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A phytochemical and chemotaxomic study on *Viburnum lancifolium*

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1. Subject and source

The genus *Viburnum* belonging to the family Adoxaceae (formerly Caprifoliaceae), comprises more than 230 species mainly distributed in the temperate or subtropical zones, from South America (Peru) to South-East Asia (Philippines, Malaysia) (Lobstein et al., 1999). Many *Viburnum* species are commonly used in folk medicine for their diuretic, antispasmodic and sedative properties, mainly as uterine excitability (British Herbal Pharmacopia, 1983; Cometa et al., 1998). The dry powder of *Viburnum tinus* leaves was reported as an effective molluscicidal agent (Ibrahim et al., 1994). In Traditional Chinese Medicine, this genus is the resource of many medicinal herbs (Tian et al., 2006). *Viburnum lancifolium* Hsu, an evergreen shrub distributed in Jiangxi, Zhejiang and Fujiang Province of China, has been used as a folk medicine in China (Tian et al., 2006).

The roots of *V. lancifolium* were collected in Linan County, Zhejiang Province, People's Republic of China, in September 2010. A voucher specimen (v431) is maintained at Jinhua College of Vocation and Technology, Jinhua, People's Republic of China.

2. Previous work

In our previous chemotaxomic studies on the genus *Viburnum*, phenolic glycosides including stilbene glycoside and lignan glycosides were isolated from *Viburnum fordiae* (Wu et al., 2008a,b,c), salicin analogous, quercetin derivatives and quinic acid derivatives isolated from *Viburnum dilatatum* (Wu et al., 2008a,b,c), lignan, flavan, monoterpene glycoside and quercetin derivatives isolated from *Viburnum erosum* (Wu et al., 2008a,b,c). Many flavonoids and biflavonoids (Glasby, 1991; Plouvier, 1992), triterpenoids, diterpenoids (Kagawa et al., 1998; Iwagawa et al., 1993; Fukuyama et al., 1999) have been isolated from this genus. This genus is also rich in iridoids and their glycosides (Bock et al., 1978; Hase et al., 1985; Jensen et al., 1985), mainly

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of the valeriana type with a β-D-glucoside linked to C-11 (El-Naggar and Beal, 1980; Boros and Stermitz, 1990, 1990; Iwagawa et al., 1990; Iwagawa and Hase, 1989). To the best of our knowledge, no phytochemical investigation on this species has been reported.

3. Present study

The shade-dried, powdered, roots (15 kg) of *V. lancifolium* were extracted at room temperature three times with methanol (3 × 20 L). The extracts were evaporated *in vacuo* to afford a gummy residue (1.2 kg). This residue was partitioned in H_2O (5 L) and extracted with petroleum ether (4 × 5 L), EtOAc (4 × 5 L) and *n*-butanol (4 × 5 L), successively. The EtOAc extract (234 g) was adsorbed onto silica gel (200 g) and subjected to chromatography over silica gel (8 × 150 cm, 1500 g, 200–300 mesh), eluting with *n*-hexane/EtOAc gradient mixtures. Nine main fractions were obtained by checking with TLC and combined. The second fraction was subjected to Sephadex LH-20 (4 × 150 cm, 300 g, Amersham) column, to afford pure compounds; β-sitosterol (10.2 mg) and stigmasterol (5.4 mg) (Ling et al., 1997; Yao et al., 2007). The fourth fraction was subjected to chromatography over silica gel (4 × 30 cm, 300 g, 200–300 mesh), eluting with *n*-hexane/EtOAc gradient mixtures to afford five subfractions. The first subfraction was purified by preparative HPLC using MeOH-H_2O (55:45) as eluent, to afford a lignan (+)-pinoresinol (14.1 mg) (Briggs et al., 1959) and a megastigmane (6*S*,9*R*)-vomifoliol (11.3 mg) (Hammami et al., 2004). The second subfraction was subjected to Sephadex LH-20 (4 × 150 cm, 300 g, Amersham) column and eluted with MeOH.

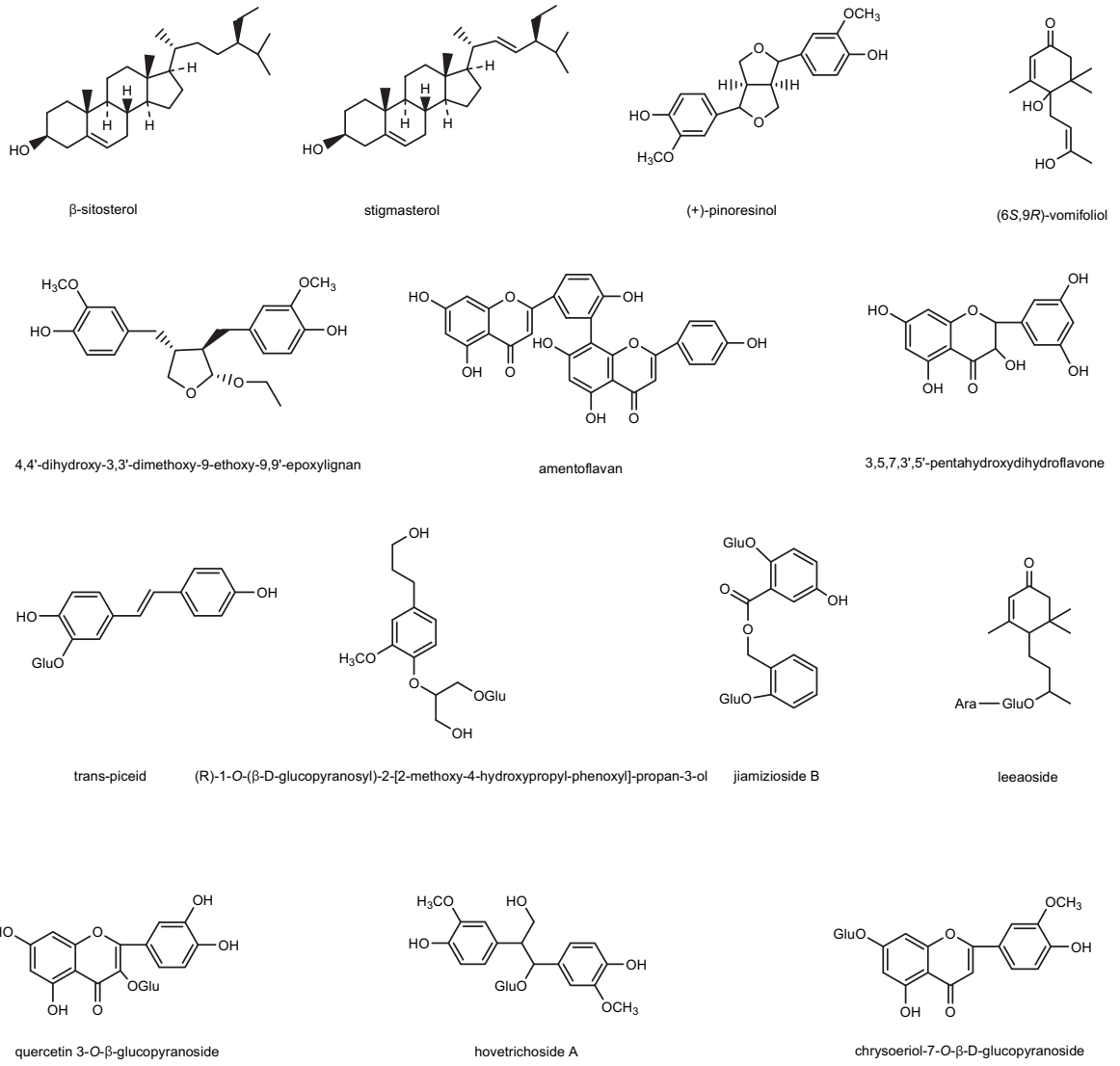

Fig. 1. The structures of all isolates.

Fractions collected were purified by preparative HPLC using MeOH-H_2O (38:62) as eluent, to afford a lignan 4,4′-dihydroxy-3,3′-dimethoxy-9-ethoxy-9,9′-epoxylignan (12.2 mg) (Liang et al., 2008) and a biflavane amentoflavan (41.0 mg) (Zhang et al., 2005). The third subfraction was subjected to Sephadex LH-20 (1.5 × 80 cm, 100 g, Amersham) column and eluted with acetone to afford a dihydroflavone, 3,5,7,3′,5′-pentahydroxydihydroflavone (12.0 mg) (Ding et al., 1997). The ninth fraction was applied to a Sephadex LH-20 column, and eluted with MeOH to yield a stilbene glucoside *trans*-piceid (7.5 mg) (Chen et al., 2000), and a monolignan glucoside (*R*)-1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-hydroxypropyl-phenoxyl]-propan-3-ol (15.1 mg) (Matsuda and Kikuchi, 1996). The *n*-butanol extract (231 g) was adsorbed onto silica gel (200 g) and subjected to chromatography over silica gel (8 × 100 cm, 1500 g, 200–300 mesh), eluting with $CHCl_3$/MeOH gradient mixtures. Six main fractions were obtained by checking with TLC and combined. The second fraction was applied to a Sephadex LH-20 column (4 × 150 cm, 300 g), and eluted with MeOH to yield a salicin adduct, jiamizioside B (7.0 mg) (Wu et al., 2008a,b,c) and a flavone glycoside chrysoeriol-7-*O*-β-D-glucopyranoside (10.0 mg) (Zhang et al., 2006). The third fraction was applied to preparative HPLC using MeOH–H_2O (20:80) as eluent, to afford quercetin 3-O-β-glucopyranoside (3.5 mg) (Kagan, 1968) and hovetrichoside A (5.8 mg) (Hsiao and Chiang, 1995). The fourth fraction was applied to pre-TLC to give pure leeaoside (6.9 mg) (Kaewkrud et al., 2007), a megastigmane diglycoside. The structures of isolates (Fig. 1) were established by analysis of their NMR and MS data, and by comparison of their spectroscopic data with literature values.

4. Chemotaxomic significance

In our previous chemotaxomic investigation on *V. erosum*, lignans, flavan and biflavan are considered to be characteristic of some species (Wu et al., 2008a,b,c). Other studies showed that the distribution of biflavonoids, such as amentoflavone, is generally considered to be a better character in Gymnospermae than Angiospermae (Lobstein et al., 1999). The occurrence of relatively high amount of amentoflavone in *V. lancifolium* supported the conclusion that the quantity of amentoflavone is a useful taxonomic character in the genus *Viburnum* at the sectional level (Lobstein et al., 1999). Although iridoids and iridoid glycosides (Bock et al., 1978; Hase et al., 1985; Jensen et al., 1985) are the main constituents of some species, they were not detected in this phytochemical study. Chemotaxonomic significance of several type compounds was summarized as below:

i) It is the first time that megastigmane and its diglycoside have been detected from the genus *Viburnum*. It could be tentatively concluded that megastigmanes might be a useful chemotaxonomic marker of the species *V. lancifolium* peculiar to China.
ii) Vibsane type of diterpenoids consisting of fumulane-type carbon skeleton with an additional isoprene unit have only been found in *Viburnum awabuki* and liverwort *Odontoschisma denudatum* (Kagawa et al., 1998; Iwagawa et al., 1993). However, salicin analogs and quercetin derivatives wildly occurring in this genus were not isolated from this species. *V. awabuki* must have a unique secondary metabolic biosynthesis route. Thus, from the chemotaxomic point of view, the taxonomical determination of *V. awabuki* remains uncertain.
iii) Stilbenes have only been detected in this plant and *V. fordiae* (Wu et al., 2008a,b,c), which can be used to differentiate *V. fordiae* and *V. lancifolium* from other *Viburnum* species.
iv) Five species of *Viburnum* including *V. fordiae*, *V. lancifolium*, *V. erosum*, *V. dilatatum* (Wu et al., 2008a,b,c) and *Viburnum henryi* (Jensen et al., 1985) were similar in the second metabolites of salicin adducts.
v) The ability to form phenolic compounds especially lignans and flavones can not be considered as a useful chemotaxonomic marker for the genus *Viburnum*, since such phenolic compounds can be formed by environmental stress (Grassmann et al., 2002).

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