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Original Research Article

Isolation and Characterization of Batatasin III and 3,4'-Dihydroxy-5-methoxybibenzyl: A Pair of Positional Isomers from *Sunipia scariosa*

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Abstract

Purpose: *To isolate and characterize chemical compounds of biological importance from the whole plant of Sunipia scariosa.*
Methods: *The whole plant of Sunipia scariosa was extracted with methanol (MeOH) and chromatographed on silica gel and sephadex LH-20 to afford the pure isolates. High perfomance liquild chromatography (HPLC) was used for further purification of the isolated compounds. Characterization of the isolated compounds was achieved by 1H and 13C nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).*
Results: *Batatasin III (3,3'-dihydroxy-5-methoxybibenzyl) and 3,4'-dihydroxy-5-methoxybibenzyl, a pair of positional isomers, were isolated from the whole plant of Sunipia scariosa. The yields of the two isomers were 60 and 40 %, respectively, from the mixture of two compounds.*
Conclusion: *Batatasin III and 3,4'-dihydroxy-5-methoxybibenzyl, a pair of positional isomers were successfully isolated from the whole plant of Sunipia scariosa for the first time.*

Keywords: *Sunipia scariosa, Batatasin III, 3,4'-Dihydroxy-5-methoxybibenzyl, Isomers*

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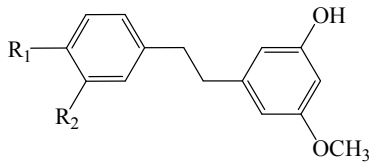
INTRODUCTION

Bibenzyls are regarded as important secondary metabolites occurring exclusively in the plants of *Orchidaceae*, which is distributed in both tropical and subtropical regions of the world, and comprises of about 700 genus and 20000 species [1]. In the plants of some genera, such as *Dendrobium*, *Bulbophyllum*, *Pholidota* and *Bletilla*, bibenzyls are dominant compounds and exhibit diverse biological activities such as anti-tumor, anti-inflammatory and platelet anti-aggregation activities [2-10].

Batatasin III (3,3'-dihydroxy-5-methoxybibenzyl, Fig 1), the most common bibenzyl isolated from many *Orchidaceae* plants, shows spasmolytic, allelopathic and inhibitory activities on germination and radicle growth [10-13]. 3,4'-dihydroxy-5-methoxybibenzyl, a positional isomer of batatasin III also displays phytotoxic activity [11,13] but both are rarely obtained from a plant simultaneously.

Sunipia scariosa is an *Orchidaceae* plant mainly distributed in India, Nepal, Sikkim, Bhutan, Burma, Thailand, Laos, Vietnam and China [14]. Previously, there has been no report on the

chemical study of the plant in the literature. In the course of our search for new bioactive compounds from the whole plant of *S. scariosa*, we study the plant and isolated batatasin III and 3,4'-dihydroxy-5-methoxybibenzyl, a pair of positional isomers from the plant.



batatasin III, R_1= H, R_2= OH

3,4′-dihydroxy-5-methoxybibenzyl, R_1= OH, R_2= H

Fig 1: Structure of batatasin III and 3,4'-dihydroxy-5-methoxybibenzyl

EXPERIMENTAL

Plant material

The whole plant of *S. scariosa* was collected from Lianghe, Yunnan Province of China in November 2008 and identified by Dr. Guangwan Hu (Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China), and the voucher specimen (No.0811005) is deposited at the herbarium of Department of Chemistry, Yunnan Normal University, Kunming, China.

Extraction

The dried powdered whole plant of *S. scariosa* (0.25 kg) was soaked with MeOH for 24 h and extracted for 6 times. The liquid extract was concentrated to dryness under reduced pressure in a rotary evaporator, yielding the dry crude extract.

Isolation of compounds

Silica gel (200-300 mesh, Qingdao Marine Chemical Co., China), and Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd.) were used for column chromatography (CC), and silica gel GF254 was used for TLC (Qingdao Marine Chemical Co., China). Solvents were of industrial purity and distilled prior to use, but MeOH used for semi-preparative HPLC was of chromatography grade and purchased from Beijing Chemical Factory, China. Semi-preparative HPLC was carried out using a system composed of a Waters 600 pump, with an Agilent 1100 detector and Sunfire C18 reversed phase column (10×150 mm, detected at UV of 254 nm).

The extract (40 g) was placed in a silica gel (400 g) column, eluting with petroleum ether containing increasing amounts of acetone to obtain 7 fractions. The fourth fraction (1.84 g, petroleum ether/acetone, 5:1, v/v portion) was purified by column chromatography, first on silica gel ($CHCl_3$/MeOH: 1:0→0:1) to afford 7 fractions, and then the third fraction (293 mg, $CHCl_3$/MeOH, 50:1, v/v portion) was purified on Sephadex LH-20 ($CHCl_3$/MeOH: 3:2) to yield a mixture of two isomers (145 mg), which was further purified on semi-preparative HPLC using MeOH–H_2O (v/v, 9:11) at 1.0 mL/min to yield 1 (41 mg) and 2 (27 mg).

Identification of compounds

Identification of compounds was carried out by MS and ^{1}H-, ^{13}C-NMR spectra. MS were determined on an API Qstar Pulsa LC/TOF mass spectrometer, and NMR spectra were measured on a Bruker DRX-500 spectrometer with CD_3OD as solvent.

RESULTS

The MeOH extract from the whole plant of *S. scariosa* was subjected to column chromatography on silica gel and Sephadex LH-20 to afford the mixture of two isomers, which was further purified by HPLC to obtain batatasin III and 3,4'-dihydroxy-5-methoxybibenzyl, a pair of positional isomers.

Compound 1: ^{1}H NMR δ: 2.77 (4H, m, CH_2), 3.69 (3H, s, 5-OCH_3), 6.20 (1H, d, J =2.0 Hz, H-4), 6.25 (2H, t, J= 2.0 Hz, H-2, 6), 6.70 (2H, dd, J= 2.0, 8.5 Hz, H-3', 5'), 6.98 (2H, dd, J= 2.0, 8.5 Hz, H-2', 6'); ^{13}C-NMR δ: 160.8 (C-5), 157.9 (C-3), 155.0 (C-4'), 144.2 (C-1), 132.7 (C-1'), 129.1 (C-2',6'), 114.6 (C-3',5'), 107.7 (C-2), 105.2 (C-6), 98.5 (C-4), 54.1 (OCH_3), 38.3 (CH_2-a'), 36.7 (CH_2-a); ESI-MS *m/z*: 245 $[M+H]^+$. Comparison with the data shown in literature [15], the compound was identified as be 3,4'-dihydroxy-5-methoxybibenzyl.

Compound 2: ^{1}H-NMR δ: 2.79 (4H, m, CH_2), 3.70 (3H, s, OCH_3), 6.20 (1H, dd, J= 2.0, 2.2 Hz, H-4), 6.24 (2H, dd, J= 2.0, 2.2 Hz, H-2, 6), 6.63 (3H, m, H-2', 4', 6'), 7.08 (1H, dd, J= 7.5, 8.0 Hz, H-5'); ^{13}C-NMR δ: 160.8 (C-5), 158.0 (C-3), 156.9 (C-3'), 144.1 (C-1), 143.3 (C-1'), 128.9 (C-5'), 119.5 (C-6'), 115.0 (C-2'), 112.4 (C-4'), 107.6 (C-2), 105.1 (C-6), 98.5 (C-4), 54.1 (OCH_3), 37.8 (CH_2-a'), 37.5 (CH_2-a). ESI-MS *m/z*: 245 $[M+H]^+$. Comparison with the data shown in literature [16], led to the compound being identified as batatasin III.

DISCUSSION

The plants of *Orchidaceae* usually contain aromatics such as phenanthrenes and bibenzyls, as characteristic compounds. Some of the isolated compounds have been found to show significant anti-tumor, anti-inflammatory and platelet anti-aggregation activities. Batatasin III, and 3,4'-dihydroxy-5-methoxybibenzyl, two positional isomers, have been isolated from *Orchidaceae* species, but both are rarely obtained from a plant simultaneously.

In the present study, the MeOH extract from the whole plant of *S. scariosa* was subjected to column chromatography on silica gel and Sephadex LH-20 to afford the mixture of two isomers. Detailed analysis of the ^{1}H and ^{13}C NMR showed that it was a dimeric bibenzyl or two similar bibenzyls in one structure. The use of HPLC, with the aid of a solvent system comprised of methanol/water (9:11, v/v) successfully led to the differentiation of two peaks. Subsequent preparative isolation by HPLC afforded two compounds 3,4'-dihydroxy-5-methoxybibenzyl and batatasin III.

CONCLUSION

Batatasin III and 3,4'-dihydroxy-5-methoxybibenzyl, a pair of positional isomers have been simultaneously isolated from the extract of *S. scariosa* for the first time. The compounds can be used for further research in pharmacological studies and as reference substances.

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