CHEMICAL CONSTITUENTS FROM FRUITS OF HYDNOCARPUS HAINANENSIS MERR. (FLACOURTIACEAE) IN VIETNAM

Nguyen Thanh Tra1,2, Truong Bich Ngan1, Doan Thi Mai Huong1, Marc Litaudon3, Nguyen Van Hung1, Do Thi Thao1, Chau Van Minh1, Pham Van Cuong1

1Institute of Marine Biochemistry - VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
2Institute of Chemistry - VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
3Institute of Natural Product Chemistry, 91190 Gif-sur-Yvette Cedex, France
4Institute of Biotechnology - VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

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Abstract

Five compounds were isolated from the fruits of Hydnocarpus hainanensis Merr. Sleum. (Flacourtiaceae). Their structures were determined by spectroscopic analysis including MS and NMR. The isolates were identified as tarakophyllin (1), hydnocarpic acid (2), 3,4-dihydroxybenzyl alcohol (3), 3,4-dihydroxybenzoic acid (4) and 3-hydroxy-4-methoxybenzoic acid (5).

Keywords. Hydnocarpus hainanensis, Flacourtiaceae, cyclopentenoid cyanohydrin glucosides.

1. INTRODUCTION

Flacourtiaceae comprises about 89 genera with 1300 species found throughout the tropical and temperate regions of the world [1]. Genus of Hydnocarpus consists of about 40 species, many of them have been used in folk medicine [2]. Previous studies showed that the genus Hydnocarpus contains flavonoligans, flavonones, phenolic and acid chaumoorgic which exhibited antibacterial, antioxidant and anticancer activities [3-7]. In continuation of our research of bioactive compounds from the plants of Flacourtiaceae family, further purification of the crude extract of Hydnocarpus hainanensis fruits has led to the isolation of five compounds 1-5.

2. MATERIAL AND METHODS

2.1. General experimental procedures

Optical rotations were recorded on a Polax-2 L polarimeter. Melting points were determined using a Buchi B-545 instrument. ESI-MS were obtained on an Agilent 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on Bruker 500.13 MHz spectrometer, operating at 500.13 MHz for $^1$H and 125.76 MHz for $^{13}$C NMR, respectively.

2.2. Plant material

Fruits of H. hainanensis Merr. were collected from Quang Tri, Vietnam in November 2006. A voucher specimen (VN-1761) was deposited at the Institute of Ecology and Biological resources, Vietnam Academy Science and Technology.

2.3. Extraction and isolation

Dry powders (0.65 kg) of the fruits of H. hainanensis were extracted with ethanol (5 x 1.5 L). The solvents were removed under diminished pressure. The residue (101.7 g) was suspended in water (0.5 L) and then partitioned successively with n-hexane, EtOAc. The n-hexane, EtOAc and water solutions were concentrated to dryness, affording 43 g, 20 g and 30 g, respectively.

n-Hexane extract (43 g) was fractionated by column chromatography (CC) on silica gel, eluting with n-hexane/EtOAc gradient to yield 8 fractions. Fractions 3 (1.7 g) was purified on silica gel CC, eluted with CH$_2$Cl$_2$/EtOAc gradient to obtain compound 5 (23 mg).

EtOAc extract (20 g) was subjected to CC on silica gel, eluted with CH$_2$Cl$_2$/MeOH gradient to
furnish 7 fractions. Fraction 3 (1.1 g) was purified by CC on silica gel (CH$_2$Cl$_2$/acetone gradient) to afford compound 7 (5 mg). Fraction 4 (1.3 g) was separated on silica gel CC (CH$_2$Cl$_2$/MeOH gradient), followed by preparative TLC (CH$_2$Cl$_2$/MeOH: 95/5) to obtain compound 8 (7 mg). Fraction 5 (1.6 g) was purified on silica gel CC (CH$_2$Cl$_2$/MeOH gradient), followed by CC on Sephadex LH-20 (MeOH) affording compound 6 (7 mg).

Water extract (30 g) was chromatographed on C-18 (MeOH/H$_2$O gradient) to give 6 fractions. Fraction 4 (1.1 g) was separated by CC on Sephadex to afford two subfractions. Subfraction 1 (0.6 g) was separated by CC on Sephadex LH-20 following by preparative TLC (CH$_2$Cl$_2$/dioxan: 9/1) to give compound 2 (7 mg) and 3 (8 mg). Subfraction 2 (0.3 g) was purified by CC on Sephadex LH-20 (MeOH) to obtain compound 1 (5 mg). Fraction 5 (1.8 g) was separated by Sephadex LH-20 CC (MeOH) yielding two subfractions. Subfraction 2 (0.6 g) was subjected to CC on silica gel (CH$_2$Cl$_2$/MeOH gradient), followed by Sephadex LH-20 CC (MeOH) to give compound 4 (5.5 mg). Fraction 3 (350 mg) was separated by CC on silica gel, eluted with n-hexane/acetone gradient to give compound 6 (59 mg).

**Tarakophyllin (1):** Colorless syrup; [a]$_D$ -181 (c 0.083, MeOH). ESI-MS (m/z): 310.0 [M+Na]$^+$. $^1$H-NMR (500 MHz, CD$_3$OD): 6.16 (1H, dd, $J = 5.5$ and 1.5 Hz, H-2), 6.25 (1H, dd, $J = 5.5$ and 2.0 Hz, H-3), 4.83 (1H, m, H-4), 2.25 (1H, dd, $J = 4.5$ and 14.5 Hz, H-5), 3.06 (1H, dd, $J = 6.0$ and 14.5 Hz, H-5), 4.69 (1H, d, $J = 8.0$ Hz, H-1’), 3.24 (1H, dd, $J = 7.5$ and 9.0 Hz, H-2’), 3.40 (1H, s, H-3’), 3.36 (1H, s, H-4’), 3.36 (1H, s, H-5’), 3.69 (1H, dd, $J = 5.0$ and 11.5 Hz, H-6’a), 3.89 (1H, dd, $J = 1.5$ and 11.5 Hz, H-6”b). $^{13}$C-NMR (125 MHz, CD$_3$OD): 82.5 (C-1), 132.9 (C-2), 142.3 (C-3), 74.1 (C-4), 47.9 (C-5), 101.4 (C-1’), 74.7 (C-2’), 77.9 (C-3’), 71.3 (C-4’), 78.2 (C-5’), 62.6 (C-6’), 120.1 (CN).

**Hydnocarpic acid (2):** Fatty oil [a]$_D$ +36 (c 0.20, CH$_2$Cl$_2$). ESI-MS (m/z): 253.0 [M+H]$^+$. $^1$H-NMR (500 MHz, CDCl$_3$): 5.68 (m, CH-5), 2.34 (t, CH$_2$-15), 2.30 (m, H-3a), 2.22 (m, H-3b), 2.00 (m, H-4a), 1.38 (m, H-4b), 1.62 (quint, $J = 7.5$ Hz, CH$_2$-14), 1.26 (8x CH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$): 179.2 (C-16), 135.5 (C-1), 130.0 (C-2), 45.6 (C-5), 36.2 (C-6), 33.9 (C-15), 32.0 (C-3), 29.9 (C-4), 29.6-28.0 (C7-C13), 24.2 (C-14).

**3,4-Dihydroxybenzyl alcohol (3):** Colorless oil. ESI-MS (m/z): 140 [M+H]$^+$. $^1$H-NMR (500 MHz, CD$_3$OD): 8.02 (d, $J = 2.5$ Hz, H-2), 7.37 (d, $J = 8.5$ Hz, H-6), 7.24 (d, $J = 2.5, 8.5$ Hz, H-5), 4.60 (CH$_2$-O).

**3,4-Dihydroxybenzoic acid (4):** White powder. $^1$H-NMR (500 MHz, CD$_3$OD): 7.45 (d, $J = 1.5$ Hz, H-2), 7.43 (dd, $J = 1.5$ Hz and 8.0, H-6), 6.80 (d, $J = 8.0$ Hz, H-5).

**3-Hydroxy-4-methoxybenzoic acid (5):** Light powder. ESI-MS (m/z): 169.1 [M+H]$^+$. $^1$H-NMR (500 MHz, CD$_3$OD): 7.58 (1H, d, $J = 1.7$ Hz, H-2), 7.55 (1H, dd, $J = 8.2$ and 1.7 Hz, H-6), 6.84 (dd, $J = 8.2$ Hz, H-5), 3.91 (3H, s, OCH$_3$).

3. RESULTS AND DISCUSSION

Compound 1 was optically active, [a]$_D$ -181 (c 0.083, MeOH). Its ESI-MS (positive) indicated the pseudomolecular ion peak at m/z 310.0 [M+Na]$^+$. The $^1$H-NMR spectrum of 1 showed the signals of oxymethylene protons from 3.24 to 4.69 ppm, two olefinic protons at $\delta$16.16 (dd, $J = 5.5$ and 1.5 Hz, H-2) and 6.25 (1H, dd, $J = 5.5$ and 2.0 Hz, H-3), and two protons of a methylene at $\delta$2.25 (dd, $J = 4.5$ and 14.5 Hz, H-5) and 3.06 (dd, $J = 6.0$ and 14.5 Hz, H-5). Analyses of the $^{13}$C-NMR and DEPT spectra with the aid of the HSQC spectrum of 1 indicated the presence of 12 carbons, including six oxymethines (one anomeric methine at $\delta$C 101.4, C-1’), two methylene groups (one of them was linked to oxygen as indicated by its chemical shifts ($\delta$C 62.6 and $\delta$H $J = 5.5$, $\delta$H $J = 6.0$ Hz). This observation suggested the presence of a sugar moiety. Analysis of the COSY spectrum of 1 revealed two spin-spin coupling systems: correlations of a sugar moiety and a connection starting from H-2 ($\delta$H 6.16) to CH$_2$-5 ($\delta$H 2.25, 3.06). Analysis of the coupling constants and chemical shifts of the sugar moiety [4.69 (d, $J = 8.0$ Hz, H-1’), 3.24 (dd, $J = 8.0$, 7.5 Hz, H-2’), 3.40 (t, $J = 7.5$ Hz, H-3’), 3.33 (overlapped, H-4’ and H-5’), 3.69 (dd, $J = 5.5$, 12.0 Hz, H-6’), 3.89 (dd, $J = 2.0$, 12.0 Hz, H-6’)] determined the presence of glucopyranose moiety in the structure of 1. In the HMBC spectrum of 1, the correlations of the protons H-2 ($\delta$H 6.16) and CH$_2$-5 ($\delta$H 2.25, 3.06) with C-1 ($\delta$C 82.5) and the nitrite carbon C-7 ($\delta$C 120.1) indicated the formation of the cycloheptene ring. The glucopyranosyl moiety was bonded to C-1 as shown by cross-peak of H-1’ ($\delta$H 4.69) with C-1 in the HMBC spectrum. The configuration of glucopyranosyl moiety was established by anti coupling constant of H-1’ ($J = 8.0$ Hz). Detailed analyses of the 2D NMR spectra and comparison of the NMR data and optical
rotation with reported values indicated the structure of 1 as taraktophyllin which was previously described [8].

Compound 2 was isolated as colorless oil and optically active, [α]D +36 (c 0.20, CH2Cl2). Its ESI-MS indicated the pseudo-molecular ion at m/z 253 [M+H]+. In the 1H NMR spectrum, the signals of two olefinic protons at δH 5.68 (H-1 and H-2) and the complex overlapped signals of protons in the aliphatic region were observed. The 13C and DEPT of 2 indicated the presence of a carboxylic carbon at δC 179.2 (C-16), two olefinic carbons at δC 135.5 (C-1), 130.0 (C-2), a sp3 methine at δC 45.6 (C-5), and twelve sp2 methylenes. Analysis of COSY spectrum of 2 defined the presence of a cyclopentene ring by a connection from H-2 (δH 5.68) to H-5 (δH 2.60) via H-2, CH2-3 and CH2-4. Thus, the remaining signals were assigned to the undecanoic acid side chain. The linkage of C-5/C-6 was determined by correlation of C-6 (δC 36.2) with H-1 in the HMBC spectrum. Complete analyses of the 2D NMR spectra allowed establishing the structure of 2 as hydnocarpic acid. This compound was previously isolated from several species of Hydnocarpus genus [9].

Compound 3 was obtained as colorless oil. The 1H NMR of 3 displayed the signals of an ABX aromatic system [δH 8.02 (d, J = 2.5 Hz, H-2), 7.37 (d, J = 8.5 Hz, H-6), 7.24 (d, J = 2.5, 8.5 Hz, H-5)] and two protons of an oxymethylene as singlet at δH 4.60. These NMR data were in agreement with those of 3,4-dihydroxybenzyl alcohol [10].

The 1H NMR spectrum of 4 also exhibited the presence of an ABX aromatic system as 3, forming from three protons at δH 7.45 (d, J = 1.5 Hz, H-2), 7.43 (dd, J = 1.5 Hz and 8.0, H-6) and 6.80 (d, J = 8.0 Hz, H-5). However, the signal of the oxymethylene was not observed in the 1H NMR spectrum of 4. This strongly suggested that the oxymethylene was oxidized into carboxylic acid group. This suggestion was confirmed by comparison of NMR data of 4 with those of previously reported for 3,4-dihydroxybenzyl acid [11, 12].

1H-NMR spectrum of 5 indicated the signals close to those of 4, except for the presence of an additional methoxy signal at δH 3.91. The ABX system was formed by signals of H-2 (δH 7.58, d, J = 1.7 Hz), H-5 (δH 7.55, dd, J = 8.2 and 1.7 Hz) and H-6 (δH 6.84, dd, J = 8.2 Hz). Comparison of the NMR data with reported data in the literature indicated the structure of 5 as 3-hydroxy-4-methoxybenzoic acid [12].

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Figure 1: Structures of compounds 1-5 isolated from the fruits of Hydnocarpus hainanensis Merr.

REFERENCES


**Corresponding author:** Pham Van Cuong  
Institute of Marine Biochemistry, VAST  
18 Hoang Quoc Viet, Cau Giay District, Hanoi, Vietnam  
E-mail: phamvc@imbc.vast.vn.