Journal of Chemistry, Vol. 48 (5), P. 539 - 545, 2010

FOUR PHENYLETHANOIDS FROM LEAVES OF *PSEUDERANTHEMUM CARRUTHERSII* (SEEM.) GUILL. VAR. *ATROPURPUREUM* (BULL.) FOSB.

Received 5 May 2009, revised 3 March 2010

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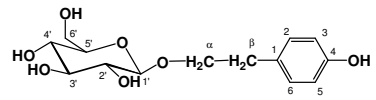
ABSTRACT

*Four phenylethanoids were isolated from the dried leaves of Pseuderanthemum carruthersii (Seem.) Guill. var. atropurpureum (Bull.) Fosb. (Acanthaceae), including salidroside (**1**), darendoside B (**2**), verbascoside (**3**) and isoverbascoside (**4**). Their chemical structures were elucidated by spectroscopic methods. These substances were isolated for the first time from this genus.*



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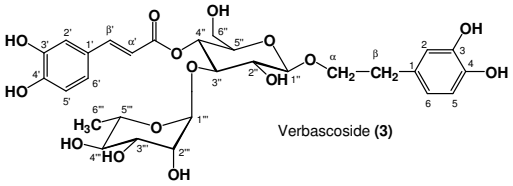

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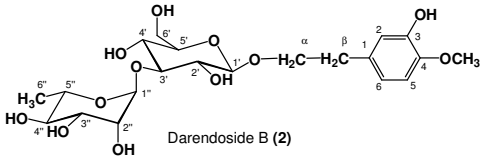
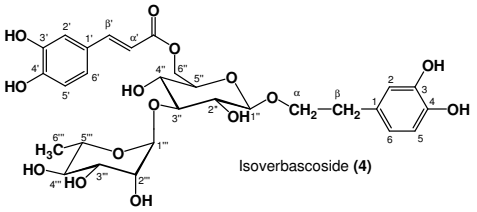


Darendoside B **(2)**



Isoverbascoside **(4)**

I - INTRODUCTION

P. carruthersii (Seem.) Guill. var. *atropurpureum* (Bull.) Fosb. (Acanthaceae) has been used in traditional medicine to heal the wound [1]. It has not yet much been studied. In this paper, from the leaves of this plant, four phenylethanoids were isolated and their chemical structures were elucidated by spectroscopic means.

II - EXPERIMENTAL

General experimental procedures

The NMR spectra were measured on a Bruker Avance spectrometer, at 500 MHz for ^{1}H and 125 MHz for ^{13}C, in the Institute of Chemistry, Vietnamese Academy of Science and Technology. The HR-ESI-MS were recorded on a HR-ESI-MS microOTOF–Q

10187 mass spectrometer in the University of Science, National University – HCM City.

Plant material

The leaves of *Pseuderanthemum carruthersii (*Seem.*)* Guill. var. *atropurpureum (*Bull.*)* Fosb., collected in June 2008 in Binh Phuoc province, were identified by Dr. Hoang Viet, Department of Biology, University of Science, National University – HCM City. A voucher specimen (US–A007) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University – HCM City.

Extraction and Isolation

The dried leaves of *P. carruthersii atropurpureum* (5 kg) were exhaustedly extracted with methanol by maceration at room temperature and the methanolic filtrate was concentrated *in vacuo* to yield a residue of 800g. This crude residue was suspended in water and partitioned between water and petroleum ether and then ethyl acetate. The remaining aqueous solution was subjected to Diaion HP–20 column chromatography, and eluted with water, 50% methanol and methanol, respectively. The 50% methanol residue (30g) was applied on a RP–18 silica gel chromatographic column eluted with water–methanol (8:1 to 2:1) to give 7 fractions. Fraction 4 (5.3g) was purified by silica gel column chromatography eluted with ethyl acetate: methanol: water 8:1:1 to afford compound **1** (51mg). Fraction 5 (3.3g) was purified by silica gel column chromatography eluted with ethyl acetate: methanol: water 8:1:1 to afford compound **2** (35mg). Fraction 6 (3.7g) was applied on a silica gel chromatographic column with chloroform:methanol:water 20:6:1 to afford compound **3** (42mg) and compound **4** (38mg).

* **Salidroside (1)**: amorphous powder; ^{1}H and ^{13}C NMR (CD_3OD) see Table 1, HR-ESI-MS *m/z* 323.1120 $[M+Na]^+$.

* **Darendoside B (2)**: amorphous powder; ^{1}H and ^{13}C NMR (CD_3OD) see table 1, HR-ESI-MS *m/z* 499.1742 $[M+Na]^+$.

* **Verbascoside (3)**: amorphous powder; ^{1}H and ^{13}C NMR (CD_3OD) see Table 2, HR-ESI-MS *m/z* 647.1972 $[M+Na]^+$.

* Isoverbascoside (4): amorphous powder; ^{1}H and ^{13}C NMR (CD_3OD) see table 2, HR-ESI-MS *m/z* 647.1985 $[M+Na]^+$.

III - RESULTS AND DISCUSSION

The methanolic extract of the dried leaves of *Pseuderanthemum carruthersii (Seem.) Guill. var. atropurpureum* (Bull.) Fosb. was separated by silica gel column chromatography to afford four compounds, **1**-**4**. Compound **1** was isolated as amorphous powder, ESI-MS showed *m/z* 323.1120 $[M+Na]^+$ supported the molecular formula $C_{14}H_{20}O_7$. The ^{1}H spectrum (table 1) of **1** showed the presence of aromatic protons at δ_H 7.08 (2H, *d*, *J* = 8.5Hz, H–2 and H–6) and 6.72 (2H, *d*, *J* = 8.5 Hz, H–3 and H–5) assigned to a *p*-substituted benzene ring. The substituent located at C–4 should be a hydroxyl group because the chemical shift value of C–4 moved to the low field at δ_C 156.8. The COSY experiment exhibited the correlation of δ_H 3.73 (1H, *m*, H–α) to δ_H 2.85 (2H, *dt*, *J* = 3.0, 8.0 Hz, H–β) proving C–α next to C–β. The HMBC correlation of H–β at δ_H 2.85 to C–1 at δ_C 130.8 showed the attachment of C–β to the aromatic ring. The above information demonstrated that **1** has a skeleton of phenylethanoid. The β–glucopyranosyl moiety was demonstrated by the presence of the anomeric proton at δ_H 4.31 (1H, *d*, *J* = 8.0 Hz, H–1') corresponding to carbon C–1' at δ_C 104.4 as well as the doublet of doublet at δ_H 3.88 (1H, *dd*, *J* = 12.0, 2.0 Hz, H–6') and δ_H 3.69 (1H, *dd*, *J* = 12.0, 5.0 Hz, H–6') corresponding to C–6' at δ_C 62.8. Additionally, ^{1}H NMR showed signals from 3.00 - 4.00 ppm corresponding to carbon signals from 60-80 ppm for carbons linking to hydroxyl groups. The HMBC correlation from the proton H–1' (δ_H 4.31) to C–α (δ_C 72.1) showed the attachment of the glucopyranosyl moiety at C–α of phenylethyl

moiety. By this information and the comparison of the published data [2], the compound **1** was identified as salidroside, known as β-(4-hydroxyphenyl)ethyl-*O*-β-D-glucopyranoside.

Table 1: 1D and 2D - NMR spectral data of **1** and **2** (CD_3OD)

No	Compound 1 (CD_3OD)			Salidroside (DMSO-d_6)[2]	Compound 2 (CD_3OD)			Darendoside B (CD_3OD)[4]
	δ_H (ppm) *J* (Hz)	δ_C (ppm)	HMBC ($^1H \rightarrow ^{13}C$)	δ_C (ppm)	δ_H (ppm) *J* (Hz)	δ_C (ppm)	HMBC ($^1H \rightarrow ^{13}C$)	δ_C (ppm)
1	-	130.8	-	128.9	-	132.9	-	132.8
2	7.08 (*d*, 8.5)	130.9	1, 3, 4, 6, β	130.1	6.75 (*d*, 2.0)	117.1	4, 6	112.8 (**)
3	6.72 (*d*, 8.5)	116.1	1, 2, 4, 5	115.3	-	147.5	-	147.4
4	-	156.8	-	155.9	-	147.4	-	147.2
5	6.72 (*d*, 8.5)	116.1	1, 3, 4, 6	115.3	6.83 (*d*, 8.5)	112.9	1, 3, 6	117.0 (**)
6	7.08 (*d*, 8.5)	130.9	1, 2, 4, 5, β	130.1	6.70 (*dd*, 8.5, 2.0)	121.2	2, 4	121.1
OCH_3					3.83(*s*)	56.5	3	56.5
α	4.05 (*m*) 3.73 (*m*)	72.1	1, 1', β	70.2	4.06 (*m*) 3.73 (*m*)	72.3	1, 1'	71.9
β	2.85 (*dt*, 3.0, 8.0)	36.4	1, α	35.1	2.82 (*dt*, 3.0, 7.5)	36.6	1, 2, 6, α	36.5
1'	4.31 (*d*, 8.0)	104.4	3', α	103.1	4.31 (*d*, 8.0)	104.2	α	104.1
2'	3.20 (*dd*, 9.0, 8.0)	75.1	1', 3'	73.7	3.28 (*)	75.6	1', 3'	75.5
3'	3.37 (*m*)	78.1	2', 4'	77.1	3.51 (*t*, 9.0)	84.5	2', 4'	84.4
4'	3.25 – 3.55	71.7	5'	70.4	3.37 (*t*, 9.0)	70.2	5', 6'	70.1
5'	3.25 – 3.55	77.9	4', 6'	77.2	3.33 (*)	77.9	3', 6'	77.7
6'a	3.88 (*dd*, 12.0, 2.0)	62.8	4', 5'	61.4	3.88 (*dd*, 12.0, 2.0)	62.7		62.6
6'b	3.69 (*dd*, 12.0, 5.0)				3.69(*)			
1''					5.17 (*d*, 1.5)	102.8	2'', 5'', 3'	102.6
2''					3.96 (*dd*, 3.0, 1.5)	72.4	4''	72.2
3''					3.71 (*)	72.0	4''	72.1
4''					3.42 (*t*, 9.5)	74.0	3'', 5''	73.9
5''					4.01 (*)	70.1		70.0
6''					1.27 (*d*, 6.0)	17.9		17.9
(*) Crowded signal, difficult to be analysed					(**) Interchangeable			

Compound **2** was isolated as amorphous powder, ESI-MS showed *m/z* 499.1742 $[M+Na]^+$ supported the molecular formula $C_{21}H_{32}O_{12}$. The ^1H spectrum (table 1) of **2**

revealed the presence of an ABX system [δ_H 6.83 (1H, *d*, 8.5, H–5), 6.75 (1H, *d*, 2.0, H–2) and 6.70 (1H, *dd*, 8.5, 2.0, H–6)] for a 1,3,4-trisubstituted benzene ring. The ^{1}H spectrum showed the presence of a methoxyl group because of δ_H 3.83 (3H, *s*). The HMBC correlation of H of methoxyl group (δ_H 3.83) to C–4 (δ_C 147.4) provided that the methoxyl group located at C–4. The chemical shift value C–3 moved to the low field at δ_C 147.5, so that the substituent at C–3 should be hydroxyl group. The COSY experiment exhibited the correlation of δ_H 3.73 (1H, *m*, H–α) to δ_H 2.82 (2H, *dt*, *J* = 3.0, 7.5 Hz, H–β) proving C–α next to C–β. The HMBC correlation of H–β at δ_H 2.82 to C–1 at δ_C 132.9 showed the attachment of C–β to the aromatic ring. The above information demonstrated that **2** is a skeleton of phenylethanoid. The β–glucopyranosyl moiety was demonstrated by the presence of the anomeric proton at δ_H 4.31 (1H, *d*, *J* = 8.0 Hz, H–1') corresponding to carbon C–1' at δ_C 104.2 as well as a doublet of doublet at δ_H 3.88 (1H, *dd*, *J* = 12.0, 2.0Hz, H–6'a) and δ_H 3.69 (1H, *m*, H–6'b) corresponding to C–6' at δ_C 62.7. The HMBC correlation from the proton H–1' (δ_H 4.31) to C–α (δ_C 72.3) showed the attachment of the glucopyranosyl moiety at C–α of phenylethyl moiety. The α–rhamnopyranosyl moiety was demonstrated by the presence of the anomeric proton at δ_H 5.17 (1H, *d*, *J* = 1.5 Hz, H–1'') corresponding to carbon C–1'' at δ_C 102.8 as well as a doublet at δ_H 1.27 (3H, *d*, *J* = 6.0 Hz, H–6'') corresponding to C–6'' at δ_C 17.9. The HMBC correlation from the proton H–1'' (δ_H 5.17) to C–3' (δ_C 84.5) showed the attachment of the rhamnopyranosyl moiety at C–3' of glucopyranosyl moiety. Based on this information and the comparison of the published data [3, 4], the compound **2** was identified as darendoside B, known as [β-(3-hydroxy-4-methoxyphenyl)ethyl]-(3'-*O*-α-L-rhamnopyranosyl)-*O*-β-D-glucopyranoside.

Compound **3** was isolated as amorphous powder, ESI-MS showed *m/z* 647.1972 $[M+Na]^+$ supported the molecular formula $C_{29}H_{36}O_{15}$. The ^{1}H spectrum (Table 2) of **3** revealed the presence of an ABX system [δ_H 6.71 (1H, *d*, 8.0, H–5), 6.72 (1H, *d*, 2.0, H–2) and 6.59 (1H, *dd*, 8.0, 2.0, H–6)] for a 1,3,4-trisubstituted benzene ring. The chemical shift values of C-3 and C–4 moved to the low field at δ_C 146.1 and 144.6, respectively, so that the substituents at C–3 and C–4 should be hydroxyl groups. The COSY experiment exhibited the correlation of δ_H 3.75 (1H, *m*, H–α) to δ_H 2.81 (2H, *dt*, *J* = 3.0, 7.5Hz, H–β) proving C–α next to C–β. The HMBC correlation of H–β at δ_H 2.81 to C–1 at δ_C 131.5 showed the attachment of C–β to the aromatic ring. The above information demonstrated that **3** has a skeleton of 3,4-dihydroxyphenylethyl moiety. The ^{1}H spectrum showed the presence of another ABX system [δ_H 7.07 (1H, *d*, 2.0, H–2'), 6.98 (1H, *dd*, 8.0, 2.0, H–6') and 6.80 (1H, *d*, 8.5, H–5')] for a 1,3,4-trisubstituted benzene ring and two *trans*-olefinic protons [δ_H 7.62 (1H, *d*, 16.0, H–β') and 6.29 (1H, *d*, 16.0, H–α')]. The chemical shift values of C–3' and C–4' moved to the low field at δ_C 146.8 and 149.1, respectively, so that the substituents at C–3' and C–4' should be hydroxyl groups. The HMBC correlation of H–β' at δ_H 7.62 to C–2' at δ_C 115.3, C–6' at δ_C 123.2 and >C=O at δ_C 168.3 proved the caffeoyl moiety. The β–glucopyranosyl moiety was demonstrated by the presence of the anomeric proton at δ_H 4.39 (1H, *d*, *J*=8.0Hz, H–1'') corresponding to carbon C–1'' at δ_C 104.2. The HMBC correlation from the proton H–1'' (δ_H 4.39) to C–α (δ_C 72.2) showed the attachment of the glucopyranosyl moiety at C–α of phenylethyl moiety. The rhamnopyranosyl moiety was demonstrated by the presence of the anomeric proton at δ_H 5.21 (1H, *d*, *J* = 1.0 Hz, H–1''') corresponding to carbon C–1''' at δ_C 103.0 as well as a doublet at δ_H 1.11 (3H, *d*, *J* = 6.0 Hz, H–6''') corresponding to C–6''' at δ_C 18.4. The HMBC correlation from the proton H–1''' (δ_H 5.21) to C–3'' (δ_C 81.6) showed the attachment of the

rhamnopyranosyl moiety at C–3'' of glucopyranosyl moiety. The HMBC correlation from H–4'' (δ_H 4.94) of glucopyranosyl moiety to >C=O (δ_C 168.3) indicated the location of the caffeoyl moiety to glucopyranosyl moiety at C–4''. Based on this information and the comparison of the published data [5], the compound **(3)** was identified as verbascoside, known as acteoside or [β-(3,4-dihydroxyphenyl)ethyl]-(3''-*O*-α-L-rhamnopyranosyl)-(4''-*O*-caffeoyl)-*O*-β-D-glucopyranoside.

Compound **4** was isolated as amorphous powder, ESI-MS showed *m/z* 647.1985 $[M+Na]^+$ supported the molecular formula $C_{29}H_{36}O_{15}$. The NMR spectra of compound **4** were similar to those of compound **3**. The ^1H spectrum (Table 2) of **4** revealed the presence of two sets of ABX systems [δ_H 6.69 (1H, *d*, 2.0, H–2), 6.66 (1H, *d*, 8.0, H–5) and 6.55 (1H, *dd*, 8.0, 2.0, H–6)] for the 3,4-dihydroxyphenylethyl moiety and [δ_H 7.05 (1H, *d*, 2.0, H–2'), 6.91 (1H, *dd*, 8.0, 2.0, H–6') and 6.80 (1H, *d*, 8.0, H–5')] for the caffeoyl moiety, two *trans*-olefinic protons [δ_H 7.58 (1H, *d*, 16.0, H–β') and 6.30 (1H, *d*, 16.0, H–α')], together with two anomeric protons at δ_H 4.35 (1H, *d*, 8.0, H–1'') for β-D-glucose and δ_H 5.20 (1H, *d*, 1.5, H–1''') for α–L–rhamnose. In the HMBC experiment, the correlations of H–1'' (δ_H 4.35) to C–α (δ_C 72.4), H–1''' (δ_H 5.20) to C–3'' (δ_C 84.0), together with chemical shift values of these protons and carbons revealed the connection among the 3,4-hydroxyphenylethyl and the two sugar moieties. So **4** is a positional isomer of **3** with the differently positional connectivity between the caffeoyl and the glucopyranosyl moieties. The HMBC correlation from H–6'' (δ_H 4.52) to >C=O (δ_C 169.1) indicated the location of the caffeoyl moiety to the glucopyranosyl moiety at C–6''. Based on this information and the comparison of the published data [6, 7], the compound **4** was identified as isoverbascoside, known as isoacteoside or [β-(3,4-dihydroxyphenyl)ethyl]-(3''-*O*-α-L-rhamnopyranosyl)-(6''-*O*-caffeoyl)-*O*-β-D-glucopyranoside.

Table 2: 1D and 2D - NMR spectral data of **3** and **4** (CD_3OD)

No	Compound 3 (CD_3OD)			Verbascoside (CD_3OD)[5]	Compound 4 (CD_3OD)			Isoverbascoside (CD_3OD) [7]
	δ_H (ppm) *J* (Hz)	δ_C (ppm)	HMBC ($^1H\rightarrow^{13}C$)	δ_C (ppm)	δ_H (ppm) *J* (Hz)	δ_C (ppm)	HMBC ($^1H\rightarrow^{13}C$)	δ_C (ppm)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	-	131.5	-	131.6	-	131.4	-	131.4
2	6.72 (*d*, 2.0)	117.1	4, 6	117.3	6.69 (*d*, 2.0)	117.0	3, 6	117.0
3	-	146.1	-	146.9	-	146.1	-	144.5 (**)
4	-	144.6	-	144.8	-	144.6	-	146.0 (**)
5	6.71 (*d*, 8.0)	116.3	1, 3	116.6	6.66 (*d*, 8.0)	116.3	1, 4	116.3
6	6.59 (*dd*, 8.0, 2.0)	121.3	2, 4	121.4	6.55 (*dd*, 8.0, 2.0)	121.3	2, 3	121.3
α	4.07 (*m*) 3.75 (*m*)	72.2	1, 1'' -	72.2	3.91 (*m*) 3.71-3.76 (*)	72.4	1, 1', β	72.4
β	2.81 (*dt*, 3.0, 7.5)	36.5	α, 1, 2, 6	36.7	2.79 (*dt*, 1.5, 6.5)	36.6	1, 2, 6, α	36.5
1'	-	127.7	-	127.8	-	127.7	-	127.6

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
2’	7.07 (*d*, 2.0)	115.3	3’, 4’, 6’	115.4	7.05 (*d*, 2.0)	115.1	1’, 3’, 6’	115.1
3’	-	146.8	-	146.2	-	146.7	-	149.5 (**)
4’	-	149.1	-	149.9	-	149.5	-	146.6 (**)
5’	6.80 (*d*, 8.5)	116.5	1’, 3’, 4’	116.5	6.80 (*d*, 8.0)	116.5	1’, 3’, 4’	116.5
6’	6.98 (*dd*, 8.0, 2.0)	123.2	β’, 2’, 4’	123.4	6.91 (*dd*, 8.0, 2.0)	123.1	2’, 3’, β’	123.1
α’	6.29 (*d*, 16.0)	114.7	1’, C=O	114.8	6.30 (*d*, 16.0)	114.8	1’, C=O	114.8
β’	7.62 (*d*, 16.0)	148.0	α’, 2’, 6’, C=O	148.2	7.58 (*d*, 16.0)	147.2	α’, 6’, C=O	147.2
C=O	-	168.3	-	168.5	-	169.1	-	169.1
1’’	4.39 (*d*, 8.0)	104.2	α	104.3	4.35 (*d*, 8.0)	104.3	α	104.2
2’’	3.41 (*dd*, 9.0, 8.0)	76.2	1’’, 3’’	76.3	3.33-3.40 (*)	75.4	1’’, 3’’	75.2
3’’	3.83 (*t*, 9.0)	81.6	1’’’, 2’’, 4’’	81.8	3.52-3.59 (*)	84.0	2’’, 4’’, 1’’’	83.9
4’’	4.94 (*t*, 9.5)	70.4	3’’, 5’’, C=O	70.5	3.40-3.50 (*)	70.0	5’’, 6’’	70.0
5’’	3.50-3.66 (*)	76.0	1’’	76.1	3.52-3.59 (*)	75.6		75.5
6’’a 6’’b	3.50-3.66 (*) 3.50-3.66 (*)	62.4	4’’	62.5	4.52 (*dd*, 12.0, 2.0) 4.38 (*dd*, 12.0, 6.0)	64.6	C=O	64.6
1’’’	5.21 (*d*, 1.0)	103.0	3’’, 2’’’, 5’’’	103.2	5.20 (*d*, 1.5)	102.7	3’’, 2’’’, 5’’’	102.6
2’’’	3.94 (*m*)	72.3	3’’’, 4’’’	72.5	3.95-4.04 (*)	72.2	3’’’	72.2
3’’’	3.50-3.66	72.0	4’’’	72.4	3.71-3.76	72.3	4’’’	72.2
4’’’	3.31 (*m*)	73.8	3’’’, 5’’’	73.9	3.40-3.50	74.0	3’’’, 5’’’	73.9
5’’’	3.50-3.66	70.6		70.7	3.95-4.04	70.4	4’’’	70.3
6’’’	1.11 (*d*, 6.0)	18.4	4’’’, 5’’’	18.6	1.29 (*d*, 6.0)	17.8	4’’’, 5’’’	17.8
(*) Crowded signal, difficult to be analysed						(**) Interchangeable		

IV - CONCLUSION

From the leaves of *Pseuderanthemum carruthersii (*Seem.*)* Guill. Var. *atropurpureum (*Bull.*)* Fosb. collected in June 2008 in Binh Phuoc province, four phenylethanoids were isolated and elucidated as salidroside **(1)**, darendoside B **(2)**, verbascoside **(3)** and isoverbascoside **(4)**. These substances were isolated for the first time from this genus. Further studies are conducting on this plant.

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BỐN PHENYLETHANOID ĐƯỢC CÔ LẬP TỪ CÂY XUÂN HOA ĐỎ, *PSEUDERANTHEMUM CARRUTHERSII* (SEEM.) GUILL. VAR. *ATROPURPUREUM* (BULL.) FOSB.

Tóm tắt:

Từ bột khô lá cây Xuân hoa đỏ, *Pseuderanthemum carruthersii* (Seem.) Guill. var. *atropurpureum* (Bull.) Fosb., họ Ô rô (Acanthaceae), đã cô lập được bốn phenylethanoid, đó là: salidroside **(1)**, darendoside B **(2)**, verbascoside **(3)** and isoverbascoside **(4)**. Cấu trúc của các hợp chất này được xác định bằng các phương pháp phổ nghiệm và so sánh với tài liệu tham khảo. Các hợp chất này được cô lập lần đầu tiên trong chi *Pseuderanthemum*.