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Cytotoxic polyacetylenes and 5-hydroxymethylfurfural from the rhizomes of *Panax stipuleanatus*

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ABSTRACT

*Phytochemical study on the hexane and butanol extracts of the rhizomes of Vietnamese Panax stipuleanatus has led to the isolation and structural elucidation of three compounds. Two of which are polyacetylenes named stipudiol (**1**) and panaxytriol (**2**). The other is characterised as 5*-Hydroxymethylfurfural *(**3**). Their structures were determined by a combination of HR-MS and 2D NMR spectroscopy. In addition, all three compounds **1-3** have cytotoxicity against KB cell line. Especially, compound **1** has the strongest ones (IC_{50} value of 8.11 μg/mL).*

Key words: *Panax stipuleanatus*, polyacetylene, cytotoxicity.

INTRODUCTION

Rhizomes of *Panax stipuleanatus* have been used as a Vietnamese traditional medicine for a long time as a tonic and in the treatment of bruises, bleeding, and muscular pain [1]. Modern pharmacological researches revealed various effects on the cardiovascular, endocrine, and central nervous systems as well as antioxidant, anti-tumor, anti-inflammatory and anti-aging properties [2]. Chemical investigation on the extract of *Panax stipuleanatus* rhizomes revealed that they possessed oleanane-type triterpenoids [3] and polyacetylenes [4] with cytotoxic activity against HL-60 (leukemia) and HCT-116 (colon cancer) cell lines. This paper describes the isolation and structural elucidation of three compounds, recently isolated from *Panax stipuleanatus* rhizomes together with their cytotoxicity.

MATERIALS AND METHODS

Materials

Rhizomes of *Panax stipuleanatus* were collected in Sapa, northern of Vietnam in June 2014. The samples were identified by Nguyen Van Anh, at National Institute of Medicinal Materials. Voucher specimens are deposited at the Faculty of Chemistry, Hanoi University of Education (NQT-1402).

Methods

General

TLC was performed on silica gel plates (Kieselgel 60 F_{254}, Merck). Preparative HPLC was performed on a Jasco PU-2087 instrument with a UV-2070 and RI-2031 detectors using a Waters 5 SL-II column (10.0 x 250 mm), flow rate of 1.0 mL/min. NMR spectra were recorded on Varian Brucker Avance 500 MHz, using $CDCl_3$ as solvent. Chemical shifts are referenced to internal TMS (0 ppm, ^1H) and $CDCl_3$ (77.0 ppm, ^{13}C), respectively. The positive ion high-resolution ESI-MS were recorded on a Bruker Apex III Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, equipped with a 7 Tesla superconducting magnet.

Extraction and isolation

Dried powders of rhizomes of *Panax stipuleanatus* (1000 g) were extracted with methanol (10L x 3). The methanolic extract was concentrated to give a residue (200 g) which was further partitioned into *n*-hexane, BuOH and water. The *n*-hexane crude extract (25 g) was chromatographied by silica gel column, eluting by n-hexane/EtOAc gradient, followed by prep. HPLC with hexane/EtOAc (3/1) to give compound **1** (7.0 mg). Furthermore, the butanol extract (50 mg) was subjected to silica gel column, eluting with $CHCl_3$/MeOH/H_2O (25/2.5/0.1) to yield six sub-fractions. Sub-Fr2 (518 mg) was purified by reversed phase silica gel (Rp-18), using MeOH/H_2O (7/3) as solvent system to afford compounds **3** (5 mg) and compound **2** (4 mg).

Compound **1**: ESI-FTICR-MS: m/z $[M-H]^-$ calcd for $C_{18}H_{25}O_2$: 273.1855; found 273.1835. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.34 (1H, dd, J = 10.0, 16.0 Hz, H-9), 5.95 (1H, m, H-2), 5.78 (1H, dd, J = 1.5, 16.0 Hz, H-8), 5.49 (1H, dd, J = 6.0, 17.0 Hz, H-1), 5.26 (1H, dd, J = 6.0, 10.5 Hz, H-1), 4.98 (1H, d, J = 5.5 Hz, H-3), 4.20 (1H, m, H-10), 1.55 (2H, m, H-11), 1.28 (12H, m, H-12 to H-17), 0.88 (3H, t, J = 7.0 Hz, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.0 (C-9), 136.1 (C-2), 117.2 (C-1), 108.1 (C-8), 80.5 (C-4), 77.6 (C-7), 74.4 (C-6), 72.1 (C-10), 70.9 (C-5), 63.7 (C-3), 36.9 (C-11), 31,8 (C-16), 29.8 (C-14), 29.4 (C-13), 29.2 (C-15), 25.3 (C-12), 22.6 (C-17), 14.1 (C-18).

Compound **2**: ESI-FTICR-MS: m/z $[M+Na]^+$ calcd for $C_{17}H_{26}O_3Na$: 301.1780; found 301.1774. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.95 (1H, ddd, J = 5, 10.5, 17 Hz, H-2), 5.47 (1H, dd, J= 1,17 Hz, H-1), 5.26 (1H, dd, J = 1, 10 Hz, H-1), 4.92 (1H, d, J = 5 Hz, H-3), 3.64 (1H, d, J = 4.5 Hz, H-9), 3.59 (1H, m, H-10), 2.58 (2H, m, H-8), 1.49 (2H, m, H-11), 1.29 (10H, m, H-13 to H-16), 0.89 (3H, t, J = 7.0 Hz, H-17). ^{13}C NMR (125 MHz, $CDCl_3$): δ 117.3 (C-1), 136.2 (C-2), 78.0 (C-4), 74.9 (C-7), 73.2 (C-10), 72.3 (C-9), 69.8 (C-5), 66.7 (C-6), 63.7 (C-3), 33.7 (C-11), 31.9 (C-15), 30.8 (C-13), 29.7 (C-14), 25.1 (C-8), 22.8 (C-16), 14.2 (C-17).

Compound **3**: ESI-MS: m/z 149.4 $[M+Na]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.52 (1H, s, H-1), 7.37 (1H, d, J = 3.5 Hz, H-3), 6.57 (1H, d, J = 3.5 Hz, H-4), 4.60 (2H, s, H-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.4 (C-1), 163.2 (C-5), 153.9 (C-2), 124.7 (C-3), 110.9 (C-4), 57.6 (C-6).

Cytotoxicity assay: Compounds **1-3** were tested against cancer cell lines from American Type Culture Collection according to the method described by Scudiero et al. [5].

RESULTS AND DISCUSSION

The hexane and butanol extracts of the rhizomes of *Panax stipuleanatus* were subjected to silica gel column chromatography and followed by prep. HPLC to give three compounds (**1**-**3**).

Compound **1** was obtained as an oil with the molecular formula of $C_{18}H_{26}O_2$, which was found by ESI-FTICR-MS (m/z $[M-H]^-$ calcd for $C_{18}H_{25}O_2$: 273.1855; found 273.1835), with 6 degrees of unsaturation. Its ^{1}H NMR spectrum has five olefinic protons (δ_H 5.26; 5.48; 5.78; 5.95 and 6.34 ppm), two protons connected to carbon-bearing oxygen at 4.98 ppm and 4.20 ppm and one methyl group at 0.88 ppm. Analysis of its ^{13}C NMR spectrum revealed the presence of 18 carbon signals, including one methyl at 14.1 ppm, seven methylenes resonanced from 22.6 ppm to 36.9 ppm, four methines at 70.9 ppm; 74.4 ppm; 77.6 ppm and 80.5 ppm, two double bond. Then, the structure of compound **1** was determined by HSQC and HMBC spectra. The long range correlations from methyl group with two methylenes at 22.6 ppm and 31.8 ppm suggested the presence of -CH_2-CH_2-CH_3 partial structure. Furthermore, H-1 and H-2 are coupled to C-3 in its HMBC; H-3 had HMBC correlations with C-2 and C-4 supporting the proposal structure. In comparison with the NMR spectral data with those of 1,8-octadecadiene-4,6-diyn-3,10-diol or stipudiol [4] revealed that compound **1** is stipudiol.

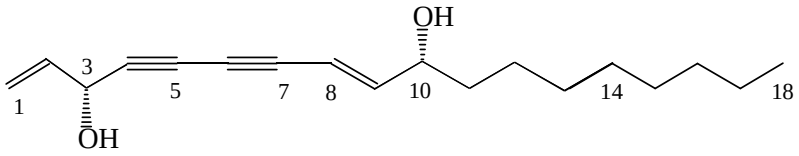
Compound **2** was isolated as an oil and showed a $[M+Na]^+$ ion peak at 301.1774 in its positive ESI-FTICR-MS, corresponding to the molecular formula of $C_{17}H_{26}O_3$. Its ^{1}H NMR spectrum showed the presence of typical polyacetylenes [4] with three olefinic protons at 5.95 ppm, 5.47 ppm, 5.26 ppm; three protons connected with carbon-bearing oxygen at 4.92 ppm (d, J = 5 Hz), 3.64 ppm (d, J = 4.5 Hz) and 3.59 ppm (m) and one methyl group at 0.89 ppm (t, J = 7 Hz). The ^{13}C NMR of compound **2** has 17 carbon signals, including one double bond (117.3 ppm and 136.2 ppm), together with two triple bonds at 78.0 ppm (C-4), 69.8 ppm (C-5), 66.7 ppm (C-6) and 74.9 ppm (C-7). The ^{1}H and ^{13}C NMR of compound **2** are identical with those of panaxytriol [6]. Consequently, compound **2** was characterized as panaxytriol as shown in Fig. 1.

Compound **3** had a molecular ion peak at m/z 149.4 $[M+Na]^+$ in ESI-MS. The ^{1}H NMR spectrum had a proton signal at 9.53 ppm, two olefinic protons at 7.37 ppm and 6.57 ppm with the small coupling constant J = 3.5 Hz. Its ^{13}C NMR spectrum exhibited the presence of six carbon signals (one aldehyde at 179.4 ppm, four olefinic carbons and one carbon-bearing oxygen). These 1D NMR spectra suggested the presence of furane ring in compound **3** [7].

Then, the structure of **3** was deduced from HSQC and HMBC spectra. There were HMBC correlations between H-1 (9.53 ppm) and C-2 (153.9 ppm); H-3, H-4 and C-2, C-5 (163.2 ppm), indicating that the aldehyde connected with C-2. In addition, H-6 (4.60 ppm) was coupled with C-4 (110.9 ppm) and C-5 (163.2 ppm) suggesting that the $-CH_2OH$ group attached with C-5 (163.2 ppm). From above discussion, compound **3** was found to be 5-Hydroxymethylfurfural [7].

Previous investigation showed that polyacetylenes isolated from *Panax* sp. exhibiting good cytotoxicity against HL-60 and HCT-116 cells [4]. In this research, all three isolated compounds (**1**-**3**) from *Panax stipuleanatus* were evaluated their cytotoxicity toward human epidermal carcinoma (KB) cells. The results showed that compounds **1** and **2** had strong activity with their IC_{50} values of 8.11 and 14.57 μg/mL, while compound **3** had weak activity (IC_{50} value of 101.97 g/mL).

Figure 1. Structures of compounds 1-3



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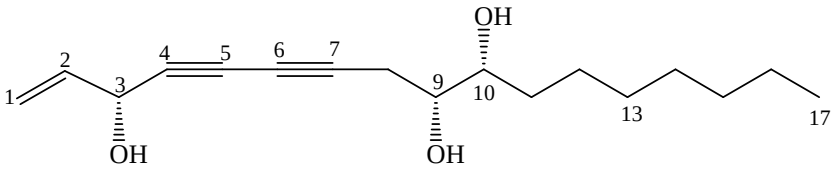

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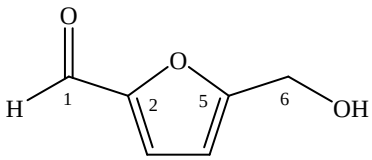

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CONCLUSION

Two polyacetylenes named stipudiol (**1**) and panaxytriol (**2**) together with 5-Hydroxymethylfurfural (**3**) were isolated from Vietnamese *Panax stipuleanatus*. All of them inhibited the growth of KB cell lines. Especially, compound **1** exhibited the strongest one (IC_{50} value of 8.11 μg/mL).

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