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THIOPHENE DERIVATIVES FROM THE AERIAL PART OF *PLUCHEA INDICA*

Yun-Qi Qiu,[1,2] Shu-Hua Qi,[1] Si Zhang,[*,1] Xin-Peng Tian, [1] Zhi-Hui Xiao,[1] Min-Yi Li,[1] and Qing-Xin Li[1]

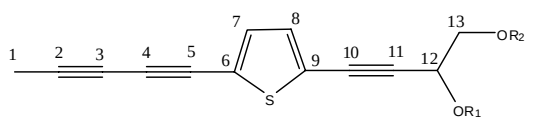
[1]Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, P. R. China; [2]Graduate School of the Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100049, P. R. China
phone: +86-20-89023103; fax: +86-20-84451672; e-mail: zhsimd@scsio.ac.cn

Abstract – Chemical investigation on the aerial part of *Pluchea indica* resulted in the isolation of two new thiophene derivatives, 2-(4-hydroxy-3- methoxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**1**) and 2-(4-*O*-β-glucopyranosyl-3-hydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**2**) along with three known thiophene derivatives 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**3**), 2-(3-acetoxy-4-hydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl) thiophene (**4**) and 2-(prop-1-yn-1-yl)-5-(6-acetoxy-5-hydroxyhexa-1,3-diyn-1-yl) thiophene (**5**). The structures of **1** and **2** were determined on the bases of extensive spectroscopic analysis, including 1D and 2D NMR data. Antimicrobial activities of compounds **1**-**5** were tested.

INTRODUCTION

Pluchea indica (Less.) (Asteraceae) is a shrub widely found in South China. In folk medicine a decoction of its root has been used to cure rheumatalgia, sciatica and cramp. The juice of its leaves has the capacity of detoxification and detumescence. It has been used for lumbago, leucorrhoea and dysentery in poultices and for the treatment of atonic and gangrenous ulcers.[1] The *P. indica* extract has been reported for its significant anti-inflammatory,[2] antiulcer, antioxidant[3] and neuropharmacological activity.[4] Some sesquiterpenes[5] and thiophene derivatives[6] have been isolated from the species. Thiophene derivates are

one of the characteristic chemical constituent of Asteraceae, and wildly exist in various species of Asteraceae. About one hundred thiophene derivates have been found in Asteraceae.[7,8] Thiophene derivates have been reported to have antibacterial, antiviral and antifungal activities.[9] Some of them exhibit their activities following photoactivation, while others, for example thiaruburine A showed insecticidal activity and cytotoxicity against hamster oocyte, *Escherichia coli*, and *Bacillus subtilis* without photoactivation.[10,11,12] In order to obtain bioactive new compounds from *P. indica*, we investigated on the chemical constituents of its aerial part, and two new thiophene derivatives, 2-(4-hydroxy-3-methoxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**1**) and 2-(4-*O*-β-glucopyranosyl-3-hydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**2**) along with other three known thiophenes, 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene[13] (**3**), 2-(3-acetoxy-4-hydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene[13] (**4**), 2-(prop-1-yn-1-yl)-5-(6-acetoxy-5-hydroxyhexa-1,3-diyn-1-yl)thiophene[6] (**5**) (Figure 1) were obtained. The structures of **1** and **2** were established by spectroscopic means. Herein, details of the isolation and structure elucidation of compounds **1** and **2**, and the antimicrobial activity of all the thiophene derivatives compounds **1**-**5** are presented.



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3 R_1=H R_2=H
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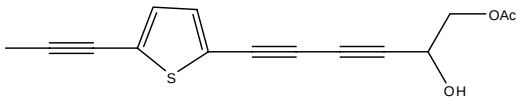

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Figure 1. Compounds **1** – **5**

RESULTS AND DISCUSSION

The ethanol extract of the plant was fractionated by successive extraction with hexane, ethyl acetate, and *n*-butyl alcohol. The hexane and ethyl acetate soluble fraction were separated by column chromatography over silica gel and Sephadex LH-20 respectively, followed by preparative thin-layer chromatography (PTLC) or HPLC when needed. The hexane part afforded **1**, **4** and **5** while the ethyl acetate part afforded **2** and **3**.

Compound **1** exhibited a molecular ion peak at *m/z* 267 $[M+Na]^+$ in its ESI-MS. Together with ^{1}H, ^{13}C NMR and DEPT spectral data (Table 1), a molecular formula of $C_{14}H_{12}O_2S$ was established and confirmed by HR-FAB-MS ($[M+Na]^+$, *m/z* 267.0468). The ^{1}H NMR spectrum exhibited signals for two methyl groups at δ_H 3.52, 2.05 (each 3H, *s*), two olefinic protons at δ_H 7.11 (1H, J = 3.8 Hz), 7.05 (1H, J = 3.8 Hz), one oxymethine at δ_H 4.29 (1H, *t*, J = 6.0 Hz), and two oxymethylenes at δ_H 3.79 (2H, *d*, J =

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1**-**3**.
(500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C NMR)[a)]

	1 (in $CDCl_3$)		**2** (in DMSO)		**3** (in Acetone)	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.05 (3H, *s*)	4.8	2.06 (3H, *s*)	4.4	2.04 (3H, *s*)	5.3
2		83.5		85.9		86
3		64.0		63.6		65.3
4		79.5		79.5		80.9
5		67.0		66.4		67.7
6		123.9		122.2		124.6
7	7.11 (1H, *d*, *J* = 3.8 Hz)	133.5	7.37 (1H, *d*, *J* = 3.7 Hz)	133.1	7.27 (1H, *d*, *J* = 3.7 Hz)	136.2
8	7.05 (1H, *d*, *J* = 3.8 Hz)	132.4	7.23 (1H, *d*, *J* = 3.7 Hz)	135.4	7.14 (1H, *d*, *J* = 3.7 Hz)	134.1
9		124.1		124.4		126.9
10		79.9		76.7		78.2
11		89.0		95.2		96.8
12	4.29 (1H, *t*, *J* = 6.0 Hz)	72.7	4.64 (1H, *dd*, *J* = 7.0, 4.3 Hz)	61.3	4.58 (1H, *d*, *J* = 6.0 Hz)	65.5
13	3.79 (2H, *d*, *J* = 6.0 Hz)	65.0	3.84 (1H, *dd*, *J* = 4.3, 10.5 Hz)	72.6	3.68 (2H, *t-like*, *J* = 6.0 Hz)	67.9
			3.58 (1H, *dd*, *J* = 7.0, 10.5 Hz)			
12-OH					4.69 (1H, *d*, *J* = 6.0 Hz)	
13-OH					4.12 (1H, *t*, *J* = 6.0 Hz)	
OMe	3.52 (3H, *s*)	57.1				
1’			4.23 (1H, *d*, *J* = 7.8 Hz)	103.5		
2’			2.96 (1H, *t*, *J* = 8.5 Hz)	73.5		
3’			3.14 (1H, *m*)	76.5		
4’			3.03 (1H, *t*, *J* = 9.0 Hz)	70.1		
5’			3.10 (1H, *m*)	77.0		
6’			3.41 (1H, *dd*, *J* = 6.0, 11.5 Hz)	61.1		
			3.64 (1H, *dd*, *J* = 2.6, 11.5 Hz)			

a) TMS was used as internal standard; δ_H in ppm, *J* values (Hz) are in parentheses. Assignments are based on HSQC and HMBC spectra.

6.0 Hz). The ^{13}C (DEPT) NMR spectra showed signals for two methyl carbons one of which was oxygenated (δ_C 4.8 and 57.1), two double bonds [δ_C 133.5 (*d*), 132.4 (*d*), 123.9 (*s*) and 124.1 (*s*)], three acetylenic bonds [δ_C 64.0 (*s*), 67.0 (*s*), 79.5 (*s*), 79.9 (*s*), 83.5 (*s*), and 89.0 (*s*)], one oxymethylene (δ_C 65.0) and one oxymethine (δ_C 72.7). These data were closely similar to those of compound **3**,[13] which suggested that **1** was a polyacetylene thiophene derivative. Analysis of the HSQC and HMBC data furnished the assignments of all ^{1}H and ^{13}C NMR signals. HMBC correlations (Figure 2) from δ_H 2.05 (3H, *s*) to δ_C 83.5 (C-2), 64.0 (C-3), 79.5 (C-4), 67.0 (C-5), 123.9 (C-6), 133.5 (C-7), from δ_H 7.11 (1H, *d*, *J* = 3.8 Hz) to δ_C 67.0 (C-5), and from δ_H 7.05 (1H, *d*, *J* = 3.8 Hz) to δ_C 123.9 (C-6) indicated a connection between the thienyl group and the methyl group through a butadiyne chain. HMBC correlations of methoxy proton δ_H 3.52 (3H, *s*) with δ_C 72.7 (C-12) suggested the methoxy group at C-12.

HMBC correlations of δ_H 3.79 (2H, *d*, *J* = 6.0 Hz) with δ_C 89.0 (C-11), 72.7 (C-12), δ_H 4.29 (1H, *t*, *J* = 6.0 Hz) with δ_C 79.9 (C-10), 89.0 (C-11), 65.0 (C-13), and δ_H 7.05 (1H, *d*, *J* = 3.8 Hz) with δ_C 79.9 (C-10) indicated the connection of 1-hydroxy-2-methoxy group with C-9 through an acetylene. Therefore, **1** was determined as 2-(4-hydroxy-3-methoxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene.

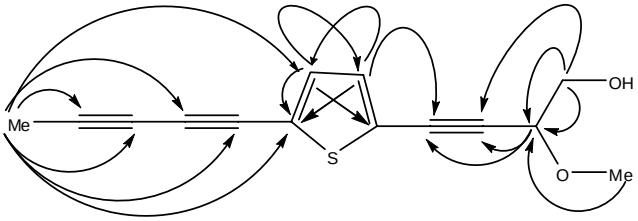

Figure 2. Key HMBC correlations for compound **1**

Compound **2** was obtained as yellow amorphous powder, and its molecular formula $C_{19}H_{20}O_7S$ was also determined by analysis of its HR-FAB-MS ($[M+Na]^+$, *m/z* 415.0818) and ^{13}C (DEPT) NMR spectra (Table 1). The ^1H and ^{13}C NMR spectral data of **2** were also closely similar to those of **3** with the only difference of an additional glucoside group. The presence of glucoside group was indicated by the ^{13}C NMR signals at δ_C 103.5, 73.5, 76.5, 70.1, 77.0, 61.1, ^1H NMR signals at δ_H 4.23 (1H, *d*, *J* = 7.8 Hz), 2.96 (1H, *t*, *J* = 8.5 Hz), 3.14 (1H, *m*), 3.03 (1H, *t*, *J* = 9.0 Hz), 3.10 (1H, *m*), 3.41 (1H, *dd*, *J* = 6.0, 11.5 Hz), 3.64 (1H, *dd*, *J* = 2.6, 11.5 Hz) and comparison with literature data.[14,15] Acid hydrolysis gave the glucose (Glc) as identified by co-TLC with an authentic glucose sample. The β-configuration at the anomeric center was assigned based on ^1H-^1H coupling constant (7.8 Hz), while the absolute configuration of the glucose wasn't determined. HMBC correlations (Figure 3.) from δ_H 4.23 (1H, *d*, *J* = 7.8 Hz) to δ_C 72.6 (C-13), and from δ_H 3.84 (1H, *dd*, *J* = 4.3, 10.5 Hz) to δ_C 103.5 (C-1') indicated the attachment of the glucopyranosyloxy group at C-13. Therefore, **2** was determined as 2-(4-*O*-β-glucopyranosyl-3-hydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene.

The spectroscopic data of compounds **3**-**5** were coincident with the known thiophene derivatives, 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene,[13] 2-(3-acetoxy-4-hydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene,[13] and 2-(prop-1-yn-1-yl)-5-(6-acetoxy-5-hydroxyhexa-1,3-diyn-1-yl)thiophene respectively.[6]

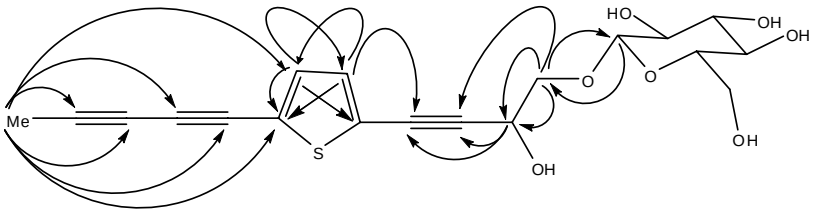

Figure 3. Key HMBC correlations for compound **2**

All thiophene derivatives were tested for antimicrobial activity against *Staphylococcus aureus, Bacillus thuringiensis, Escherichia coli, Saccharomyces cerevisiae, Rhizopus stolonifer,* and *Bacillus subtilis* using standard disc diffusion assay. The bioassay results revealed that compounds **1** - **5** all could selectively inhibit the growth of several bacteria (Table 2), which further proved that thiophene derivates had potent antimicrobial activities.[9] Not all tested microorganisms were sensitive to the positive controls (nalidixic acid and nystatin).

Table 2. Antimicrobial activities of compounds **1** - **5**

Compounds		Inhibiton zones (mm)[a)]				
	μg/disk	*E. coli.*	*S. cerevisiae*	*S. aureus*	*B. thuringiensis*	*B. subtilis*
1	100	4.25 ± 0.25 (clear)	1.00 ± 0.00 (clear)	1.88 ± 0.22 (clear)	2.63 ± 0.22	3.33 ± 1.25 (clear)
2	100	-	-	1.17 ± 0.24	-	1.00 ± 0.00
	200		1.13 ± 0.22	1.33 ± 0.47	1.67 ± 0.24	11.50 ± 0.50
3	100		1.00 ± 0.00	1.25 ± 0.43	-	9.50 ± 0.50
	50		-	1.50 ± 0.41	-	1.13 ± 0.22
4	100	-	-	-	1.00 ± 0.00	-
5	200	2.88 ± 0.54 (clear)	2.00 ± 0.82	1.75 ± 0.75 (clear)	2.38 ± 0.41 (clear)	2.17 ± 0.62 (clear)
	100	1.67 ± 0.47	1.00 ± 0.41	1.38 ± 0.41	2.00 ± 0.61	-
	50	1.00 ± 0.41	-	-	1.50 ± 0.61	-
nystatin		2.25 ± 2.28	7.50 ± 0.50	-	-	3.50 ± 3.04
nalidixic acid		9.5 ± 0.71	-	-	4.00 ± 0.00	9.75 ± 1.09

[a)]Paper disks (Φ 5mm), impregnated with these compounds, were incubated on agar plates containing microorganisms. Controls were nalidixic acid 125 μg/disc and nystatin 500 μg/disc. Data presented are the mean width of the inhibition zone (mm) ± standard error (*SE*) of three replicates. Clean and faint indicate complete growth inhibition and incomplete growth inhibition, respectively. Inactive (-).

EXPERIMENTAL

General Macroporous resin D101 (Nankai University Chemical Plant, Nankai, P. R. China), silica gel (200-300 mesh, Qingdao Haiyang Chemical Plant, Qingdao, P. R. China), and RP-18 silica gel (40-60 mesh, Merck) were used in Column chromatography (CC). Precoated silica gel G plates (Qingdao Haiyang Chemical Plant, Qingdao, P. R. China) was used in thin layer chromatography (TLC). HPLC was performed on a ODS column (250 × 10 mm i.d., YMC) with a Waters 996 photodiode array detector. NMR Spectra were recorded on a Bruker DRX-500 spectrometer with TMS as internal standard. Optical rotations were measured with a Perkin Elmer 341 high resolution polarimeter. UV spectra were recorded with a Varian cary 100 conc UV-Visible spectrophotometer. IR spectra were recorded with a Bruker VECTOR22 infrared spectrophotometer, in cm^{-1}. HR-FAB-MS were recorded with a MAT 95XP (Thermo) High Resolution mass spectrometer.

Plant Material The aerial part of *Pluchea indica* was collected in April 2005 from Sanya, Hainan province, P. R. China. The specimen was identified by Professor *Si Zhang*, the South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen has been deposited in the South China Sea Institute of Oceanoloy, Chinese Academy of Sciences (accession number: 0501).

Extraction and isolation The air-dried and powdered plant material (5 kg) was extracted with 95% ethanol at rt. After evaporation of EtOH under reduced pressure, 280 g of viscous residue was obtained. The residue was then suspended in water and extracted successively with hexane, EtOAc and *n*-BuOH. The hexane extract (70 g) was fractionated by column chromatography (CC) (Si gel, 0→50% EtOAc/petroleum ether) to yield Fractions 1-13 (H-Fr. 1-13). H-Fr. 7 (3 g) was fractionated by CC (Si gel, 10→50% EtOAc/petroleum ether) to yield H-Fr. 7h and then fractionated by CC (Sephadex LH-20, $CHCl_3$/MeOH 1:1) to yield H-Fr. 7hc then by CC (Si-gel, $CHCl_3$), then purified by preparative thin layer column (PTLC, Si-gel, 20% EtOAc/petroleum ether) to afford **1** (2 mg) and **4** (3 mg). H-Fr. 8 (2.2 g) was fractionated by CC (Si-gel, 10→50% EtOAc/petroleum ether) to yield H-Fr. 8d and then fractionated by CC (Sephadex LH-20, $CHCl_3$/MeOH 1:1) to yield H-Fr. 8dc then by CC (Si-gel, 10% acetone/petroleum ether) to afford **5** (6 mg). The EtOAc extract (66.5 g) was fractionated by CC (Si-gel, 40→50% EtOAc/petroleum ether, 10→50%Acetone/$CHCl_3$, 10→50% MeOH/ $CHCl_3$) to yield Fractions 1-12 (E-Fr. 1-12). E-Fr. 4 (17g) was fractionated by CC (Si-gel, 20→50% acetone/petroleum ether, 10→50% acetone/$CHCl_3$) to yield E-Fr. 4f and then fractionated by CC (Sephadex LH-20, $CHCl_3$/MeOH 1:1) to yield E-Fr. 4fc then by CC (Si-gel. 10% acetone/$CHCl_3$), finally purified by recrystallization to afford **3** (15 mg). E-Fr. 8 (3.7 g) was fractionated by CC (Si-gel, 7.5%→50% MeOH/ $CHCl_3$) to yield E-Fr. 8c and then fractionated by CC (Sephadex LH-20, $CHCl_3$/MeOH 1:1) to yield E-Fr. 8cd then by HPLC (ODS, 75% MeOH/H_2O) to afford **2** (2 mg).

2-(4-Hydroxy-3-methoxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (1): Yellow amorphous powder. UV λ_{max} (MeOH) nm: 319.7, 339.9, 247.6. IR (KBr) cm^{-1}: 3585, 2240. $[\alpha]_D^{20}$ +7.5°(*c* 0.0013, $CHCl_3$). ESI-MS *m/z*: 267 $[M+Na]^+$, 213 $[M-OMe]^+$. HR-FAB-MS: 267.0468 ($[M+Na]^+$, $C_{14}H_{12}NaO_2S^+$, calc. 267.0456). ^1H and ^{13}C NMR: Table 1.

2-(4-*O*-β-Glucopyranosyl-3-hydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (2): Yellow amorphous powder. UV λ_{max} (MeOH) nm: 319.7, 339.9, 247.5. IR (KBr) cm^{-1}: 3600, 2825, 2230. $[\alpha]_D^{20}$ -43.5°(*c* 0.0013, MeOH). ESI-MS *m/z*: 415 $[M+Na]^+$. HR-FAB-MS: 415.0818 ($[M+Na]^+$, $C_{19}H_{20}NaO_7S^+$, calc. 415.0827). ^1H and ^{13}C NMR: Table 1.

Acidic hydrolysis of compound 2. Compound **2** (1 mg) was dissolved in 5% HCl aqueous solution, and then heated in a boiling water bath for 5 h. After cooling, the reaction mixtures were neutralized with 10% aqueous Na_2CO_3 and glucose was identified by co-TLC with an authentic β-D-glucose sample (EtOAc-MeOH-H_2O-HOAc, 13:3:3:4; *Rf* 0.46).

Antimicrobial Activity Antimicrobial activity was determined by the paper disk method.[16] The test strains were six common microorganisms as gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus thuringiensis*, gram-negative bacterium *Escherichia coli*, and fungi *Saccharomyces cerevisiae* and *Rhizopus stolonifer*. Zero point two ml of the suspension of each strain (1×10^6 CFU/ML) was spread on an ISP2 agar plate. DMSO solutions of compounds **1** to **5** were prepared at concentrations of 20 mg/mL for **1**, **2**, and **4**, 40 mg/mL for **3**, 20 and 40 mg/mL for **5**. Each solution (5 μL) was added to one sterile paper disk (Φ = 5 mm) while using DMSO as negative control and nalidixic acid (5 μL, 25 mg/mL), nystatin (5 μL, 100 mg/mL) as positive control. The paper disks with three copies for each susceptible strain were placed on the inoculated ISP2 medium plates. The plates were incubated at 25 °C for 24 h. The diameters of inhibition zones were measured to determine the inhibition effects of test compounds.

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