

## Chemical constituents of *Humboldtia vahliana*

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### Abstract

From aerial parts of *Humboldtia vahliana*, 3 $\alpha$ -methoxy friedelan,  $\beta$ -sitosterol, sitosterol-3-O- $\beta$ -D-glucopyranoside and 5,7,4'-trihydroxyflavone (apigenin) were isolated for the first time. All constituents were identified by spectral studies.

**Key words :** *Humboldtia vahliana*, phytochemical analysis, spectral studies.

### Introduction

*Humboldtia vahliana* Wight (Fabaceae) is a medium size tree with rough, dark brown bark mottled with grey. Its bark is used for the treatment of ulcers, biliousness, leprosy and epilepsy [2]. The genus *Humboldtia* has not been subjected to detailed phytochemical studies. The only phytochemical report available is on *H. laurifolia* by Samaraweera *et al.* [5]. In the present study, we report isolation of 3 $\alpha$ -methoxyfriedelan,  $\beta$ -sitosterol, sitosterol-3-O- $\beta$ -D-glucopyranoside and 5,7,4'-trihydroxyflavone (apigenin) from the aerial parts of *Humboldtia vahliana*, for the first time.

### Materials and methods

Aerial parts of *Humboldtia vahliana* were collected from the premises of Neyyar dam (Kerala) and identified by Dr. N. Sasidharan, Senior Scientist, Kerala Forest Research Institute, Peechi (Kerala), where a voucher specimen has been deposited.

For column chromatographic separation of compounds, silica gel (60-120 mesh) of E-Merck was used. Silica gel-G containing 13% calcium sulphate as binder was used for TLC. IR spectra were recorded on Shimadzu FT-IR 8101-A spectrometer, as KBr pellets. <sup>1</sup>H NMR (300 MHz) and <sup>13</sup>C NMR (75 MHz) spectra were recorded using Bruker spectrometer, in CDCl<sub>3</sub> and DMSO-*d*<sub>6</sub>, using TMS as internal standard. Chemical shifts are in ppm ( $\delta$  values). EI-Mass spectra were

recorded by means of direct insertion probe at an ionisation energy of 70 eV.

### Isolation of compounds

Shade-dried plant material (1 kg) was soxhlet-extracted successively with hexane (60-80°C), chloroform and methanol for 30 hours in each case. The extracts were separately concentrated to dryness and subjected to column chromatography over silica gel.

The residue (20 g) from the hexane extract was subjected to column chromatography over silica gel (400 g) and eluted with hexane followed by hexane-ethyl acetate mixtures of increasing polarity and finally with ethyl acetate. Fractions of 100 ml were collected, concentrated and similar fractions as monitored by TLC were combined.

Fractions eluted with hexane-ethyl acetate (98:2), upon repeated column chromatography yielded compound I. Elution of the column with hexane-ethyl acetate (95:5) yielded compound II.

The chloroform extract (12 g) by usual chromatographic work-up on a silica gel column (240g) and elution with hexane-ethyl acetate (70:30) yielded compound III.

The methanol extract was separated into ethyl acetate soluble and insoluble fractions by extraction with ethyl acetate. The residue (3 gm) from ethyl acetate soluble fraction was subjected to usual column

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chromatographic work-up and elution with hexane-ethyl acetate (50:50) yielded compound IV.

## Results and discussion

### Compound I

Compound I was obtained as colourless needles (115 mg) from chloroform, mp 298-300°C (lit. mp 308°C) [5]. Compound I showed positive reaction to Liebermann-Burchard test indicating triterpene nature. It was identified as 3 $\alpha$ -methoxy friedelan by comparison of its IR, <sup>1</sup>H NMR and MS with that reported earlier [1, 5]. However its <sup>13</sup>C NMR data were not reported. It is reported here for the first time.

<sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>):  $\delta$ (ppm): 80.95(C-3), 60.03(C-10), 56.28(-OCH<sub>3</sub>), 52.90(C-8), 51.39(C-4), 42.77(C-18), 41.36(C-6), 39.64(C-13), 39.21(C-22), 38.25(C-14), 38.03(C-5), 36.96(C-9), 36.00(C-16), 35.48(C-11), 35.29(C-30), 35.02(C-19), 32.75(C-21), 32.30(C-15), 32.07(C-28), 31.76(C-29), 30.56(C-12), 29.96(C-17), 29.64(C-2), 28.14(C-20), 20.14(C-27), 19.25(C-1), 18.36(C-26), 18.12(C-25), 17.82(C-7), 14.56(C-24), 9.82(C-23).

### Compound II

The fractions eluted with hexane-ethyl acetate (95:5) yielded colourless needles (48 mg) from chloroform, mp 136°C, which was identified as  $\beta$ -sitosterol by comparison with an authentic sample (m.m.p, ir, co-tlc) and literature data [1, 3].

### Compound III

Compound III was obtained as white amorphous powder (53 mg) from chloroform-methanol, mp 278°C (lit. mp 282-284°C) [1]. It gave positive reaction to Liebermann-Burchard test for sterols and Molisch's test for glycosides. Hydrolysis of this compound with 10% methanolic sulphuric acid followed by usual work-up gave an aglycone, which was characterized as  $\beta$ -

sitosterol and the sugar part was identified as glucose by paper chromatography (n-butanol:acetic acid:water, 4:1:5). Hence Compound III was identified as sitosterol-3-O- $\beta$ -D-glucopyranoside.

### Compound IV

Compound IV (18 mg), mp 345°C (lit. mp 347°C) was obtained as pale yellow powder. It gave red colour to Mg-HCl test for flavonoids [5] and was identified as 5,7,4'-trihydroxyflavone (apigenin) by comparison of its UV, IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and EI-MS with that reported earlier [4, 5].

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