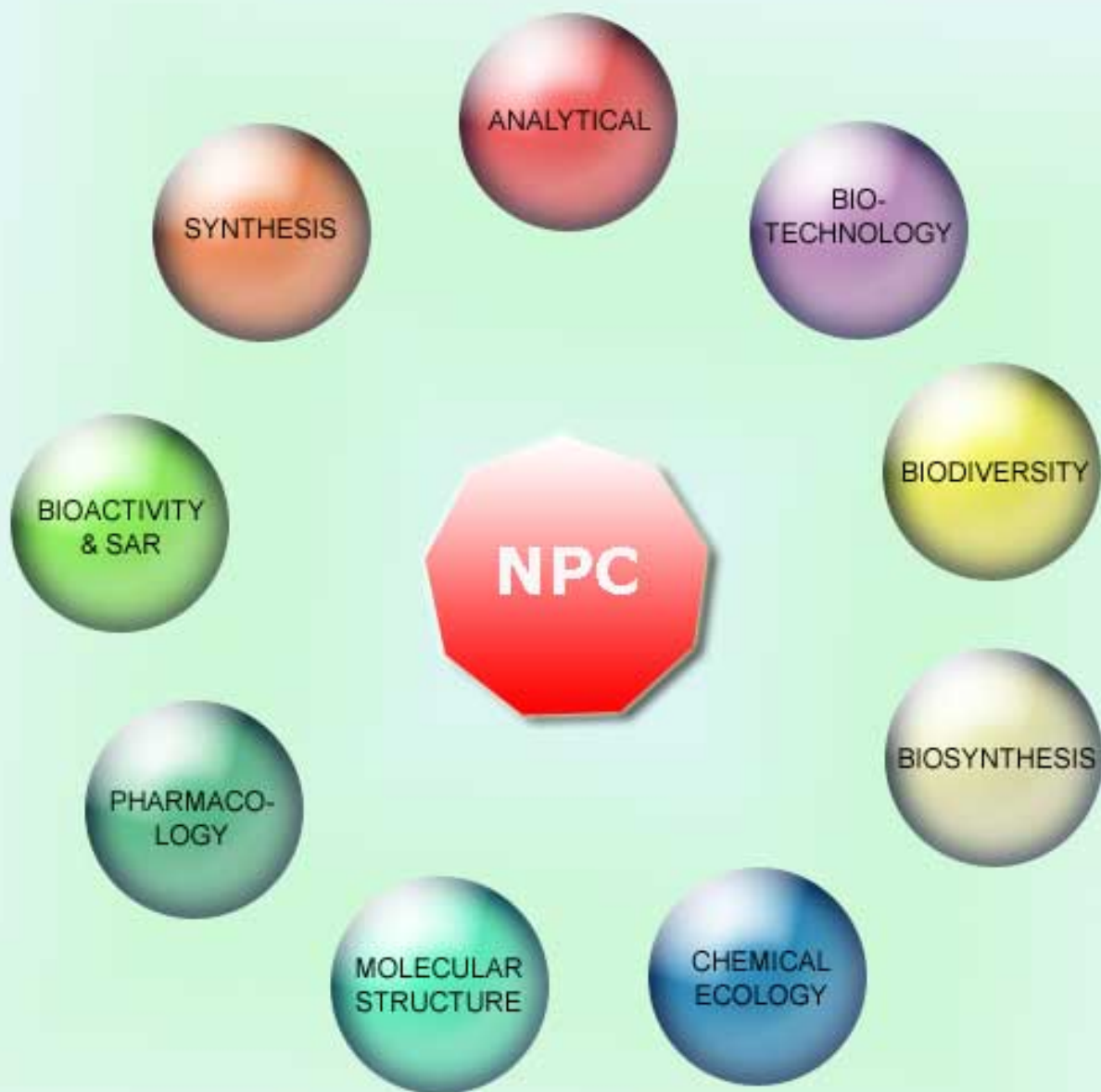


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## Two New Megastigmane Sulphonoglucosides from *Mallotus anisopodus*

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Phytochemical study of the methanol extract of *Mallotus anisopodus* led to the isolation of two new megastigmane sulphonoglucosides, namely anisoposides A (**1**) and B (**2**), along with junipetriolide A (**3**), bergenin (**4**),  $\alpha$ -tocopherol, and *N*<sup>1</sup>-methyl-2-pyridone-5-carboxamide. Their structures were deduced by spectroscopic and spectrometric methods including 1D-, 2D-NMR, ESI-MS, and HRESI-MS.

**Keywords:** *Mallotus anisopodus*, Euphobiaceae, megastigmane sulphonoglucosides, anisoposides A and B.

*Mallotus* is a large genus of the spurge family, Euphorbiaceae. About 140 species are found in East and South-east Asia and from Indo-Malaysia to New Caledonia and Fiji, northern and eastern Australia. Only two species are found in tropical Africa and Madagascar. Thirty-seven species have been recorded in Vietnam, of which seven are endemic.

*Mallotus* species usually grow in rain, evergreen, primary or secondary forests at an altitude less than 1000 m. *M. anisopodus* (Gagnep.) Airy-Shaw is distributed mainly in Vietnam, Laos, and Cambodia. In Vietnam, the plant grows sparsely in semi-deciduous forests, along stream-sides, and in peat-soils [1]. In the course of our continuing studies of the chemical components of *Mallotus* species, we have isolated and identified new megastigmane sulphonoglucosides, namely anisoposides A (**1**) and B (**2**), along with junipetriolide A, bergenin,  $\alpha$ -tocopherol, and *N*<sup>1</sup>-methyl-2-pyridone-5-carboxamide from the methanol extract of *M. anisopodus*.

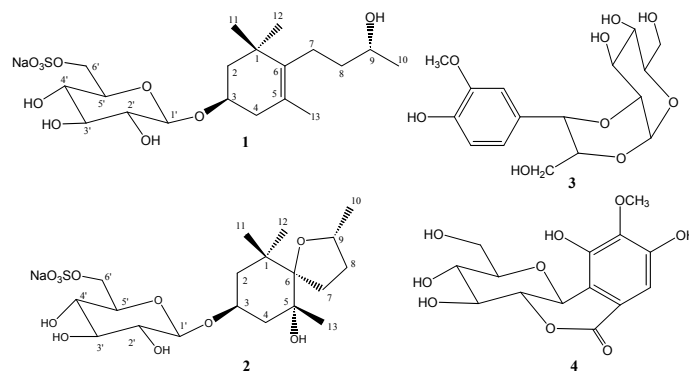


Figure 1: The structures of compounds 1 – 4.

Compound **1** was obtained as a white amorphous powder. Spectroscopic analyses revealed that **1** has the common megastigmane-type aglycone, megastigman-5-en-3,9-diol [2], which contained characteristic signals of one fully substituted double bond at  $\delta_C$  124.98 (C-5)/138.58 (C-6), two oxymethines at  $\delta_C$  73.50 (C-3) and 69.18 (C-9), three tertiary and one secondary methyls at  $\delta_C$  28.81 (C-11), 30.24 (C-12), 19.99 (C-13), and 23.21 (C-10),

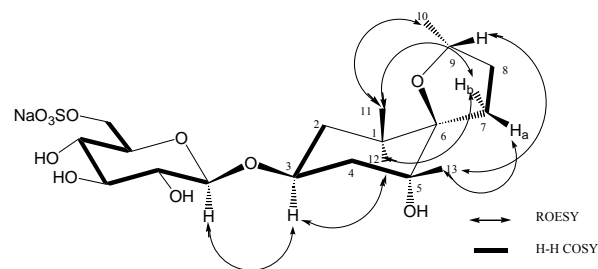
**Table 1:** The NMR spectral data of **1** and **2**<sup>#</sup>.

No.	<b>1</b>		<b>2</b>	
	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult. <i>J</i> in Hz)	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult. <i>J</i> in Hz)
1	38.75	-	40.20	-
2	47.47	1.50 (dd, 12, 4) 1.87 (t, 12)	44.77	( $\alpha$ ) 1.57 (dd, 12, 4) ( $\beta$ ) 1.65 (t, 12)
3	73.50	4.04 (tt, 12, 4)	73.69	4.14 (tt, 12, 4)
4	39.76	2.34 (dd, 16, 4.5) 2.02 (dd, 16, 9.5)	43.68	( $\alpha$ ) 1.97 (br d, 13) ( $\beta$ ) 1.77 (dd, 13, 12)
5	124.98	-	78.65	-
6	138.58	-	91.00	-
7	25.52	2.22 (m) 1.94 (m)	27.99	H <sub>a</sub> 2.12 (dt, 14, 10) H <sub>b</sub> 1.93 (m)
8	40.71	2.00 (m) 1.50 (m)	36.32	2.05 (m) 1.48 (m)
9	69.18	3.72 (dd, 12.5, 6)	77.90	4.09 (m)
10	23.21	1.19 (d, 6)	21.14	1.21 (d, 6)
11	28.81	1.07 (s)	28.94	0.91 (s)
12	30.24	1.07 (s)	26.22	1.22 (s)
13	19.99	1.66 (s)	27.88	1.20 (s)
1'	102.43	4.44 (d, 8)	102.55	4.40 (d, 8)
2'	75.13	3.18 (dd, 8, 9)	74.97	3.16 (dd, 8, 9)
3'	77.78	3.38 (t, 9)	77.65	3.39 (t, 9)
4'	71.46	3.36 (t, 9)	71.31	3.40 (t, 9)
5'	75.91	3.50 (m)	75.83	3.47 (m)
6'	68.27	4.15 (dd, 12, 5) 4.33 (dd, 12, 2)	68.13	4.19 (dd, 12, 5) 4.30 (dd, 12, 2)

<sup>a</sup>recorded in CD<sub>3</sub>OD, <sup>b</sup>at 125 MHz, <sup>c</sup>at 500 MHz, <sup>#</sup>all the data were assigned by H-H COSY, HSQC, HMBC, and ROESY experiments.

respectively. In the <sup>13</sup>C NMR spectrum, the presence of a glucopyranosyl moiety was suggested by signals at  $\delta$  102.43 (CH, C-1'), 75.13 (CH, C-2'), 77.78 (CH, C-3'), 71.46 (CH, C-4'), 75.91 (CH, C-5'), and 68.27 (CH<sub>2</sub>, C-6'). Acid hydrolysis of **1** provided D-glucopyranose. All the carbons were assigned to relevant protons by HSQC and H-H COSY experiments and the results are summarized in Table 1. The large coupling constant of the anomeric proton H-1' at  $\delta$  4.44 (*J* = 8.0 Hz) confirmed the  $\beta$ -configuration of the sugar moiety.

The NMR spectral data and stereochemistry of **1** were in excellent agreement with those of linarioside A [2]. The differences between the two compounds were only observed in the signals of the oxymethylene chemical shifts, suggesting that **1** is an isomer of linarioside A at C-6'. In addition, the structure of **1** was further confirmed by the HMBC spectrum. The attached position of the sugar moiety at C-3 was identified by the HMBC cross peak from the anomeric proton ( $\delta$  4.44) to carbon C-3 ( $\delta$  73.50). Based on the above assigned structure, the molecular mass of the compound should be 374 (C<sub>19</sub>H<sub>34</sub>O<sub>7</sub>), which was the same as that of linarioside A. However, the ESI mass spectra at *m/z* 499 [M + Na]<sup>+</sup> (positive) and 453 [M - Na]<sup>-</sup> (negative) suggested the molecular mass to be 476. This difference of 102 mass units could, however, be accommodated by

**Figure 2:** Probable conformation and cross-peaks in ROESY and H-H COSY of **2**.

assuming that the compound is a sulfate sodium salt and suggested the molecular formula of C<sub>19</sub>H<sub>33</sub>O<sub>10</sub>SNa, which was confirmed by HRESI-MS (found *m/z* 499.1573 [M + Na]<sup>+</sup>, calcd C<sub>19</sub>H<sub>33</sub>O<sub>10</sub>SNa<sub>2</sub> for 499.1570). Careful analysis of the <sup>13</sup>C NMR chemical shift of carbon C-6' revealed that the methylene carbons (C-6') of the glucopyranose units of **1** and bacopaside I were similar, but experienced deshielding by 5.5 ppm compared with that of linarioside A (without sulfate group at C-6') [3], demonstrating that the sulfate group must be linked to C-6' of **1**. This was also supported by the observed up-field shift of the C-5' signal by 2.0 ppm ( $\gamma$  effect), as against that of linarioside A [2]. Based on all the above evidence, the structure of **1** was deduced as the sodium salt of (3*S*)-megastigman-5-en-3,9-diol 3-*O*-(6-*O*-sulphonyl- $\beta$ -D-glucopyranoside), a new compound named as anisoposide A.

Compound **2** was also isolated as a white amorphous powder. The molecular formula was suggested as C<sub>19</sub>H<sub>33</sub>O<sub>11</sub>SNa by ESI-MS at *m/z* 515 [M + Na]<sup>+</sup>, 493 [M + H]<sup>+</sup>, 413 [M - SO<sub>3</sub>Na + H + Na]<sup>+</sup> (positive), and 469 [M - Na]<sup>-</sup> (negative), which was confirmed by HRESI-MS (found *m/z* 515.1541 [M + Na]<sup>+</sup>, calcd C<sub>19</sub>H<sub>33</sub>O<sub>11</sub>SNa<sub>2</sub> for 515.1544). The NMR spectral data of **2** were similar to those of **1** (Table 1). The easily visible changes were the absence of the double bond and additional signals of two oxygenated quaternary carbons at  $\delta$  78.65 (C-5) and 91.00 (C-6) in **2** compared with those in **1**. These data suggested hydroxylated positions at C-5 and C-6 of **2**. Based on the molecular formula, the unsaturation number, and the strong downfield shift of the resonance at  $\delta$  77.90 (C-9) compared with the corresponding value for **1**, the aglycone of **2** was considered to form a bicyclic structure with the side chain retaining a methylfuran ring, which shared C-6 ( $\delta$  91.00) with a six-membered ring. The NMR data of **2** were compared with those of scorospiroside [4] and found to match well, except for the downfield shift of the resonances

of C-5' and C-6' of the glucopyranose, suggesting that **2** has a sulfate sodium salt group attached to C-6', as is the case of **1**. The structure of **2** was further confirmed by HSQC, HMBC, H-H COSY, and ROESY experiments. The HMBC cross peaks from the methyl protons H-11 (0.91)/H-12 (1.22) to carbon C-6 ( $\delta$  91.00), from H-13 (1.20) to C-5 ( $\delta$  78.65) and C-6, from H-10 ( $\delta$  1.21) to C-9 ( $\delta$  77.90) and C-8, from H-9 ( $\delta$  4.09) to C-6, as well as from H-1' ( $\delta$  4.40) to C-3 ( $\delta$  73.69) confirmed the structure of **1** as shown in Figure 1. Acid hydrolysis of **2** provided D-glucopyranose. Furthermore, the steric relation of the two rings was confirmed by cross peaks between H-3/H-12, H-10/H-11, H<sub>a</sub>-7/H-13, H<sub>b</sub>-7/H-12 and H-9/H-13 in the ROESY spectrum, and the D-glucopyranose was attached to 3 $\beta$ -OH with a  $\beta$ -linkage based on a coupling constant of H-1' ( $J = 8$  Hz) and ROESY cross peak between H-1' and H-3 $\alpha$  (Figure 2). From these data, the structure of **2** was deduced to be a new compound, the sodium salt of scorospiroside, and named as anisoposide B.

By detailed analyses of the 1D and 2D NMR spectral data and comparison of these with those reported, the other compounds were identified to be junipetriolide A (**3**) [5], bergenin (**4**) [6],  $\alpha$ -tocopherol [7], and *N*<sup>1</sup>-methyl-2-pyridone-5-carboxamide [8].

## Experimental

**General:** Optical rotations were determined on a JASCO DIP-1000 KUY polarimet. All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C), and chemical shifts ( $\delta$ ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. The ESI-MS was obtained on an AGILENT 1200 SERIES LC-MSD Trap spectrometer. The HRESI-MS were obtained using a JEOL JMS-AX505 HR-5890 series spectrometer. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0.040 - 0.063 mm, Merck) or YMC RP-18 resins (30 - 50  $\mu$ m, Fujisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F<sub>254</sub> (Merck 1.05715) or RP<sub>18</sub> F<sub>254s</sub> (Merck) plates. Compounds were visualized by spraying with 10% H<sub>2</sub>SO<sub>4</sub> aqueous and heating for 5 minutes.

**Plant materials:** The branches and leaves of *M. anisopodus* were collected in Dakrong, Quang Tri Province, Vietnam during July, 2007 and identified

by one of us, Prof. Nguyen Nghia Thin. A voucher specimen (No NNT-DR2007) was deposited at the Herbarium of the Institute.

**Extraction and isolation:** The air dried branches and leaves of *M. anisopodus* (5 kg) were powdered and extracted with hot MeOH (50°C) to give the methanol extract (200 g). This was suspended in water and partitioned in turn with chloroform and *n*-butanol to obtain corresponding extracts: chloroform (C, 50 g), *n*-butanol (B, 70 g), and water (W, 80 g). The W extract was submitted to a dianion HP-20 column ( $\phi = 10$  cm, L = 80 cm) using a stepwise gradient of MeOH in water (0%, 25%, 50%, 75% and 100%) to give five fraction W1 - W5. Fraction W4 (10 g) was separated using reverse phase CC ( $\phi = 5$  cm, L = 100 cm) eluting with acetone-water 7/1 to give ten subfractions W4A - W4J. From subfraction W4C (1 g), compounds **1** (20 mg) and **2** (25 mg) were purified as white amorphous powders by normal phase CC, eluting with chloroform-methanol-water (7/1/0.1), followed by normal and reverse phase preparative TLC. Similarly, **3** (15 mg) and **4** (10 mg) were obtained from the *n*-butanol extract as white amorphous powders. From the chloroform extract,  $\alpha$ -tocopherol (white crystals, 30 mg) and *N*<sup>1</sup>-methyl-2-pyridone-5-carboxamide (white amorphous powder, 7 mg) were isolated by combined chromatographic methods.

### Anisoposide A (**1**)

$[\alpha]_D^{20}$ : -49.5 (*c* 1.0, MeOH).

Rf: 0.35 (acetone-water, 7:1).

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): see Table 1.

<sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): see Table 1.

ESIMS: *m/z* 499 [M + Na]<sup>+</sup> (positive), 453 [M - Na]<sup>-</sup> (negative).

HRESI-MS: *m/z* 449.1573 [M + Na]<sup>+</sup> (calcd C<sub>19</sub>H<sub>33</sub>O<sub>10</sub>Na<sub>2</sub> for 449.1570).

20 mg ( $4 \times 10^{-4}$  % of dried weight).

### Anisoposide B (**2**)

$[\alpha]_D^{20}$ : -27.6 (*c* 1.0, MeOH).

Rf: 0.40 (acetone-water, 7:1).

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): Table 1.

<sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): Table 1.

ESI-MS: *m/z* 514.9 [M + Na]<sup>+</sup>, 493 [M + H]<sup>+</sup>, 413 [M - SO<sub>3</sub>Na + H + Na]<sup>+</sup> (positive), 469 [M - Na]<sup>-</sup> (negative).

HRESI-MS: *m/z* 515.1541 [M + Na]<sup>+</sup> (calcd C<sub>19</sub>H<sub>33</sub>O<sub>11</sub>SN<sub>2</sub> for 515.1544).

25 mg ( $5 \times 10^{-4}$  % of dried weight).

**Acid hydrolysis of 1 and 2:** Each compound (2.0 mg) was dissolved in 1.0 N HCl (dioxane/H<sub>2</sub>O, 1:1, v/v, 1.0 mL) and then heated to 80°C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven off under N<sub>2</sub> overnight. After extraction with CHCl<sub>3</sub>, the aqueous layer was concentrated to dryness using N<sub>2</sub> gas. The residue was dissolved in 0.1 mL of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. The reaction mixture was heated at 60°C for 2 h, before 0.1 mL of trimethylsilylimidazole solution was added, followed by heating at 60°C for 1.5 h. The dried product was partitioned with *n*-hexane and H<sub>2</sub>O (0.1 mL, each),

and the organic layer was analyzed by gas liquid chromatography (GC): Column: column SPB-1 (0.25 mm × 30 m); detector FID, column temp 210°C, injector temp 270°C, detector temp 300°C, carrier gas He (30 mL/min). The retention time of the persilylated sugar was 14.11 min. (The retention time of persilylated D-glucopyranose prepared under the same conditions was 14.11 min).

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