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Lignan and megastigmane glycosides from *Sauropus androgynus*

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Dedicated to Professor Vichiara Jirawongse on the occasion of his 85th birthday

Abstract

A lignan diglycoside, (−)-isolariciresinol 3α-*O*-β-apiofuranosyl-(1→2)-*O*-β-glucopyranoside, and a megastigmane glucoside, sauroposide, were isolated from the aerial part of *Sauropus androgynus* together with (+)-isolariciresinol 3α-*O*-β-glucopyranoside, (−)-isolariciresinol 3α-*O*-β-glucopyranoside, (+)-syringaresinol di-*O*-β-glucopyranoside, guanosine and corchoionoside C. The structural elucidations were bases on analyses of physical and spectroscopic data.
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Keywords: *Sauropus androgynus*; Euphorbiaceae; Lignan glycoside; Megastigmane glycoside; Isolariciresinol glycoside; Sauroposide

1. Introduction

Sauropus androgynus Merr. (Euphorbiaceae, Thai name: Pak-Waan-Bann) is a shrub widely distributed in South and Southeast Asia. The leaves of this plant are a common nutritious vegetable in Thailand (Padmavathi and Rao, 1990). In Thai traditional medicine, the roots are used as an anti-fever preparation, as an antidote for food poisoning, and an antiseptic agent. In preliminary investigations, the leaves were found to contain the alkaloid papaverine (Bender and Ismail, 1973, 1975). In the course of continuing studies on Thai Medicinal plants, the constituents of this plant were investigated, following plant collection in Phetcha-Buri Province, Thailand. The present study deals with the isolation and structural determination of seven compounds (**1**–**7**) including a new lignan diglycoside (**3**), a new megastigmane glucoside sauroposide (**7**), along with five known compounds, three lignan glycosides (**1**, **2**, **4**), a purine nucleoside (**5**) and a megastigmane glucoside, corchoionoside C (**6**), from the aerial part of this plant.

S. androgynus was also introduced to Taiwan, and became a popular body-weight-reducing vegetable. The leaves and stems are homogenized and consumed as a vegetable juice (Chen et al., 1996). In 1995, several cases of apparent poisoning related to consumption of the uncooked extract were reported (Lai et al., 1996).

2. Results and discussion

The methanolic extract of the aerial part of *S. androgynus* was partitioned between Et_2O and H_2O. The H_2O soluble fraction was purified by using highly porous synthetic resin, silica gel, ODS, and prep. HPLC-ODS column chromatographies to give seven compounds. Five were identified as known compounds, (+)-isolariciresinol 3α-*O*-β-glucopyranoside (**1**), (−)-isolariciresinol 3α-*O*-β-glucopyranoside (**2**) (Achenbach et al., 1992), (+)-syringaresinol di-*O*-β-glucopyranoside

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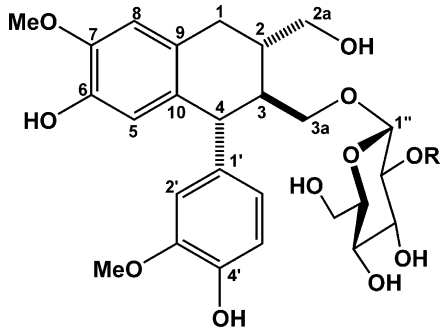

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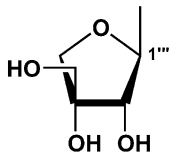

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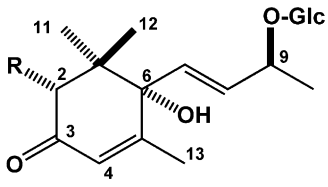
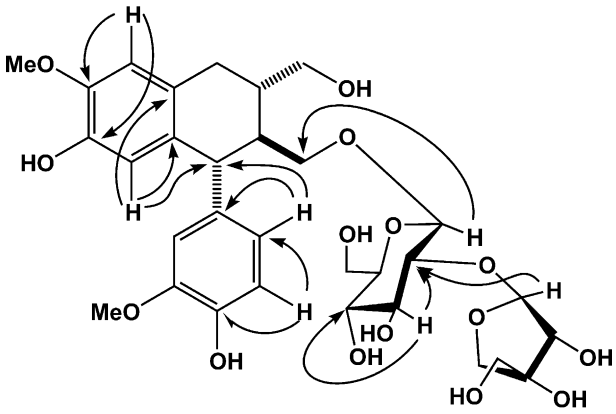


R

6 H

7 OH

Fig. 1. The significant HMBC correlations of compound **3**.



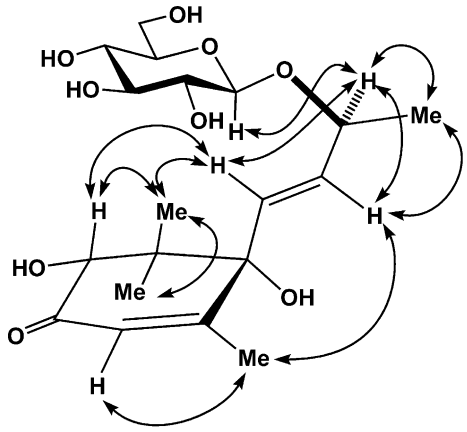


Fig. 2. The NOESY correlations of sauroposide (**7**).

(**4**, liriodendrin) (Kobayashi et al., 1985), guanosine (**5**) (Jones et al., 1970) and corchoionoside C (**6**) (Yoshikawa et al., 1997) by comparison of physical data with literature values and from spectroscopic evidence.

The molecular formula of compound **3** was determined as $C_{31}H_{42}O_{15}$ by HR-FAB mass spectrometry Fig. 1. The ^{1}H and ^{13}C NMR spectra of **3** were in part very similar to those of **2**. In addition, the signals of one β-apiofuranosyl unit were observed. Enzymatic hydrolysis of **3** with crude hesperidinase (Kohda and Tanaka, 1975) gave (−)-isolariciresinol, identified by analyses of both physical and spectral data (Urones et al., 1987). Comparison of the ^{13}C NMR spectral data of **3** with those of **2** revealed the downfield shift of C-2″ (5.2 ppm) and the upfield shifts of C-1″ (0.7 ppm) and C-3″ (0.3 ppm) of the glucosyl moiety, indicating that an additional sugar was located at C-2″ of the glucosyl moiety. The chemical shifts of the sugar moiety coincided with those reported of an β-apiofuranosyl-(1→2)-β-glucopyranoyl unit (Zhong et al., 1998). Moreover, the HMBC spectrum provided confirmation of the structure of compound **3** as (−)-isolariciresinol 3α-*O*-β-apiofuranosyl-(1-2)-*O*-β-glucopyranoside.

The molecular formula of compound **7** was determined as $C_{19}H_{30}O_9$ by HR-FAB mass spectrometry. The ^{13}C NMR spectral data showed the presence of a β-glucopyranosyl unit, and the remaining 13 carbon signals suggested the presence of a megastigmane skeleton. These signals were assigned to four methyls, five methines, and four quaternary carbons. The chemical shifts were closely related to those of corchoionoside C (**6**). However, the HR-FAB mass spectrometry revealed one more additional oxygen atom than **6**, and the chemical shifts of **7** at C-1, C-2 were significantly changed to δ 49.5 and δ 78.7, respectively, indicating the presence of an hydroxyl groups at C-2. The relative configuration of this hydroxyl group was examined by phase-sensitive NOESY as shown in Fig. 2., clearly demonstrating the position of the hydroxyl group at C-2. The absolute configuration of C-6 was determined to be *S* from the CD spectrum, in which extreme values were observed at $\Delta\varepsilon$ (nm) $+14.8^{\circ}$ (243) and -1.6° (317) (Otsuka et al., 1995); and in turn, that of C-2 to be *R*. The remaining chiral center at C-9 was assigned as S-configuration based on the ^{13}C NMR chemical shift of C-9 (δ 74.7) and C-10 (δ 22.2) being similar to those of **6**, which was

known to have the *S*-configuration (Yoshikawa et al., 1997; Pabst et al., 1992). Consequently, the structure of compound **7** was established as (2*R*, 6*S*, 9*S*, 7*E*)-trihydroxymegastigmane-4, 7-dien-3-one 9-*O*-β-glucopyranoside, namely sauroposide.

3. Experimental

3.1. General

NMR, MS, HPLC and CC were performed as described (Kanchanapoom et al., 2002). The CD and UV spectra were recorded on JASCO J-702 spectropolarimeter and a JASCO V-520 spectrophotometer, respectively. The solvent systems were: (I) EtOAc–MeOH (9:1), (II) EtOAc–MeOH–H_2O (40:10:1), (III) EtOAc–MeOH–H_2O (70:30:3), (IV) EtOAc–MeOH–H_2O (6:4:1), (V) 10–50% aq. MeOH, and (VI) 15% aq. MeCN. The spraying reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. Plant material

S. androgynus Merr. was collected in September 2001 from Phetcha-Buri Province. The identification was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. A voucher sample (KKU 0042) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried aerial part of *S. androgynus* was extracted with hot MeOH (10 l×4, continuous). The MeOH extract was concentrated to dryness (256.0 g) and partitioned between Et_2O and H_2O. The aqueous layer was applied to a column of highly porous copolymer resin of styrene and divinylbenzene and eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (25.2 g) was subjected to a silica gel column (solvent systems I, II, III and IV), providing seven fractions. Fraction 2 (800 mg) was purified by a column of ODS (solvent system IV) to afford compounds **6** (31 mg) and **7** (51 mg). Fraction 3 (1.7 g) was applied to a column of ODS (solvent system IV) to give 14 fractions. Fraction 3-12 was purified by prep. HPLC-ODS (solvent system V) to afford compounds **1** (8 mg) and **2** (12 mg). Fraction 4 (2.5 g) was similarly purified by an ODS column, then followed by prep. HPLC-ODS to provide compound **3** (58 mg). Fraction 5 (1.5 g) was separated by using a column of ODS (solvent system IV) to give crystal of compound **5** (1.1 g) from fraction 5-2. Fraction 6 was further separated in a similar manner to fraction 3 to give compound **4** (20 mg).

3.4. (−)-Isolariciresinol 3a-O-β-apiofuranosyl-(1→2)-O-β-glucopyranoside (3)

Amorphous powder, $[\alpha]_D^{28}$ −48.0° (MeOH, *c* 1.71); UV λ_{max}^{MeOH} nm (log ε): 282 (3.74), 215 (4.15); ^1H and ^{13}C NMR (CD_3OD) spectra: Table 1; CD (MeOH, *c* 4.12×10^{-5} M) Δε (λ nm): −4.8° (238), −2.6° (275), +3.3° (292); negative HR-FAB-MS, *m*/*z*: 653.2491 ($C_{31}H_{41}O_{15}$ required 653.2445).

3.5. Enzymatic hydrolysis of compound 3

A sample of compound **3** (25 mg) was dissolved in 0.5 ml MeOH. A solution of crude herperidinase (100 mg in 20 ml of H_2O) was then added. After stirring at 37 °C for 5 days, the mixture was extracted with EtOAc, and concentrated to dryness, affording (−)-isolariciresinol (11 mg). The structure was identified by ^1H and ^{13}C NMR spectral analyses and comparison of physical data with literature values (Urones et al., 1987).

Table 1
NMR spectral data of compound **3** (100 MHz for ^{13}C NMR and 400 MHz for ^1H NMR, CD_3OD)

No.	C	H
1	33.6	2.70 (1H, *dd*, *J* = 15.1, 4.6 Hz)
		2.79 (1H, *dd*, *J* = 15.1, 10.3 Hz)
2	40.8	1.96 (1H, *m*)
2a	65.8	3.50[a] (1H) 3.67[a] (1H)
3	45.7	1.89 (1H, *m*)
3a	70.6	3.50[a] (1H) 3.78[a] (1H)
4	48.3	3.72[a] (1H)
5	117.4	6.17 (1H, *s*)
6	145.8	
7	147.1	
8	112.4	6.60 (1H, *s*)
9	129.2	
10	133.8	
1′	138.7	
2′	114.0	6.64 (1H, *d*, *J* = 2.0 Hz)
3′	148.9	
4′	145.9	
5′	116.0	6.70 (1H, *d*, *J* = 8.1 Hz)
6′	123.4	6.60 (1H, *dd*, *J* = 8.1, 2.0 Hz)
7-OMe	56.4	3.75 (3H, *s*)
3′OMe	56.6	3.73 (3H, *s*)
Glc-1″	103.1	4.14 (1H, *d*, *J* = 7.8 Hz)
2″	80.2	3.27 (1H, *dd*, *J* = 9.0, 7.8 Hz)
3″	77.6	3.39 (1H, *dd*, *J* = 9.2, 9.0 Hz)
4″	71.6	3.28 (1H, *dd*, *J* = 9.5, 9.2 Hz)
5″	78.2	3.10 (1H, *ddd*, *J* = 9.5, 5.1, 2.4 Hz)
6″	62.6	3.63 (1H, *d*, *J* = 12.0, 5.1 Hz)
		3.78 (1H, *d*, *J* = 12.0, 2.4 Hz)
Api-1‴	111.0	5.28 (1H, *d*, *J* = 2.2 Hz)
2‴	77.9	3.90 (1H, *d*, *J* = 2.2 Hz)
3‴	80.4	
4‴	75.1	3.65 (1H, *d*, *J* = 9.5 Hz)
		3.90 (1H, *d*, *J* = 9.5 Hz)
5‴	65.7	3.49 (2H, *s*)

[a] Chemical shift obtained approximately from HSQC.

Table 2
NMR spectral data of compound **7** (100 MHz for ^{13}C NMR and 400 MHz for ^{1}H NMR, CD_3OD)

No.	C	H
1	49.5	
2	78.7	4.17 (1H, *s*)
3	200.5	
4	125.4	5.91 (1H, *d*, *J* = 1.2 Hz)
5	166.2	
6	81.0	
7	133.4	5.89 (1H, *d*, *J* = 16.1 Hz)
8	134.0	5.65 (1H, *dd*, *J* = 16.1, 7.1 Hz)
9	74.7	4.82 (1H, *dq*, *J* = 7.1, 6.6 Hz)
10	22.2	1.24 (3H, *d*, *J* = 6.6 Hz)
11	16.2	0.82 (3H, *s*)
12	20.9	1.05 (3H, *s*)
13	19.4	1.88 (3H, *d*, *J* = 1.2 Hz)
Glc-1″	101.2	4.23 (1H, *d*, *J* = 7.8 Hz)
2″	74.9	3.15 (1H, *dd*, *J* = 8.8, 7.8 Hz)
3″	78.2	3.27 (1H, *dd*, *J* = 8.8, 8.5 Hz)
4″	71.6	3.21 (1H, *dd*, *J* = 9.3, 8.5 Hz)
5″	78.1	3.11 (1H, *m*)
6″	62.8	3.59 (1H, *d*, *J* = 12.0, 6.1 Hz)
		3.81 (1H, *d*, *J* = 12.0, 2.2 Hz)

3.6. Sauroposide (7)

Amorphous powder, $[\alpha]_D^{31}$ −37.9° (MeOH, *c* 1.11); UV λ_{max}^{MeOH} nm (log ε): 231 (3.94); ^{1}H and ^{13}C NMR (CD_3OD) spectra: Table 2; CD (MeOH, *c* 5.54×10^{-5} M) $\Delta\varepsilon$ (λ nm): +14.8° (243), −1.6° (317); negative HR-FAB-MS, *m*/*z*: 401.1783 ($C_{19}H_{29}O_9$ required 401.1811).

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