010), α-amyrin (**13**, 115 mg) (Seo et al., 1981), 2α,3β,11α,23-tetrahydroxyurs-12-en-28-oic acid (**14**, 17.6 mg) (Acebey-Castellon et al., 2011), and 3-epi-ternstroemic acid (**15**, 4.6 mg) (Akira et al., 2003).

The *n*-BuOH extract (230 g) was chromatographed over Sephadex LH-20 ($MeOH/H_2O$, gradient 3:7, 6:4, 9:1) to afford six fractions, Fr.1 ~ Fr.6. Fr.3 (17 g, 30% MeOH) was further submitted to Sephadex LH-20 ($MeOH/H_2O$; gradient 3:7, 6:4, 9:1), and the concentrated 30% M/W part (2.5 g) was applied to repeated silica gel column ($CHCl_3/MeOH/H_2O$, 8:2:0.2, petroleum ether/Me_2CO 1:5, and petroleum ether/isopropyl alcohol 3:1) to yield craiobiotoxin I (**9**, 5.5 mg) (Zhang et al., 2005), craiobioside A (**10**, 4.5 mg) (Zhang et al., 2005), grayanoside (**11**, 5.5 mg) (Zhang et al., 2005) and 3,4-dihydroxyphenylethanol (**23**, 4.4 mg) (Yukiko et al., 2010).

All the isolated compounds (Fig. 1) were identified by a series of spectrometric methods, such as MS, 1D and 2D NMR experiments, as well as comparison with the data reported in the literatures.

4. Chemotaxonomic significance

Species of the family Ericaceae can be toxic or non-toxic depending on their chemistry (Chen and Zheng, 1987). Many species within the family contain grayanane diterpenoids which possess a specialized carbon skeletons with highly oxygenated functionalities, the compounds occur mainly in the genera *Kalmia*, *Leucothoe*, *Lyonia*, *Pieris*, and *Rhododendron* (Wang and Qin, 1997). Grayanane diterpenoids can be toxic to mammals, especially to the heart and nervous systems, and are thought to be the toxic constituents in those species within the Ericaceae family reported to be toxic (Wang et al., 1997).

Our research group has systematically carried out the phytochemical study on seven toxic and non-toxic plants of the family Ericaceae, including the genus *Rhododendron*, *Lyonia*, *Pieris*, and *Crabiodendron*.

During our investigation of the non-toxic species of this family, showed that *R. spinuliferum* (Luo et al., 2009; Wang et al., 2010a, b, c; Wu et al., 2011c), *R. delavayi* (Song et al., 2009a, b) and *R. alutaceum* (Li et al., 2012) did not contain grayanane diterpenoid and the main compounds were flavonoids.

In contrast, three highly oxidized grayanane diterpenoids, lyonin A and secorhodomollolides A and D, were isolated from the branches and leaves of the toxic *L. avalifolia* (Wu et al., 2011b). Investigations into chemical constituents of the toxic *P. fomosa* resulted in the isolation of forty-one grayanane diterpenoids, mainly highly acylated grayanane diterpenoids (Li et al., 2011; Wang et al., 2010a, b, c, 2012; Wu et al., 2011a, c, 2012a, b). The present study reports the isolation of nine grayanane diterpenoids (**1**–**9**), two grayanane diterpenoid glycosides (**10**, **11**), four flavonoids and flavonoid glycosides (**16**–**19**), four triterpenoids (**12**–**15**), and six others type compounds (**20**–**25**) from the toxic leaves of *C. yannanense*.

According to our phytochemical investigation on the toxic and non-toxic plants of the family Ericaceae and literatures, we may state the following conclusions:

(1) All well-defined taxa consist of species of the family Ericaceae consistently have maxima in the diterpene and flavanone components, thus, diterpenoids and their glycosides, flavanones and their glycosides can be chemotaxonomic markers for the family Ericaceae.
(2) Grayanane diterpenoids can be a useful taxonomic tool for the classification of toxic and non-toxic species of the family Ericaceae. Besides, the toxic of plants could be estimated by the content of grayanane diterpenoids and the oxidizability of grayananes [e.g. 2,3-epoxy and acylated at C-6,14 (Chen et al., 1992)]. Flavanones, e.g. quercetin, quercetin derivatives and quercetin nucleoside, were mainly got from the non-toxic plants.