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Stilbenes from *Paphiopedilum exul* roots

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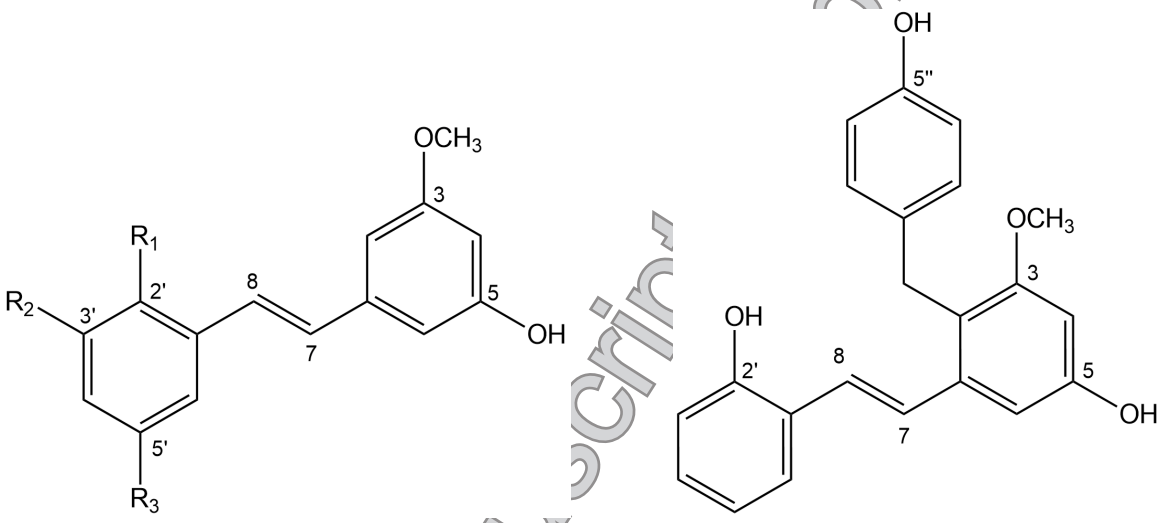
Five *trans*-stilbenes including (*E*)-3-methoxy-5-hydroxystilbene (**1**), (*E*)-3,5'-dimethoxy-5,2'-dihydroxystilbene (**2**), thunalbene (**3**), (*E*)-3-methoxy-5,2'-dihydroxystilbene (**4**) and (*E*)-3-methoxy-2-(4-hydroxybenzyl)-5,2'-dihydroxystilbene (**5**) were isolated from the roots of *Paphiopedilum exul* (family Orchidaceae). Their chemical structures were identified by spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR and 2D-NMR. This study is the first report of chemical constituents of this lady's slipper orchid native to the south of Thailand.



	R_1	R_2	R_3
1	H	H	H
2	OH	H	OCH_3
3	H	OH	H
4	OH	H	H

5

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Introduction

The family Orchidaceae is one of the biggest flowering plant families. Many bioactive phytochemicals such as stilbenoids, flavonoids, terpenoids and alkaloids have been discovered from orchids.[1-2] *Paphiopedilum*, which is a genus of subfamily Cypripedioideae, consists of several lady's slipper orchids native to Thailand. Although these orchids are widely grown for their characteristic, beautiful flowers, their chemical constituents are very rarely investigated. This study aims to explore chemical constituents of the roots of *Paphiopedilum exul,* a terrestrial lady's slipper orchid endemic to southern Thailand.

Material and methods

General procedures: ^{1}H-NMR (300 MHz), ^{13}C-NMR (75 MHz), and 2D-NMR spectra were obtained on a Bruker Avance DPX-300 NMR spectrometer (Bruker Corp., Billerica, MA, USA), in $CDCl_3$ or acetone-d_6 with tetramethylsilane

as an internal standard. Silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) were used for TLC analysis. Column chromatography (CC) was performed using either silica gel 60 (230-400 mesh, Merck) as the adsorbent or Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as the gel filter.

***Plant material*:** The whole plants of *Paphiopedilum exul* (Ridl.) Rolfe were purchased from Chatuchak market, Bangkok, in January 2015. Plant identification was performed by comparison with authentic specimen (QBG No. 13143) at the herbarium of the Botanical Garden Organization, Ministry of Natural Resources and Environment, Thailand. The fresh roots were separated from the plants, cleaned and dried at temperature not more than 50°C.

Extraction and isolation: Dried roots of *P. exul* (380 g) were ground and macerated three times with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure and combined to give 100 g of crude MeOH extract. A portion of the extract (50 g) was separated on a silica gel column (1.25 kg, 10 × 40 cm), washed down with *n*-hexane/acetone (3:1), to yield eight fractions (A-H). Fraction C (2.9 g) was chromatographed on a silica gel column (150 g, 4.5 × 19 cm), eluted with CH_2Cl_2/acetone (40:1), to provide eight subfractions (C1-C8). Subfraction C2 (890 mg) was subjected to a Sephadex LH-20 CC using MeOH as the eluent to yield three subfractions (C21-C23). Purification of subfraction C23 (300 mg) by repeated silica gel CC, using CH_2Cl_2/acetone (40:1) as the eluent, afforded compound **1** (44 mg). Fraction E (9.9 g) was separated on a silica gel column (300 g, 4.5 × 40 cm) washed down with *n*-hexane/acetone (2:1) to give six subfractions (E1-E6). Subfraction E3 (3.4 g) was subjected to silica gel CC (175 g, 4.5 × 26 cm) eluted with CH_2Cl_2/acetone (30:1) to give nine subfractions (E31-E39). Compound **2** (32 mg) was obtained from subfraction E38. Subfraction E35 (1.4 g) was chromatographed on a silica gel column (70 g, 3 × 33 cm) eluted with CH_2Cl_2/acetone (30:1) to give three subfractions (E351-E353). Silica gel CC (55 g, 3 × 26 cm) of subfraction E352 (1.1 g), eluted with CH_2Cl_2/acetone (30:1), afforded five subfractions (E3521-E3525). A mixture of compounds **3** and **4** (72 mg) was obtained from subfraction E3525. Gel filtration of fraction E5 (780 mg) on a Sephadex LH-20 column eluted with MeOH yielded four subfractions (E51-E54). Subfraction E53 (290 mg) was chromatographed on a silica gel column (15 g, 2 × 14 cm), eluted with CH_2Cl_2/acetone (20:1), to give six subfractions (E531-E536). Compound **5** (58 mg) was obtained from subfraction E535.

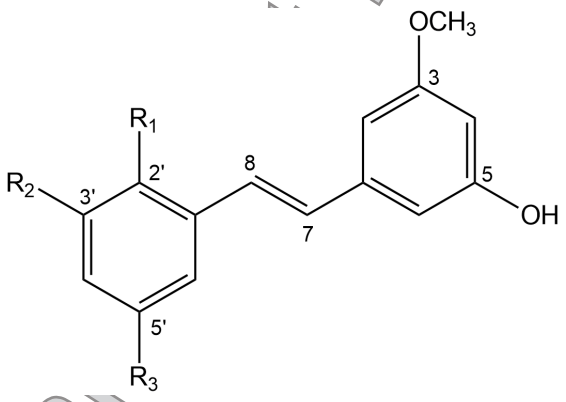

Figure 1. Chemical structures of compounds **1**-**5**

Results

Five *trans*-stilbenes were isolated from the MeOH extract of the roots of *Paphiopedilum exul*. They were identified as (*E*)-3-methoxy-5-hydroxystilbene (**1**), (*E*)-3,5'-dimethoxy-5,2'-dihydroxystilbene (**2**), thunalbene (**3**), (*E*)-3-methoxy-5,2'-dihydroxystilbene (**4**), and (*E*)-3-methoxy-2-(4-hydroxybenzyl)-5,2'-dihydroxystilbene (**5**), respectively, by comparison of their ^{1}H-NMR, ^{13}C-NMR (see **Table 1**) and 2D-NMR data with previous reports.[3-5]

Discussion

The stilbenes isolated in this study represented interesting chemotaxonomic significance. Among these *trans*-stilbenes, (*E*)-3-methoxy-5-hydroxystilbene (**1**) is the most widely found one. It has been reported as a constituent of pteridophytes, gymnosperms, monocots and dicots,[6-13] and has been reported to display various biological activities including anti-inflammatory, antimicrobial, antifungal and antiproliferative activities[(6),14-17] Its wide distribution might be due to its chemical structure, which is basic *trans*-stilbene with biosynthetically common oxy-substitutions at positions 3 and 5. On the other hand, thunalbene (**3**), with another hydroxy substitution at position 3', occurred more limitedly in nature and has been previously found in only two monocot families, i.e. Orchidaceae and Dioscoreaceae.[5,18-19] The

compound was also reported to be anti-inflammatory.[20] The structures of the other three isolated stilbenes from *P. exul*, i.e. (*E*)-3,5'-dimethoxy-5,2'-dihydroxystilbene (**2**), (*E*)-3-methoxy-5,2'-dihydroxystilbene (**4**), and (*E*)-3-methoxy-2-(4-hydroxybenzyl)-5,2'-dihydroxystilbene (**5**), similarly possess 2'-hydroxy substitution. They have once been isolated from a *Phragmipedium* orchid, which belongs to the same subfamily (Cypripedioideae) as *Paphiopedilum*.[4] This position of the hydroxy group is conveniently located for the formation of 2-phenylbenzofuran stilbenes found in the roots of another *Paphiopedilum* species, *P. godefroyae*.[21]

Table 1. ^{13}C-NMR (75 MHz) spectral data of compounds **1**-**5** (in $CDCl_3$)

Position	1	2	3	4	5*
1	139.7	139.8	138.8	140.0	139.3
2	104.9	105.1	105.0	105.0	119.7
3	161.1	161.1	161.1	161.1	159.5
4	101.0	101.1	101.1	101.0	99.0
5	156.9	156.9	156.8	156.8	157.3
6	105.9	105.9	106.0	105.9	104.4
7	128.3	129.7	129.0	127.2	127.0
8	129.4	123.7	128.8	124.4	125.7
1'	137.0	125.1	139.5	123.7	125.3
2'	126.6	147.3	113.0	153.1	155.5
3'	128.7	116.9	155.8	116.0	120.4
4'	127.8	114.7	114.9	129.6	129.1
5'	128.7	153.9	129.9	121.1	116.4
6'	126.6	111.6	119.5	128.8	127.3
3-OCH_3	55.4	55.4	55.4	55.4	55.6
5'-OCH_3		55.4			
1''					30.0
2''					133.3
3''/7''					129.7
4''/6''					115.5
5''					155.8

* in acetone-d_6

Conclusion

Five *trans*-stilbenes were obtained from the roots of a lady's slipper orchid, *Paphiopedilum exul*. The 2'-hydroxy substitution in three of these stilbenes appears to be characteristic of orchids in the subfamily Cypripodioideae.

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