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Investigation on Chemical Constituents of the *Brugmansia Suaveolens* Flowers

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***Abstract*—Chemical constituents from the flowers of *Brugmansia suaveolens* were investigated by chromatographic methods. Besides the presence of scopolamine alkaloids, our results obtained the isolation of scopoletin 7-O-β-D-galactopyranoside (1), 20-hydroxyecdysone (2), acanthoside B (3), and kaemferol (4). their chemical structure was confirmed by HR-ESI-MS, NMR spectral data, and as well as comparison with reported literature. This is the first report on the isolation of compounds 1-3 from the *Brugmansia suaveolens* species.**

Keywords*—*Brugmansia suaveolens; acanthoside B; 20-hydroxyecdysone, scopoletin 7-O-β-D-galactopyranoside.

I. INTRODUCTION

Brugmansia suaveolens is known as angel's trumpet flower. This plant is widely distributed in the tropical climate regions. Aqueous extract of *B. suaveolens* flowers was reported potential analgesic and antinociceptive effect [1, 2]. Its chemical constituents were reported including flavonol glycosides and alkaloids as well as rich of tropane alkaloid called scopolamine [2, 3]. In our previous report, the content of scopolamine in the flowers was found higher in the leaves of this plant [4]. In the aim of clarification of chemical constituents of *B. suaveolens* flowers this work deals with the isolation, chemical structure determination of four compounds including scopoletin 7-O-β-D-galactopyranoside (**1**), 20-hydroxyecdysone (**2**), acanthoside B (**3**), and kaemferol (**4**) from the flowers of *B. suaveolens*.

II. MATERIALS AND METHODS

A. General experiment procedures

Optical rotation was measured on a Jasco P2000 polarimeter. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. HR-ESI-MS were carried out on an Agilent 6530 Accurate Mass QTOF LC/MS system. Column chromatography was performed using silica gel and reverse phase C18 resins. Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} (0.25 mm) and RP-C18 F_{254S} plates (0.25 mm). Spots were visualized under UV radiation (254 and 365 nm) and sprayed with aqueous solution of H_2SO_4 (10%), heating with a heat gun.

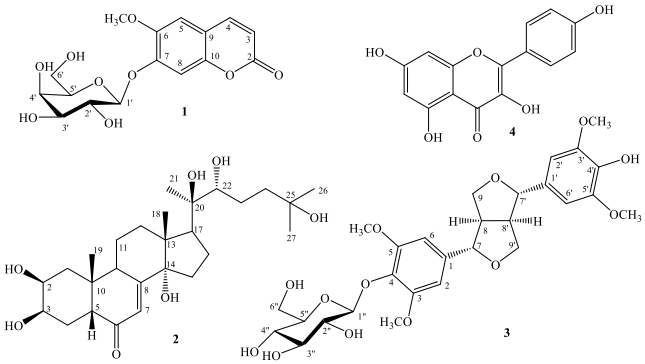

Fig. 1. *Chemical structure of compounds (**1**-**4**) isolated from the flowers of Brugmansia suaveolens*

B. Plant materials

The flowers of *Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl were collected at Lao Cai Province, Vietnam in April 2017. Its scientific name was identified by Dr. Bui Van Thanh, Institute of Ecology and Biological Resources, Hanoi, Vietnam. A voucher specimen (No. 201704BS) was kept at the Faculty of Basic Science, University of Transport and Communications.

C. Extraction and isolation

The dried powdered flowers of *B. suaveolens* (800 g) was extracted with methanol in the ultrasonic bath (3 times, each 3 L in 30 minutes at 40°C). After removal of methanol *in vacuo*, the crude extract (53 g) was subjected on a silica gel column and eluted with gradient solvent system of *n*-hexane/acetone (0-100% volume of acetone) to give eight fractions (BSF1-BSF8). The BSF3 fraction was repeatedly chromatographed on a silica gel column, eluting with *n*-hexane/ ethyl acetate (4/1, v/v) to obtain five fractions BSF3A-BSF3E. The BSF3C fraction was chromatographed on a silica gel column, eluting with dichloromethane/methanol (7/1, v/v) to give compound **4** (32 mg). Fraction BSF3D was first separated on a silica gel column, eluting with dichloromethane/ methanol (6/1, v/v) and then purified on a reverse phase (RP-C18) column eluting with methanol/water (1/1, v/v) to yield compound **2** (17 mg). Fraction BSF5 was chromatographed on a silica gel column, eluting with acetone/ dichloromethane/ water (3/1/0.1, v/v/v) to give four fractions BSF5A-BSF5D. Fraction BSF5B

was purified on a silica gel column, eluting with dichloromethane/ methanol/ water (5/1/0.1, v/v/v) to give compound **1** (14 mg). Finally, compound **3** (21 mg) was isolated from fraction BSF5C using silica gel column and solvent system of dichloromethane/ methanol/ water (4/1/0.1, v/v/v) as an eluent.

- Scopoletin 7-O-β-D-galactopyranoside (**1**) : Yellowish amorphous powder; $[\alpha]_D^{25}$ = -41.8 (c 0.25, MeOH); Molecular formula: $C_{16}H_{18}O_9$; HR-ESI-MS *m/z*: 377.0835 $[M+Na]^+$ (calcd for $C_{16}H_{18}O_9Na$, 377.0849); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) are given in the Table I.

- 20-hydroxyecdysone (**2**) : White amorphous powder; $[\alpha]_D^{25}$ = + 57.4 (c 0.15, MeOH); Molecular formula: $C_{27}H_{44}O_7$; HR-ESI-MS *m/z*: 515.2790 $[M+Cl]^-$ (calcd for $C_{27}H_{44}O_7Cl$, 515.2776); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) are given in the Table II.

- Acanthoside B (**3**) : Yellowish amorphous powder; $[\alpha]_D^{25}$ = -23.6 (c 0.1, MeOH); Molecular formula: $C_{28}H_{36}O_{13}$; HR-ESI-MS *m/z*: 603.2045 $[M+Na]^+$ (calcd for $C_{28}H_{36}O_{13}Na$, 603.2054); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) are given in the Table II.

- Kaempferol (**4**): Yellowish amorphous powder; Molecular formula: $C_{15}H_{10}O_6$; HR-ESI-MS *m/z*: 287.0552 $[M+H]^+$ (calcd for $C_{15}H_{11}O_6$, 287.0556); ^{1}H-NMR (CD_3OD, 500 MHz) δ_H ppm: 8.08 (d, *J* = 8.5 Hz, H-2', H-6'), 6.92 (d, *J* = 8.5 Hz, H-3', H-5'), 6.40 (br s, H-8), 6.20 (br s, H-6); and ^{13}C-NMR (CD_3OD, 125 MHz) δ_C ppm: 177.3 (C-4), 165.5 (C-7), 162.4 (C-5), 160.5 (C-4'), 158.2 (C-9), 148.1 (C-2), 137.1 (C-3), 130.7 (C-2', C-6'), 123.7 (C-1'), 116.3 (C-3', C-5'), 104.5 (C-10), 99.3 (C-6), 94.5 (C-8).

III. Results and Discussion

Compound **1** was isolated as a yellowish amorphous powder. Its molecular formula was deduced as $C_{16}H_{18}O_9$ by HR-ESI-MS at *m/z* 377.0835 $[M+Na]^+$ (calcd for $C_{16}H_{18}O_9Na$, 377.0849) and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **1** showed signal of four aromatic protons [δ_H 7.92 (d, *J* = 9.5 Hz), 7.22 (s), 7.19 (s), 6.32 (d, *J* = 9.5 Hz)], a methoxy group [δ_H 3.92 (s)], and an anomeric proton [δ_H 5.40 (d, *J* = 8.0 Hz)]. The ^{13}C-NMR spectrum of **1** revealed signal of 16 carbons. Among them, six carbinol carbon at δ_C 100.3, 76.0, 73.0, 71.9, 68.5, 62.7 were assigned for sugar moiety in term of hexose. A methoxy group was observed by signals at δ_C 57.1. Remaining 9 aromatic carbons at δ_C 163.6, 152.1, 150.7, 148.3, 145.7, 114.6, 114.5, 110.8, 105.1 suggested for a coumarin skeleton. A pair of ortho coupled protons at δ_H 7.92 and 6.32 were assigned for H-4 and H-3. The HMBC correlations between H-4 (δ_H 7.92) and C-5 (δ_C 110.8)/ C-9 (δ_C 150.7)/ C-10 (δ_C 114.6) supported for the assignment of C-5 and H-5. And HMBC correlations between H-5 (δ_H 7.22)/ anomeric proton (δ_H 5.40) and C-7 (δ_C 152.1) indicated O-glycosidic linkage at C-7. Thus, remaining singlet aromatic proton δ_H 7.19 was assigned for H-8. The HMBC correlations between H-8 (δ_H 7.19)/ methoxy protons (δ_H 3.92) and C-6 (δ_C 148.3) indicated location of methoxy group at C-6. The NMR data corresponding to sugar moiety, especially multiplicity of H-4' (t, *J* = 3.0 Hz) suggested for the presence of galactopyranosyl group. Consequently, compound **1** was determined as scopoletin 7-O-β-D-galactopyranoside. To date, this compound has not been isolated from natural sources so far, except from the *Angelica dahurica* species [5].

TABLE I. ^{1}H- AND ^{13}C-NMR SPECTRAL DATA OF COMPOUND **1**

No.	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult., *J* in Hz)
2	163.6	-
3	114.5	6.32 (d, 9.5)
4	145.7	7.92 (d, 9.5)
5	110.8	7.22 (s)
6	148.3	-
7	152.1	-
8	105.1	7.19 (s)
9	150.7	-
10	114.5	-
OGal		
1'	100.3	5.40 (d, 8.0)
2'	68.5	3.64 (dd, 8.0, 9.5)
3'	71.9	3.70 (dd, 3.0, 9.5)
4'	73.0	4.19 (dd, 3.0, 3.0)
5'	76.0	3.94 (m)
6'	62.7	3.92 (dd, 2.5, 12.5) 3.72 (dd, 3.0, 12.5)
OCH_3	57.1	3.92 (s)

Compound **2** was isolated as a white amorphous powder. Its molecular formula was deduced as $C_{27}H_{44}O_7$ by HR-ESI-MS at *m/z* 515.2790 $[M+Cl]^-$ (calcd for $C_{27}H_{44}O_7Cl$, 515.2776) and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **2** showed signal of an olefinic proton (δ_H 5.84), three oxygenated methins (δ_H 3.98, 3.87, 3.36), and five methyl groups (δ_H 0.91, 0.99, 1.21, 1.22, and 1.23). The ^{13}C-NMR and DEPT spectra of **2** revealed signals of 27 carbons including one carbonyl carbon, 2 olefinic carbons, five non-protonated carbons, 6 methins, 8 methylenes, and 5 methyl groups. The NMR spectral data of **2** suggested for the structure of sterol. A singlet methyl signal of H_3-21 (δ_H 1.22) and the HMBC correlations between H_3-21 and C-17 (δ_C 50.4)/C-20 (δ_C 77.9)/C-22 (δ_C 78.3) indicated the presence of two hydroxy groups at C-20 and C-22. Additionally, HMBC correlations between H-5 (δ_H 2.40) and C-6 (δ_C 206.5)/ C-7 (δ_C 122.1), H-7 (δ_H 5.84) and C-5 (δ_C 51.7)/ C-9 (δ_C 35.0)/ C-14 (δ_C 85.2) indicated location of oxo functional group at C-6 and a double bond at C-7/C-8. The HMBC correlations between H-1 (δ_H 1.45, 1.81) and C-2 (δ_C 68.6)/ C-3 (δ_C

TABLE II. ^{1}H- AND ^{13}C-NMR SPECTRAL DATA OF COMPOUNDS **2** AND **3**

No.	Comp. 2			Comp. 3	
	δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)	No.	δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)
1	37.3	1.45 (m)/ 1.81 (m)	1	135.5	-
2	68.6	3.87 (dt, 4.0, 12,0)	2	104.8	6.74 (s)
3	68.4	3.98 (br s)	3	154.3	-
4	32.7	1.73 (m)	4	139.5	-
5	51.7	2.40 (dd, 5.0, 13.0)	5	154.3	-
6	206.5	-	6	104.8	6.74 (s)
7	122.1	5.84 (d, 2.5)	7	87.1	4.79 (d, 4.0)
8	168.0	-	8	55.4	3.16 (m)
9	35.0	3.17 (t, 8.5)	9	72.9	4.31*/ 3.93*
10	39.2	-	1'	133.0	-
11	21.5	1.73 (m)/ 1.82 (m)	2'	104.4	6.67 (s)
12	32.4	1.90 (m)/ 2.14 (m)	3'	149.3	-
13	48.6	-	4'	136.2	-
14	85.2	-	5'	149.3	-
15	31.7	1.63 (m)/ 1.98 (m)	6'	104.5	6.67 (s)
16	21.5	1.75 (m)/ 2.00 (m)	7'	87.5	4.74 (d, 4.5)
17	50.4	2.39 (m)	8'	55.6	3.16 (m)
18	18.0	0.91 (s)	9'	72.8	4.31*/ 3.93*
16	24.4	0.99 (s)	3,5-OCH_3	57.0	3.88 (s)
20	77.9	-	3',5'-OCH_3	56.8	3.87 (s)
21	21.1	1.22 (s)	OGlc		
22	78.3	3.36*	1"	105.3	4.85 (d, 8.0)
23	27.3	1.31 (m)/ 1.70 (m)	2"	75.6	3.49 (m)
24	42.3	1.45 (m)/ 1.81 (m)	3"	77.7	3.46 (m)
25	71.3	-	4"	71.3	3.47 (m)
26	29.0	1.21 (s)	5"	78.3	3.22 (m)
27	29.7	1.23 (s)	6"	62.5	3.79 (dd, 2.0, 12.0) 3.68 (dd, 5.0, 12.0)

Assignments were done by DEPT, HSQC and HMBC experiments, *)Overlapped signals.

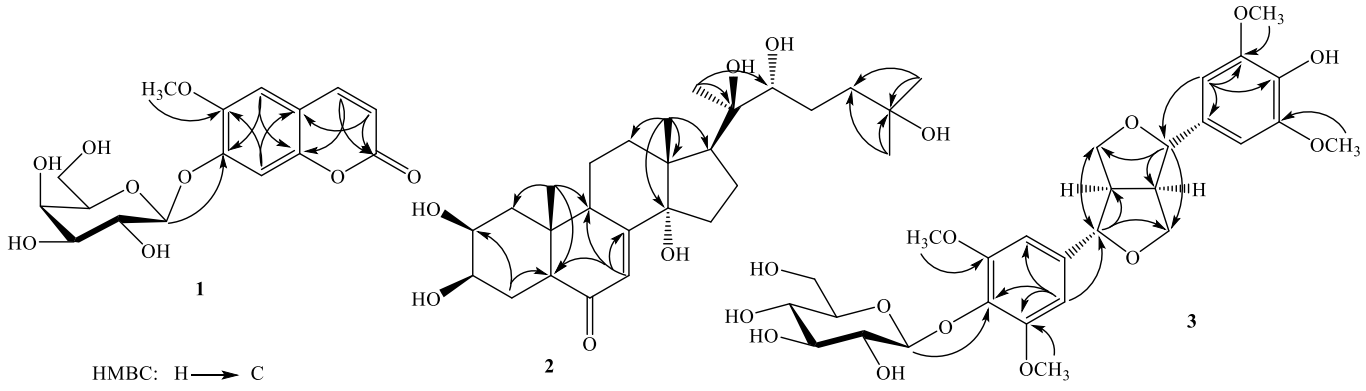

Fig. 2. *Keys HMBC correlation of compounds (**1-3**)*

68.4), H-3 (δ_H 3.98) and C-5 (δ_C 51.7) demonstrated the presence of two hydroxy group at C-2 and C-3. And the HMBC correlations between H-18 (δ_H 0.91) and C-12 (δ_C 32.4)/ C-13 (δ_C 48.6)/ C-14 (δ_C 85.2)/ C-17 (δ_C 50.4), H-26 (δ_H 1.21)/H-27 (δ_H 1.23) and C-24 (δ_C 42.3)/C-25 (δ_C 71.3) confirmed others hydroxy

groups at C-14 and C-25. Thus, compound **2** was determined to be 2,3,14,20,22,25-hexahydroxycholest-7-en-6-one, having trivial name as 20-hydroxyecdysone. The NMR spectral data of **2** were well consited with those reported for 20-hydroxyecdysone in the literature [6].

Compound **3** was isolated as a yellowish amorphous powder. The HR-ESI-MS analysis of **3** showed quasi-molecular ion peak at *m/z*: 603.2045 $[M+Na]^+$, indicating molecular formula of $C_{28}H_{36}O_{13}$ (calcd for $C_{28}H_{36}O_{13}Na$, 603.2054). The ^{1}H-NMR spectrum showed symmetric structure including for aromatic protons (δ_H 6.74 and 6.67, each 2H), four methoxy groups (δ_H 3.87 and 3.88, each 6H), an anomeric proton (δ_H 4.85). The ^{13}C-NMR and DEPT spectra of **3** contained signals of 28 carbons. Among them, six carbinol carbons at δ_C 105.3, 78.3, 77.7, 75.6, 71.3, 62.5 characterized for glucopyranosyl group. Carbon signals at δ_C 57.0 and 56.8 were assigned for methoxy groups. Remaining 18 carbon signals including 12 aromatic carbons and 6 sp3 hybridized carbons suggested for a lignan compound. Furthermore, a pair of singlet aromatic protons (δ_H 6.74 and 6.67, each 2H) and two set of aromatic carbon signals [(δ_C 135.5, 104.8, 154.3, 139.5) and (δ_C 133.0, 104.4, 149.3, 136.2)] suggested for the presence of two 1,3,4,5-tetrasubstitutedbenzene structure fragment. Other two set of propane backbone [(δ_C 87.1, 72.9, 55.4) and (δ_C 87.5, 72.8, 55.6)] expected for a 7,9':9,7'-diepoxylignan. The HMBC correlations between H-7' (δ_H 4.74) and C-9 (δ_C 72.9), H-7 (δ_H 4.79) and C-9' (δ_C 72.8), H-2/ H-6 (δ_H 6.74) and C-7' (δ_C 87.5), H-2'/ H-6' (δ_H 6.67) and C-7 (δ_C 87.1) were further confirmed the presence of two epoxy bridges (7,9':9,7') and the binding of two 1,3,4,5-tetrasubstitutedbenzene at C-7, C-7'. In addition, HMBC correlations between H-2/ H-6 (δ_H 6.74) and C-4 (δ_C 139.5), H-2'/ H-6' (δ_H 6.67) and C-4' (δ_C 136.2), 3,5-OMe (δ_H 3.88) and C-3/C-5 (δ_C 154.3), 3',5'-OMe (δ_H 3.87) and C-3'/C-5' (δ_C 149.3) suggested for location of methoxy groups at C-3, C-5, C-3', C-5' and a hydroxy group at C-4'. The location of O-glucopyranosyl group at C-4 was confirmed by HMBC correlation between anomeric proton (δ_H 4.85) and C-4 (δ_C 139.5). Thus, chemical structure of **3** was established and determined to be acanthoside B by the agreement of the NMR data with those reported in the literature.

The NMR data of compound **4** suggested it to be kaempferol [7], a well-known flavonoid naturally occurring in many plants.

In conclusion, chemical study of the flowers of *B. suaveolens* resulted isolation of scopoletin 7-O-β-D-galactopyranoside (**1**), 20-hydroxyecdysone (**2**), acanthoside B (**3**), and kaemferol (**4**). Of these, compounds **1**-**3** were reported for the first time from the *Brugmansia suaveolens* species.

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