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GLUCOSIDES AND UREA DERIVATIVES FROM THE SEEDS OF *SCAPHIUM MACROPODUM* (MIQ.) BEUMÉE

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ABSTRACT

Five known compounds {carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic acid] dimethyl ester (**1**), (1'*R*,3'*S*,5'*R*,8'*S*,2*E*,4*E*)-dihydrophaseic acid 3'-*O*-*β*-D-glucopyranoside (**2**), 3-methylbutan-1-ol *β*-D-glucopyranoside (**3**), astragalin (**4**) and daucosterol (**5**) were isolated from the methanol extract of the seeds of *Scaphium macropodum* (Miq.) Beumée. The structures of the isolated compounds were elucidated by spectroscopic methods including NMR and MS, and also by comparison with the literature data. Compounds **1-3** were isolated from this plant for the first time.

Keywords: *Scaphium macropodum*, Sterculiaceae, glucosides, urea derivative

1. INTRODUCTION

Scaphium macropodum (Miq.) Beumée ex K. Heyne (Sterculiaceae) also known as malva nut (English) or uoi (Vietnam), is a large tree up to 30 m in height. They are widely distributed in tropical rainforests in Vietnam, Myanmar, Cambodia, Thailand, Malaysia and Indonesia. The seeds of this plant have been used in Vietnam for the treatment of heatdisease, dry cough, sore throat, toothache, sore redeyes and dysentery. It also has cooling properties [1]. The chemical constituents of the seeds and the stem bark of this plant such as alkaloids, cerebrosides, flavonoids, triterpenes, steroids and sesquiterpenes have been reported [2 – 4]. In this article, we report the isolation and structural elucidation of five known compounds **1–5** from the seeds of *S.macropodum* in Vietnam. Compounds **1-3** have not been previously isolated from this species.

2. EXPERIMENTAL

2.1. General experimental procedures

The ESI-MS was measured on Agilent 1260 series single quadrupole LC/MS systems. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, Billerica, MA, U.S.A.) using TMS as an internal standard. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) or YMC RP-18 resins (30 - 50 μm, Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3–5 minutes.

2.2. Plant material

The samples of the plant *Scaphium macropodum* (Miq.) Beumée were collected in May 2013 at Da Huoai, Lam Dong and identified by Dr. Nong Van Duy from the Tay Nguyen Institute for Scientific Research, VAST. A voucher specimen (No. TN3/309) was deposited at the Tay Nguyen Institute for Scientific Research, VAST.

2.3. Extraction and isolation

The air dried and powdered seeds of *S. macropodum* (4.5 kg) were extracted with methanol at 40 °C three times. Methanolic extracts were combined and evaporated under vacuum. This extract (500 g) was suspended in water and partitioned in turn with *n*-hexane, CH_2Cl_2, and EtOAc to provide the corresponding extracts: *n*-hexane (H, 80 g), CH_2Cl_2 (D, 7.5 g), EtOAc (E, 3.5 g) and a water layer.

The D extract was subjected to silica gel CC using stepwise elution of n-hexane/acetone (from 10:0 to 0:10) to afford 8 fractions D1 – D8, respectively. Fraction D6 was further separated by silica gel CC eluting with CH_2Cl_2/MeOH (40:1) to give compound **1** (10 mg).

The E extract was chromatographed over a column of Sephadex LH-20, eluting with MeOH/H_2O (1:1) to afford six fractions (E1-E6). Fraction E6 (155 mg) was further purified YMC RP-18 CC eluting with MeOH/H_2O (1:1) to give compounds **5** (12 mg) and **4** (6 mg). T 1. Positive ESI-MS *m/z* 387 $[M+H]^+$.

(1′*R*,3′*S*,5′*R*,8′*S*,2*E*,4*E*)-dihydrophaseic acid 3′-*O*-*β*-D-glucopyranoside (**2**): Colorless amophous powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) (see Table 2). Positive ESI-MS *m/z*467 $[M+Na]^+$.

3-methylbutan-1-ol *β*-D-glucopyranoside (**3**): Colorless needles; ^{1}H-NMR (500 MHz, CD_3OD) δ 3.96 (1H, m, H-1a), 3.59 (1H, m, H-1b), 1.54 (2H, dd, J = 7.0,J = 13.5 Hz, H-2), 1.77 (1H, m, H-3), 0.95 (3H, s, H-4), 0.93 (3H, s, H-5), 4.26 (1H, d, J = 8.0 Hz, H-1'), 3.18 (1H, dd, J= 8.0, J = 9.0 Hz, H-2'), 3.36 (1H, t, J = 8.5 Hz, H-3'), 3.31 (1H, m, H-4'), 3.27 (1H, m, H-5'), 3.69 (1H, dd, J = 5.5,J = 12.0 Hz, H-6'a), and 3.89 (1H, dd, J = 2.0,J = 12.0 Hz, H-6'b); ^{13}C-NMR (125 MHz, CD_3OD) δ 22.98 (C-5), 23.02 (C-4), 26.06 (C-3), 39.66 (C-2), 62.81 (C-6'), 69.28 (C-1), 71.71 (C-4'), 75.14 (C-2'), 77.92 (C-5'), 78.17 (C-3'), and 104.41 (C-1'). Positive ESI-MS *m/z* 251 $[M+H]^+$.

Astragaline (**4**): Yellow powder. ^{1}H-NMR (500 MHz, CD_3OD) δ 6.18 (1H, d, J = 2.0 Hz, H-6), 6.37 (1H, d, J = 2.0 Hz, H-8), 7.93 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.90 (2H, d, J = 8.5 Hz, H-3′, H-5′), 5.47 (1H, d, J = 7.5 Hz, H-1″), 4.32 (1H, m, H-2″), 3.90 (1H, m, Hz, H-3″), 3.82 (1H, m, H-4″), 3.48 (1H, m, H-5″), 3.80 (1H, dd, J = 2.5, J = 12.0 Hz, H_a-6″), and 3.55 (1H, dd, J = 4.5, J = 12.0 Hz, H_b-6″); ^{13}C-NMR (125MHz, CD_3OD) δ: 158.51 (C-2), 135.46 (C-3), 179.53 (C-4), 163.09 (C-5), 99.87 (C-6), 165.97 (C-7), 94.74 (C-8), 159.09 (C-9), 105.75 (C-

10), 122.80 (C-1'), 132.27 (C-2', C-6'), 116.07 (C-3', C-5'), 161.56 (C-4'), 104.07 (C-1''), 75.74 (C-2''), 78.05 (C-3''), 71.37 (C-4''), 78.43 (C-5''), and 62.64 (C-6''). Positive ESI-MS *m/z* 471 $[M+Na]^+$.

Daucosterol (**5**): White crystals.

3. RESULTS AND DISCUSSION

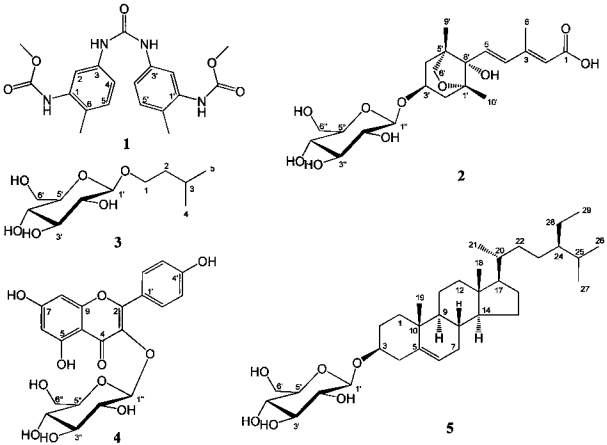

Figure 1. Structure of compounds **1** – **5.**

Compound **1** (Fig. 1) was obtained as white powder. Its molecular formula was established as $C_{19}H_{22}N_4O_5$ on the basis of a ion peak $[M + H]^+$ at *m/z* 387 in ESI-MS. The ^{13}C and DEPT NMR spectra showed only 10 resonance signals, including two amide carbonyls (δ_C 152.4 and 154.74), one methyl, one methoxy, three sp^2 methynes and three sp^2 quaternary carbons, which suggested that **1** might have a symmetrical structure. The ^1H NMR spectrum displayed signals for an ABX coupling system at δ_H7.50 (1H, br s, H-2, H-2'), 7.13 (1H, dd, $J = 2.0$, $J = 8.0$ Hz, H-4, H-4'), 7.05 (1H, d, $J = 8.0$ Hz, H-5, H-5') indicating the typical 1,3,4-trisubstituted benzene ring. The structure of benzene ring was further established by HMBC correlations (Fig. 2) between H-2 and C-3, C-4, C-6, and between H-4 and C-2, C-3, C-6, and between H-5 and C-1, C-3. Moreover, two singlet signals at δ_H 8.50 (1H) and 8.77 (1H) were assignable to two amine group, which were attached to C-3 and C-1 respectively, on the basis of the long-range correlations from proton 3-NH to C-2, C-3, C-4, carbon NHCONH, and from proton 1-NH to C-1, C-2, C-6. In the aliphatic region, a singlet at δ_H 2.12 (3H) and an other singlet at δ_H 3.65 (3H)

were assigned to methyl group 6-Me and methyl ester group respectively, due to the HMBC cross peaks from proton 6-Me to C-1, C-5, C-6 and from proton OMe to carbon NHCOO. On the basis of the above evidence, the structure of **1** was identified as {carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic acid] dimethyl ester by comparison of spectral data with those reported in the literature [5].

Table 1. The NMR data of compound **1.**

C	$^{\#}\delta_C$ [a]	δ_C [a,b]	δ_H [a,c]	C	$^{\#}\delta_C$ [a]	δ_C [a,b]	δ_H [a,c]
1, 1'	136.7	136.48	-	Me-6	17.3	17.07	2.12 (3H, s)
2, 2'	114.6	114.38	7.50 (1H, br s)	MeO	51.8	51.62	3.65 (3H, s)
3, 3'	137.9	137.72	-	NHCONH	152.6	152.43	-
4, 4'	115.0	114.79	7 13 (1H, dd, 2.0; 8.0)	NHCOO	154.9	154.74	-
5, 5'	130.5	130.29	7.05 (1H, d, 8.0)	NHCONH	-	-	8.50 (1H, s)
6, 6'	124.8	124.64	-	NHCOO	-	-	8.77 (1H, s)

[a] *recorded in DMSO-d_6,* [b] *125 MHz,* [c] *500 MHz,*

[#] *δ_C of {carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic acid] dimethyl ester[5]*

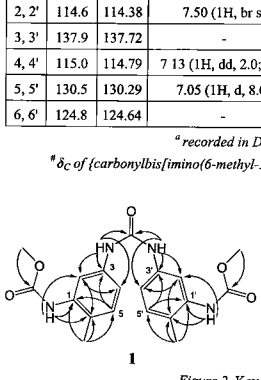

Figure 2. Key HMBC correlations of compounds **1-2.**

Compound **2** (Fig. 1) was isolated as colorless amorphous powder. A molecular formula of $C_{21}H_{32}O_{10}$ was determined for compound **2** on the basis of the observation of a molecular ion peak $[M + Na]^+$ at *m/z* 467 in ESI-MS. The ^1H and ^{13}C NMR spectra in combination with the HSQC spectrum of **2** exhibited signals for an acid carbonyl group at δ_C 177.20, a tertiary methyl group at δ_H 2.03 (3H, s, H-6)/δ_C 20.82, two *trans* olefinic signals at δ_H 7.88 (1H, d, J = 16.0 Hz, H-4)/δ_C 132.63 and δ_H 6.37 (1H, d, J = 16.0 Hz, H-5)/δ_C 132.02, one trisubstituted double bond signal at δ_H 5.85 (1H, br s)/δ_C 125.20 and δ_C 144.80, suggesting the presence of a 3-methyl-penta-2,4-dienoic acid moiety. This moiety was confirmed by the HMBC cross peaks (fig. 2) from H-2 to C-1, C-6, C-4, from H-6 to C-2, C-3, C-4 and from H-4 to C-5, C-2, C-6. In the ^1H and ^{13}C NMR spectra, the signals of two tertiary methyl groups, two methylenes, one oxymethine, one oxymethylene, two oxygenated quaternary carbons, and one quaternary carbon were attributable to a bicyclohexane ring. This suggestion was proved by the HMBC correlations from H-9' to C-4', C-5', C-6', C-8', from H-10' to C-1', C-2', C-8', from H-2' to C-1', C-3', C-4', C-8', C-10', from H-4' to C-2', C-6', C-8', C-9' and from H-6' to C-1', C-4', C-5', C-8', C-9'. Moreover, the signals of a β-D-glucopyranosyl unit were observed at δ_H 4.38 (1H, d, J =

8.0Hz, H-1")/δ_C 103.13, δ_H 3.17 (1H, t, J = 8.5 Hz, H-2")/δ_C 75.13, δ_H 3.37 (1H, m, H-3")/δ_C 78.10, δ_H 3.31 (1H, m, H-4")/δ_C 71.68, δ_H 3.30 (1H, m, H-5")/δ_C 77.97, δ_H 3.67 (1H, dd, J = 4.0, 11.5 Hz, H-6"a), δ_H 3.89 (1H, d, J = 11.5 Hz, H-6"b)/δ_C 62.78. The long-range correlations between H-1" and C-3', and between H-5 and C-1', C-5', C-8' indicated that the sugar linked at C-3' and the 3-methyl-penta-2,4-dienoic acid moiety was attached to C-8'. On the basis of the results described above and comparison of the NMR data with those in literature, the structure of **2** was determined to be (1'*R*,3'*S*,5'*R*,8'*S*,2*E*,4*E*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside [6].

Table 2. The NMR data of compound **2.**

C	$^{\#}\delta_C$ [a]	δ_C [a,b]	δ_H [a,c]	C	$^{\#}\delta_C$ [a]	δ_C [a,b]	δ_H [a,c]
1	nd	177.20		6'	77.2	77.15	3.76 (1H, m) 3.82 (1H, m)
2	126.9	125.20	5.85 (1H, br s)	8'	83.4	83.29	-
3	143.3	144.80	-	9'	19.9	19.73	1 18 (3H, s)
4	131.2	132.63	7.88 (1H, d, 16.0)	10'	16.5	16.37	0.95 (3H, s)
5	132.9	132.02	6.37 (1H, d, 16.0)	1"	103.3	103.13	4.38 (1H, d, 8.0)
6	20.8	20.82	2 03 (3H, s)	2"	75.2	75.13	3.17 (1H, t, 8.5)
1'	49.5	49.20	-	3"	78.2	78.10	3.37 (1H, m)
2'	43.0	42.88	1.81 (1H, m) 2.00 (1H, dd, 7.0, 13.5)	4"	71.8	71.68	3.31 (1H, m)
3'	74.2	74.00	4.27 (1H, m)	5"	78.1	77.97	3.30 (1H, m)
4'	42.9	42.82	1.82 (1H, m) 2.22 (1H, dd, 7.0; 13.5)	6"	62.9	62.78	3.67 (1H, dd, 4.0; 11.5) 3.89 (1H, d, 11 5)
5'	87.7	87.62	-	-	-	-	-

[a] *recorded in CD_3OD,* [b] *125 MHz,* [c] *500 MHz, nd: Not detected*

[#] *δ_C of (1'R,3'S,5'R,8'S,2E,4E)-dihydrophaseic acid 3'-O-β-D-glucopyranoside[6]*

Compounds **3-5** were identified as 3-methylbutan-1-ol β-D-glucopyranoside (**3**)[7], astragalin (**4**) [8] and daucosterol (**5**) [9] by comparing their NMR spectral data with those of reported in literature.

4. CONCLUSION

The phytochemical investigation of the seeds of *Scaphium macropodum* (Miq.) Beumée resulted in the isolation of five known compounds including{carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic acid] dimethyl ester (**1**), (1'*R*,3'*S*,5'*R*,8'*S*,2*E*,4*E*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside (**2**), 3-methylbutan-1-ol β-D-glucopyranoside (**3**), astragalin (**4**) and daucosterol (**5**). Their structures were identified by comparison of their spectroscopic data with those reported in the literature. This is the first report for the isolation of compounds **1-3** from this species.

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TÓM TẮT

CÁC GLUCOSIDE VÀ DẪN XUẤT CỦA URE PHÂN LẬP TỪ QUẢ ƯƠI *SCAPHIUM MACROPODUM* (MIQ.) BEUMÉE

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Sử dụng các phương pháp sắc kí kết hợp, năm hợp chất {carbonylbis[imino(6-methyl-3,1-phenylen)]}bis[carbamic acid] dimethyl ester (**1**), (1'*R*,3'*S*,5'*R*,8'*S*,2*E*,4*E*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside (**2**), 3-methylbutan-1-ol β-D-glucopyranoside (**3**), astragalin (**4**) và daucosterol (**5**) đã được phân lập từ cặn chiết metanol của quả ươi - *Scaphium macropodum* (Miq.) Beumée. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ cộng hưởng từ hạt nhân và phổ khối lượng kết hợp so sánh với các số liệu phổ đã được công bố. Các hợp chất **1-3** lần đầu tiên được phân lập từ quả ươi.

Từ khóa: *Scaphium macropodum*, Sterculiaceae, glucosides, urea derivative.