

CHEMICAL CONSTITUENTS FROM THE CHLOROFORM EXTRACT OF THE STEM OF MAHONIA NEPALENSIS DC., BERBERIDACEAE

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TÓM TẮT

THÀNH PHẦN HÓA HỌC CAO CHLOROFORM CỦA THÂN CÂY MẬT GẤU (MAHONIA NEPALENSIS) Họ Hoàng Liên Gai (BERBERIDACEAE)

From the stems of *Mahonia nepalensis*, five compounds were isolated: 1-C-syringylglycerol 4-O- β -D-glucopyranoside (**1**), 5,6-dihydro-2,3,9,12-tetramethoxydibenzo quinolizinium (**2**), 7-hydroxy-6-methoxyisochroman-1-one (**3**) and 6,7-dimethoxyisochroman-1-one (**4**), 4',7-dihydroxyflavanone (**5**). The chemical structure of these compounds were elucidated by their NMR spectra and comparison with references.

Keywords: *Mahonia nepalensis*, isochroman-1-one, flavanone, dibenzoquinolizinium

1. INTRODUCTION

Mahonia nepalensis belongs to the Berberidaceae family, is widely distributed in the high mountainous areas at altitude of about 1700-1900 m of VietNam as Lai Chau, Cao Bang, Ha Giang, Lam Đông provinces [1]. It is medium sized fully hardy perennial evergreen shrub with yellow flowers in winter. The stems and woods of *M. Nepalensis* have anti-inflammatory, anti-bacterial, anti-fungal activities. It is particularly used for treatment of skin diseases like eczema, psoriasis, etc. This plant contains alkaloids as the main compounds which belong to two major classes the protoberberines and the bisbenzylisoquinolines [2,3]. In this research, we reported the isolation and structure elucidation of five compounds: 1-C-syringylglycerol 4-O- β -D-glucopyranoside (**1**), 5,6-dihydro-2,3,9,12-tetramethoxydibenzo quinolizinium (**2**), 7-hydroxy-6-methoxyisochroman-1-one (**3**), 6,7-

dimethoxyisochroman-1-one (**4**) and 4',7-dihydroxyflavanone (**5**) from the stems of *M. Nepalensis*.

2. EXPERIMENTAL

2.1. General

NMR spectra were taken on a Bruker Avance III 500 spectrometer, at 500 MHz for ^1H and 125 MHz for ^{13}C . All spectra were recorded at the Central of Laboratory for Analysis, University of Science, VietNam National University – HCM city. Column chromatography was performed with silica gel (Kieselgel 60, 40-63 μm , Merck), and Lichroprep RP₁₈ (40-63 μm , Merck). Analytical and preparative TLC were carried out on precoated Kieselgel 60F₂₅₄ or RP18 plates (0.25 mm, Merck).

2.2. Plant material

Stems of *Mahonia Nepalensis* were collected in Dak Lak province, Viet Nam in May 2017. The scientific name of plant was identified by Master Hoang Viet, Faculty of Biology, University of Science, Ho Chi Minh city.

2.3. Extraction and isolation

Fresh stems were washed, dried, grounded into powder (10.0 kg); and then each 500g of each was exhaustively extracted with MeOH (3.5L, reflux, 3hx2) in a 5L round bottom flask to yield a MeOH extract (400g). The MeOH was suspended in H₂O and then successively partitioned in petroleum ether, CHCl₃, EtOAc and MeOH to yield petroleum ether extract (70g), CHCl₃ extract (100g), EtOAc (120g) and MeOH extract (50g), respectively. The CHCl₃ was repeatedly chromatographed over silica gel eluted with CHCl₃-MeOH in order to increasing polarity to give 8 fractions (fr.1-fr.8). Fraction 3 (3.5g) was rechromatographed on silica gel column (0-30% MeOH/CHCl₃) to yield 5 subfractions (fr.3.1- fr.3.5). Subfraction 3.2 (500 mg) was rechromatographed on silica gel (0-20% MeOH/CHCl₃), followed preparative TLC with a mixture of CHCl₃:EtOAc (3:1) to give **(1)** (5 mg) and **(2)** (6 mg). Fraction 5 (2.7 g) was rechromatographed on silica gel column (0-100% CHCl₃/EtOAc) to give 7 subfractions (fr.5.1-fr.5.7). Subfraction 5.5 (620 mg) was rechromatographed on silica gel (0-20% MeOH/CHCl₃), followed preparative TLC with a mixture of CHCl₃:EtOAc (5:1) to yield **(3)** (5 mg), **(4)** (7 mg) and **(5)** (6 mg).

1-C-syringylglycerol

4-O- β -D-glucopyranoside (1). White amorphous powder ¹H-NMR (500MHz, DMSO-*d*₆): δ _H 6.65 (s, H-2); δ _H 6.65 (s, H-6), 3.76 (s, 6H), 4.66 (H-7), 3.09 (H-8), 3.84 (H-9a), 4.20 (H-9b), 4.87 (H-1'), 3.15 (H-2'), 3.20 (H-3'), 3.22 (H-4'), 3.04 (H-5'), 3.40 (H-6'a), 3.60 (H-6'b). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ _C 56.4 (3-OCH₃), 56.4 (5-OCH₃), 85.0 (C-7), 53.6 (C-8), 71.3 (C-9), 137.1 (C-1), 104.3 (C-2,6), 152.6 (C-3,5), 102.7 (C-1'), 69.9 (C-2'), 74.1 (C-3'), 76.5 (C-4'), 60.9 (C-6').

5,6-Dihydro-2,3,9,12-tetramethoxydibenzo

quinolizinium (2). Yellow amorphous powder. ¹H-NMR (500MHz, DMSO-*d*₆): δ _H 8.20 (d, J=9 Hz, H-10), 8.02 (d, J=9 Hz, H-11), 7.70 (s, H-1), 7.10 (s, H-4), 9.86 (s, H-8), 9.00 (s, H-13), 3.22 (t, J=6 Hz, 2H-5), 4.94 (t, J=6 Hz,

2H-6), 3.93 (3H; s), 3.87 (3H; s), 4.10 (3H, s), 4.07 (3H, s). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ _C 108.8 (C-1), 148.8 (C-2), 151.6 (C-3), 111.4 (C-4), 128.7 (C-4a), 26.0 (C-5), 55.9 (C-6), 145.4 (C-8), 133.1 (C-8a), 143.7 (C-9), 126.9 (C-10), 123.4 (C-11), 150.3 (C-12), 121.4 (C-12a), 119.9 (C-13), 137.7 (C-14), 118.9 (C-14a), 56.2 (2-OCH₃), 55.4 (3-OCH₃), 61.9 (9-OCH₃), 57.1 (12-OCH₃).

7-Hydroxy-6-methoxyisochroman-1-one (3).

Yellow oil. ¹H-NMR (500MHz, DMSO-*d*₆): δ _H 3.30 (m, 2H-3), 2.70 (t, J=6.5, 2H-4), 6.54 (s, H-5), 7.34 (s, H-8), 3.80 (s, 6-OCH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ _C 164.9 (C-1), 27.1 (C-4), 40.0 (C-3), 110.5 (C-5), 149.9 (C-6), 146.4 (C-7), 114.0 (C-8), 120.5 (C-9), 133.0 (C-10), 55.6 (6-OCH₃).

6,7-Dimethoxyisochroman-1-one (4).

Yellow oil. ¹H-NMR (500MHz, DMSO-*d*₆): δ _H 3.30 (m, 2H-3), 2.80 (t, J=6.5, 2H-4), 6.88 (s, H-5), 7.35 (s, H-8), 3.80 (6H, s, 6,7-OCH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ _C 164.9 (C-1), 27.1 (C-4), 40.0 (C-3), 110.5 (C-5), 149.9 (C-6), 146.4 (C-7), 114.0 (C-8), 120.5 (C-9), 133.0 (C-10), 55.6 (6-OCH₃).

4',7-Dihydroxyflavanone (5).

Yellow oil. ¹H-NMR (500MHz, acetone-*d*₆): 5.44 (dd, 12.8 & 3.0, H-2), 2.66 (dd, 17.0 & 3.0, H-3a), 3.03 (dd, 17.0 & 13.0, H-3b), 7.71 (d, 8.4, H-5), 6.56 (dd, 8.4 & 2.4, H-6), 6.41 (d, 2.4, H-8), 7.39 (d, 8.4, H-2',6'), 6.89 (d, 8.4, H-3',5'). ¹³C-NMR (125 MHz, acetone-*d*₆): 80.5 (C-2), 44.7 (C-3), 191.7 (C-4), 116.1 (C-4a), 129.4 (C-5), 111.3 (C-6), 164.0 (C-7), 103.7 (C-8), 164.1 (C-8a), 130.2 (C-1'), 128.9 (C-2',6'), 116.1 (C-3',5'), 158.6 (C-4').

3. RESULTS AND DISCUSSION

Compound **(1)** was isolated as a white amorphous powder. The ¹H-NMR of spectrum compound **(1)** showed the signals of two meta-coupled proton of tetra-substituted symmetrical benzene ring [δ _H 6.65 (s, H-2); δ _H 6.65 (s, H-6)], a 1,2,3-trihydroxypropan-1-yl group [4.66 (H-7), 3.09 (H-8), 3.84 (H-9a), 4.20 (H-9b)] and a sugar unit [4.87 (H-1'), 3.15 (H-2'), 3.2 (H-3'), 3.22 (H-4'), 3.04 (H-5'), 3.40 (H-6'a), 3.60 (H-6'b)]. The position of the 1,2,3-

trihydroxylpropan-1-yl group at C-1 and the sugar unit at C-4 were also indicated by HMBC correlations between proton H-7/C-1 and H-1'/C-4, respectively. Moreover, NMR data of **(1)** showed good compatibility to the ones in literature [4] so compound **(1)** were proposed to be 1-*C*-syringylglycerol 4-*O*- β -D-glucopyranoside.

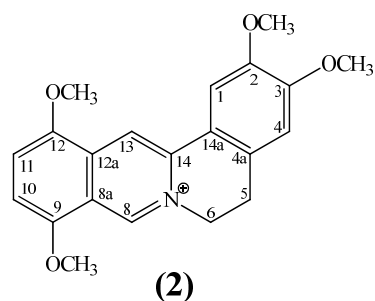
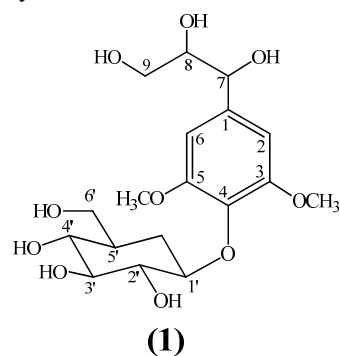
Compound **(2)** was obtained as a yellow amorphous powder. The $^1\text{H-NMR}$ spectrum showed two adjacent aromatic protons at δ_{H} 8.20 (d, $J=9.0$ Hz, H-10), 8.02 (d, $J=9.0$ Hz, H-11), four isolated aromatic protons at δ_{H} 7.70 (s, H-1), 7.10 (s, H-4), 9.86 (s, H-8), 9.0 (s, H-13) and two methylene groups [3.22 (t, $J=6$ Hz, 2H-5), 4.94 (t, $J=6$ Hz, 2H-6)]. The $^{13}\text{C-NMR}$ showed 15 signals including 9 aromatic quaternary carbons [C-2, C-3, C-4a, C-8a, C-9, C-12, C-12a, C-14, C-14a] and six aromatic methine carbons signals at [C-1, C-4, C-8, C-10, C-11, C-13]. Base on these characteristics, we suggested that **(2)** was an isoquinoline alkaloid. Besides, the HMBC spectrum showed cross-peak of correlations between H-1 and C-4; H-6 and C-8, C-4, C-4a. Through comparison of NMR data with the ones in the literature [5], **(2)** was identified as 5,6-dihydro-2,3,9,12-tetramethoxydibenzoquinolizinium.

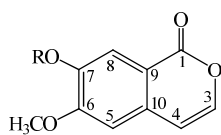
Compound **(3)** was obtained as a yellow oil. The $^1\text{H-NMR}$ spectrum of compound **(3)** showed two aromatic singlets [at δ 6.54 (s, H-5) and 7.34 (s, H-8)], a methylene protons [δ_{H} 3.30 (m, 2H-3)] and an oxymethylene protons [2.70 (t, $J=6.5$, 2H-4)]. The $^{13}\text{C-NMR}$ together with HSQC spectra showed the presence of 10 carbons signals including of six aromatic carbons, a carbonyl carbon, a methylene carbon and an oxymethylene carbon. The HMBC spectrum showed correlations between H-5 and C-4, C-7, C-9; H-8 and C-1, C-7, C-9; H-4 and C-5, C-9. All above data suggested **(3)**'s skeleton was isochroman-1-one. Besides, HMBC correlations also indicated the position of two methoxyl groups at C-6 and C-7. The comparison of NMR data of compound **(3)**

with published report [6] showed that **(3)** was 7-hydroxy-6-methoxyisochroman-1-one.

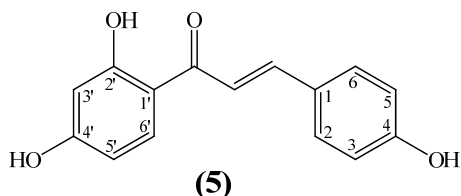
Compound **(4)** was also obtained as a yellow oil. Spectroscopic data of compound **(4)** showed that it was also an isochroman-1-one because of the similarity in NMR spectra of **(4)** and those of **(3)**. However, the ^1H and $^{13}\text{C-NMR}$ spectra of **(4)** showed that compound lost one oxymethyl group signal. Moreover, NMR data of **(4)** showed good compatibility to the ones in literature [7] so compound **(4)** was proposed to be 6,7-dimethoxyisochroman-1-one.

Compound **(5)** was obtained as a yellow oil. The $^1\text{H-NMR}$ of compound **(5)** showed the presence of an ABX system [7.71 (d, 8.4, H-5), 6.56 (dd, 8.4 & 2.4, H-6), 6.41 (d, 2.4, H-8)] for a tri-substituted benzene ring, a para-substituted benzene ring [7.39 (d, 8.4, H-2',6'), 6.89 (d, 8.4, H-3',5')]. Moreover, the presence of a methylene proton [2.66 (dd, 17.0 & 3.0, H-3a), 3.03 (dd, 17.0 & 13.0, H-3b)] and an oxymethine proton [5.44 (dd, 12.8 & 3.0, H-2)] revealed that compound **(5)** is a flavanone. This conclusion was in agreement with the observed $^{13}\text{C-NMR}$ data [8]. These was further confirmed by the HMBC correlation and verified the compound **(5)** is 4',7-dihydroxyflavanone.





(3) R = H
(4) R = CH₃



(5)

4. CONCLUSION

From the chloroform extract of the stem of *Mahonia nepalensis*, five compounds were isolated including: 1-*C*-syringylglycerol 4-*O*- β -D-glucopyranoside (1), 5,6-dihydro-2,3,9,12-tetramethoxydibenzo quinolizinium (2), 7-hydroxy-6-methoxyisochroman-1-one (3), 6,7-dimethoxyisochroman-1-one (4) and 4',7-dihydroxyflavanone (5). These chemical structures were identified based on NMR spectroscopic analysis and comparison between these data and those of reported in the literatures. In addition, these compounds were different from previous reports about chemical constituents of *Mahonia nepalensis* in the world.

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