

THREE PHENOLIC COMPOUNDS FROM THE BARK OF *AEGICERAS FLORIDUM* ROEM. & SCHULT.

● LUU HUYNH VAN LONG* - NGUYEN KIM PHI PHUNG

ABSTRACT:

From the *n*-hexane and ethyl acetate extracts of *Aegiceras floridum* bark, three phenolic compounds were isolated, namely 3-methoxy-5-octylphenol (1), 2-methyl-5-nonylbenzene-1,3-diol (2) and methyl-3,4-dihydroxybenzoate (3). Their structures were determined through the interpretation of their NMR data and compared with the ones reported in the literature. Although these compounds were reported in other plants, it is the first time that they are known in this species.

Keywords: *Aegiceras floridum*, phenolic compounds, primulaceae.

1. Introduction

The Vietnamese Can Gio forest is one of big mangrove forests of the country, providing many medicinal plants which have both ecological and economic benefits [1,2]. *Aegiceras floridum* Roem. & Schult., one of two species of the genus (family Primulaceae) distributed in the high salinity areas in the Philippines, Indonesia, East Malaysia, and South Vietnam [3]. In previous work, six compounds were determined [4]. As part of our continuing research on the chemical constituents of *Aegiceras floridum*, three phenolic compounds, including 3-methoxy-5-octylphenol (1), 2-methyl-5-nonylbenzene-1,3-diol (2), and methyl-3,4-dihydroxybenzoate (3) were isolated from the bark of this species (Figure 1).

2. Materials and methods

2.1. General experimental procedures

The ESI-MS spectra were obtained from a MSQ plus Mass. The ¹H-NMR (500 MHz), ¹³C-NMR (125 MHz) spectra were recorded on a Bruker Avance 500 MHz. Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F₂₅₄ (0.2 mm) (Merck Co., Germany) and compounds were detected under UV (254/365 nm) and visualized by spraying with 10% H₂SO₄ aqueous solution, followed by heating. Silica gel 60 (0.040-0.063 mm, Merck Co., Germany) was used for normal phase column chromatography (CC).

2.2. Plant material

The bark of *Aegiceras floridum* was collected

in the Can Gio mangrove forest (15°11'00" N, 106°14'00" E), Ho Chi Minh City, Vietnam in April 2015 at an altitude of 109 m. The scientific name of the plant was authenticated by Dr. Pham Van Ngot, Department of Biology, Ho Chi Minh University of Education. A voucher specimen (US-B016) was deposited at the herbarium of the Department of Organic Chemistry, University of Science, Viet Nam National University - Ho Chi Minh City, Vietnam.

2.3. Extraction and isolation

The dried powder of the bark (20.5 kg) was macerated with methanol at room temperature (2 x 20 L). After filtration, the solvent was evaporated under reduced pressure to dryness to afford the crude methanol residue (950 g). This dry residue was subsequently re-extracted using solvents of increasing polarities: *n*-hexane (H, 150 g), EtOAc (EA, 250 g) and methanol (M, 500 g).

The extract H was applied to silica gel column chromatography (CC) and eluted with the solvent systems of *n*-hexane-chloroform (stepwise, 9:1 to 0:10, v/v) to give 6 fractions, namely H1-H6, then eluted with ethyl acetate-methanol (9:1, v/v) to give fraction H7. The fraction H3 (20.5 g) was rechromatographed by silica gel CC, eluted with *n*-hexane-chloroform (9:1-3:7, v/v) to provide 7 fractions H3.1-7. Fraction H3.3 (2.5 g) was applied to Sephadex LH-20 chromatography, eluted with chloroform-methanol (1:1, v/v) to provide three fractions H3.3.1-3. Fraction H3.3.1 (125 mg) was applied to silica gel CC, eluted with the solvent system of chloroform-methanol (99:1 to 97:3, v/v) to obtain compound 1 (2.0 mg).

The extract EA was applied to silica gel column chromatography (CC) and eluted with the solvent systems of *n*-hexane-ethyl acetate (stepwise, 9:1 to 0:10, v/v) to give 7 fractions, namely EA1-EA7, then eluted with ethyl acetate-methanol (9:1, v/v) to give fraction EA8. The fraction EA2 (21.5 g) was rechromatographed by silica gel CC, eluted with *n*-hexane-ethyl acetate (9:1-3:7, v/v) to provide 7 fractions EA2.1-7.

Fraction EA2.2 (2.1 g) was applied to Sephadex LH-20 chromatography, eluted with chloroform-methanol (1:1, v/v) to provide four fractions EA2.2.1-4. Fraction EA2.2.2 (350 mg) was applied to silica gel CC, eluted with the solvent system of chloroform-methanol (99:1 to 92:8, v/v) to obtain compound 2 (4.5 mg), 3 (15.5 mg).

3-Methoxy-5-octylphenol (1): White amorphous powder; C₁₅H₂₄O₂; HR-ESI-MS *m/z* 259.16633 [M+Na]⁺; ¹H and ¹³C-NMR (CDCl₃), see table 1.

2-Methyl-5-nonylbenzene-1,3-diol (2): White amorphous powder; C₁₆H₂₆O₂; ESI-MS *m/z* 249.54 [M-H]⁻; ¹H and ¹³C-NMR (CDCl₃), see Table 1.

Methyl-3,4-dihydroxybenzoate (3): White solid C₈H₈O₄; ESI-MS *m/z* 166.96 [M-H]⁻; ¹H and ¹³C-NMR (CD₃OD), see table 1.

3. Results and discussion

Compound 1 was obtained as white amorphous powder. Its molecular formula was determined as C₁₅H₂₄O₂ from the observation of the protonated ion peak at *m/z* 259.16633 [M+Na]⁺ in HR-ESI-MS (calcd. for C₁₅H₂₄O₂+Na, 259.16740). The ¹H-NMR spectrum displayed signals for one 1,3,5-trisubstituted benzene ring with three aromatic protons (δ_H 6.33, 6.26, and 6.23, each with a small coupling constant around 2.0 Hz), a methoxy group (δ_H 3.77, s), and one aliphatic side chain having some methylene groups (δ_H 2.51, 2H, *t*, *J* = 7.5 Hz; 1.54, 2H, *m*; and a broad signal in the range of 1.25-1.43 ppm) and a terminal methyl group (δ_H 0.88, 3H, *t*, *J* = 7.0 Hz). The ¹³C-NMR and HSQC spectra in accordance with MS data exhibited 15 carbon signals including three aromatic methines (δ_C 98.8, 107.0, 108.0) and three quaternary aromatic carbons (δ_C 145.9, 156.6, and 160.9) of the trisubstituted benzene ring, a methoxy carbon (δ_C 55.4) and the up-field signals of a side chain in the range of 14.3-36.2 ppm.

Assistance of HSQC and HMBC experiments

three of these methylenes were assigned to carbons C-1' (δ_C 36.2), C-2' (δ_C 31.0), and C-7' (δ_C 22.7). Evident correlations in HMBC experiments of H-1' to C-4, C-5, and C-6 allowed us to anchor the octyl moiety to C-5 of the aromatic ring (Figure 2). The comparison of its spectral data with the one published in the literature, 1 was confirmed as 3-methoxy-5-octylphenol.[5]

Compound 2 was isolated as white amorphous powder. Its molecular formula was established as $C_{16}H_{26}O_2$ on the basis of a pseudomolecular ion peak $[M-H]^-$ at m/z 249.54 in the negative ESI-MS. The 1H -NMR spectrum displayed signal for one 1,2,3,5-tetrasubstituted benzene ring with a singlet aromatic proton signal, integrated two protons at δ_H 6.24, and one aliphatic side chain having some methylene groups (δ_H 2.45, 2H, *t*, $J = 7.5$ Hz; 1.54, 2H, *m*; and a broad signal in the range of 1.27–1.29 ppm) and a terminal methyl group (δ_H 0.89, 3H, *t*, $J = 7.0$ Hz). Besides, 2 showed signals of a methyl group at δ_H 2.11 (s) and δ_C 7.7 (CH_3). The ^{13}C -NMR spectra exhibited 4 carbon signals of aromatic ring (δ_C 154.5, 142.1, 107.9, and 107.4) in which two signals at δ_C 154.5 and 107.9 appearing as double intensity. It suggested that the 1,2,3,5-tetrasubstituted

benzene ring was symmetry. Based on the NMR data and comparison of data given in the literature, the chemical structure of compound 2 was 2-methyl-5-nonylbenzene-1,3-diol.[6]

Compound 3 was isolated as a white solid. Its molecular formula was established as $C_8H_8O_4$, which was deduced from the ESI-MS with m/z 166.96 $[M-H]^-$. The 1H -NMR spectrum of 3 exhibited signals at δ_H 7.41 (1H, *dd*, $J = 9.0$; 2.0 Hz, H-6), 7.42 (1H, *brs*, H-2) and 6.80 (1H, *dd*, $J = 7.5$; 1.0 Hz, H-5) defined a 1,3,4-trisubstituted aromatic ring. This was confirmed by the ^{13}C -NMR spectrum revealing three quaternary aromatic carbons at δ_C 122.7 (C-1), 146.2 (C-3) and 151.7 (C-4) and three methines at δ_C 117.5 (C-2), 115.9 (C-5) and 123.7 (C-6). Besides, the 1H -NMR spectrum of compound 3 showed signal of a methyl ester group at δ_H 3.83. The ^{13}C -NMR spectrum of 3 indicated the existence of a carbonyl carbon of the ester at δ_C 168.9. Therefore, compound 3 was suggested to be a three-substituted benzene with a methyl ester and two hydroxyl groups. Based on the compatibility of the above-mentioned data with those given in the literature, 3 was identified as methyl-3,4-dihydroxybenzoate.[7]

Figure 1: Structure of isolated compounds 1-3

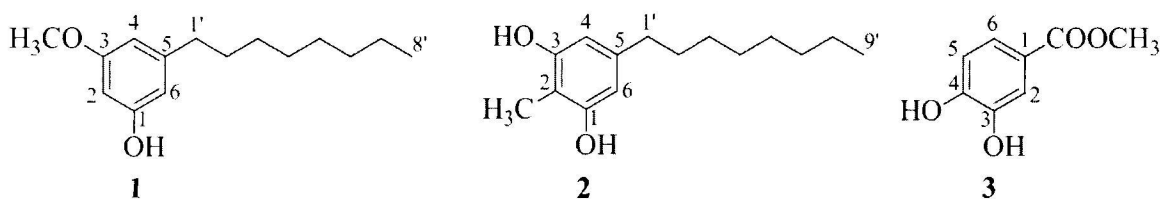


Figure 2: Selected HMBC correlations of 1

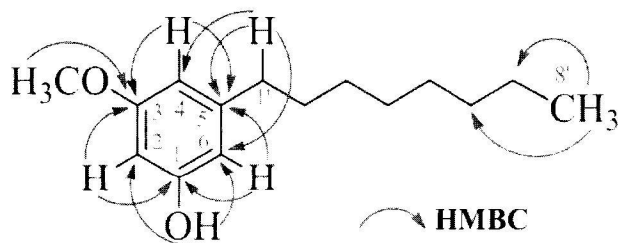


Table 1: ^1H and ^{13}C NMR data for compounds 1-3

Position	1(CDCl ₃)		2(CDCl ₃)		3 (CD ₃ OD)	
	δ_{H} mult. (<i>J</i> in Hz)	δ_{C}	δ_{H} mult. (<i>J</i> in Hz)	δ_{C}	δ_{H} mult. (<i>J</i> in Hz)	δ_{C}
1		156.6		154.5		122.7
2	6.23 (<i>dd</i> , 2.0, 2.0)	98.8		142.1	7.42 (<i>brs</i>)	117.5
3		160.9		154.5		146.2
4	6.33 (<i>brs</i>)	107.0	6.24 (<i>s</i>)	107.9		151.7
5		145.9		107.4	6.80 (<i>dd</i> ; 7.5; 1.0)	115.9
6	6.26 (<i>brs</i>)	108.0	6.24 (<i>s</i>)	107.9	7.41 (<i>dd</i> ; 9.0; 2.0)	123.7
1'	2.51 (<i>t</i> , 7.5)	36.2	2.45 (<i>t</i> , 7.5)	32.0		
2'	1.54 (<i>m</i>)	31.0	1.54 (<i>m</i>)	31.2		
3'	1.25–1.43 (<i>m</i>)	29.5–29.9	1.27–1.29 (<i>m</i>)	29.3–29.7		
4'						
5'						
6'						
7'					22.7	
8'	0.88 (<i>t</i> , 7.0)	14.3		22.7		
9'			0.89 (<i>t</i> , 7.0)	14.1		
-OCH ₃	3.77 (<i>s</i>)	55.4				
-CH ₃			2.11 (<i>s</i>)	7.7		
-COO						168.9
-COOCH ₃					3.83 (<i>s</i>)	52.2
1-OH	4.67 (<i>s</i>)		4.78 (<i>s</i>)			
3-OH			4.78 (<i>s</i>)			
4-OH						

4. Conclusion

In the investigation of the chemical constituents of bark of *Aegiceras floridum* collected in Can Gio mangrove forest, Ho Chi Minh City, three phenolic compounds, including 3-methoxy-5-octylphenol

(1), 2-methyl-5-nonylbenzene-1,3-diol (2) and methyl-3,4-dihydroxybenzoate (3) were isolated and structural elucidated. All three isolated compounds were reported in this species for the first time ■

Acknowledgement

This research was funded by Thu Dau Mot University under grant number DT.19.1-017. The authors would like to thank Dr. Pham Van Ngot for the taxonomic authentication of the studied object *Aegiceras floridum* Roem. & Schult.

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Ngày nhận bài: 12/1/2021

Ngày phản biện đánh giá và sửa chữa: 22/1/2021

Ngày chấp nhận đăng bài: 3/2/2021

Author information:

Ph.D student LUU HUYNH VAN LONG^{1,2}

Prof. Ph.D. NGUYEN KIM PHI PHUNG¹

¹ Department of Organic Chemistry

University of Science, Vietnam National University - Ho Chi Minh City

² Faculty of Food Technology

University of Thu Dau Mot, Binh Duong Province

BA HỢP CHẤT PHENOL TỪ VỎ CÂY *AEGICERAS FLORIDUM* ROEM. & SCHULT.

● **LƯU HUYNH VAN LONG^{1,2}**

● **NGUYỄN KIM PHI PHỤNG¹**

¹ Bộ môn Hóa hữu cơ,

Trường ĐH Khoa học tự nhiên, ĐHQG TP. Hồ Chí Minh

² Khoa Công nghệ thực phẩm,

Trường ĐH Thủ Dầu Một, tỉnh Bình Dương

TÓM TẮT:

Từ các cao trích *n*-hexane và ethyl acetate của vỏ cây *Aegiceras floridum*, ba hợp chất phenol đã được phân lập với tên gọi là 3-methoxy-5-octylphenol (1), 2-methyl-5-nonylbenzene-1,3-diol (2) và methyl-3,4-dihydroxybenzoate (3). Cấu trúc các hợp chất được xác định bằng phân tích phổ cộng hưởng từ hạt nhân (NMR) và so sánh với dữ liệu phổ đã công bố từ các tài liệu tham khảo. Mặc dù các hợp chất này đã được công bố từ những loài cây khác, nhưng đối với loài cây *Aegiceras floridum* là lần đầu tiên được biết đến.

Từ khóa: *Aegiceras floridum*, phenolic compounds, primulaceae.

ĐÍNH CHÍNH

Tạp chí Công Thương xin đính chính phần lỗi của bài báo "NGHIÊN CỨU VỀ ĐẦU TƯ VÀ HIỆU QUẢ HOẠT ĐỘNG CỦA CÁC DOANH NGHIỆP NIÊM YẾT TẠI VIỆT NAM", trên Tạp chí Công Thương - Các Kết quả nghiên cứu khoa học và ứng dụng công nghệ, Số 29 + 30 - Tháng 12/2020 (từ trang 90 đến trang 94). Cụ thể:

Vị trí lỗi	Lỗi	Đúng
Trang 4, 90 và 94 của bài báo - phần Thông tin tác giả	Nguyễn Công Thành	Nguyễn Công Thành
	Nguyễn Thị Nha	Nguyễn Thị Nha
	Phạm Thị Anh Thư	Nguyễn Trọng Nghĩa

Trân trọng!