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Echinopsacetylenes A and B, New Thiophenes from *Echinops transiliensis*

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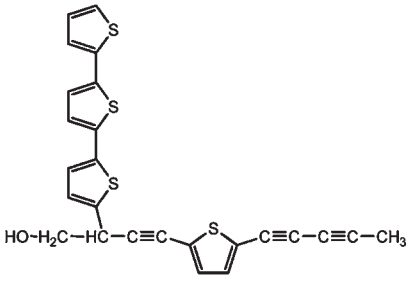
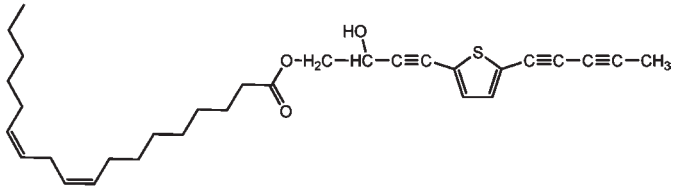
ABSTRACT



echinopsacetylene A (**1**)



echinopsacetylene B (**2**)

Two new polyacetylene thiophenes, echinopsacetylenes A and B (1 and 2), were isolated from the roots of *Echinops transiliensis*. The structures of 1 and 2 were elucidated on the basis of spectroscopic analyses and chemical transformations. Echinopsacetylenes A (1) is the first natural product possessing an α-terthienyl moiety covalently linked with another thiophene moiety. Echinopsacetylenes B (2) is the first natural thiophene conjugated with a fatty acid moiety. Echinopsacetylene A (1) showed toxicity against the Formosoan subterranean termite (*Coptotermes formosanus*).

Many *Echinops* and *Tagetes* species have been investigated resulting in the isolation of many bioactive thiophenes.[1] In our continuing study of the thiophenes from the *Echinops* species,[2] we isolated echinopsacetylenes A and B (**1** and **2**) possessing an α-terthienyl covalently linked with another thiophene moiety and a thiophene conjugated with a fatty acid moiety, respectively. In this paper, we describe the isolation and structure elucidation of **1** and **2**.

The roots (0.5 kg) of *Echinops transiliensis* were extracted with 3.5 L of DCM for 24 h at room temperature providing 8.2 g of DCM extractables. A portion of the DCM extract (3.0 g) was subjected to normal phase column chromatography followed by normal phase HPLC

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(3) Echinopsacetylene A (**1**): yellow solid; $[\alpha]^{25}_{D}$ −50.7° (*c* 0.073, $CHCl_3$); UV ($CHCl_3$) λ_{max} 325 and 345 nm (ε 41 800 and 48 000); IR (NaCl) ν_{max} 3401, 3069, 2926, 2234, 2151, 1721, 1509, 1448, 1425, 1374, 1333, 1208, 1057, 836, 795, 757, and 695 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 461.01853 $(M+H)^+$ (calcd for $C_{25}H_{16}OS_4$, 461.01806).

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Table 1. NMR Spectroscopic Data (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR, $CDCl_3$) for Echinopsacetylenes A (**1**) and B (**2**)

	1					**2**				
position	δC,	mult.[a]	δH	(*J* in Hz)	HMBC[b]	δC,	mult.[a]	δH	(*J* in Hz)	HMBC[b]
1	5.0,	CH_3	2.02,	s	2, 3, 4, 5, 6, 7	4.8,	CH_3	2.02,	s	2, 3, 4, 5, 6, 7
2	83.4,	C				83.6,	C			
3	64.4,	C				64.1,	C			
4	79.3,	C				79.6,	C			
5	66.8,	C				66.3,	C			
6	123.6,	C				124.3,	C			
7	133.6,	CH	7.09,	d (3.8)	5, 8	133.5,	CH	7.08,	d (3.8)	5, 6, 8, 9
8	132.0,	CH	7.04,	d (3.8)	7, 10	132.4,	CH	7.01,	d (3.8)	6, 7, 9, 10
9	124.5,	C				123.6,	C			
10	77.8,	C				79.0,	C			
11	92.2,	C				90.7,	C			
12	37.9,	CH	4.29,	t (6.4)	10, 11, 13, 14, 15	61.8,	CH	4.80,	m	10, 11, 13
13	67.3,	CH_2	3.94,	dd (10.7,6.4)	11, 12, 14	66.9,	CH	4.28,	d (5.3)	11, 12, 14
			3.90,	dd (10.7,6.4)	11, 12, 14					
14	139.4,	C				173.8,	C			
15	126.5,	CH	6.96,	d (3.5)	14	34.1,	CH_2	2.36,	t (7.5)	14, 16
16	123.4,	CH	7.03,	d (3.5)		24.9,	CH_2	1.62,	m	14, 15
17	136.8,	C				29.1–29.6,	CH_2	1.23–1.28,	m	
18	135.9,	C				29.1–29.6,	CH_2	1.23–1.28,	m	
19	124.3,	CH	7.03,	d (7.0)		29.1–29.6,	CH_2	1.23–1.28,	m	
20	124.3,	CH	7.05,	d (7.0)		29.1–29.6,	CH_2	1.23–1.28,	m	
21	136.3,	C				27.2,	CH_2	2.03,	m	22, 23
22	137.0,	C				128.0,	CH	5.31,	m	
23	123.7,	CH	7.15,	dd (3.5, 1.2)		130.2,	CH	5.36,	m	
24	127.9,	CH	7.00,	dd (3.5, 1.2)	22, 24	25.6,	CH_2	2.75,	t (6.7)	22, 23, 25, 26
25	124.5,	CH	7.20,	dd (5.1, 3.5)	22, 23, 24	130.0,	CH	5.36,	m	
26						127.9,	CH	5.31,	m	
27						27.2,	CH_2	2.03,	m	25, 26
28						29.1–29.6,	CH_2	1.23–1.28,	m	
29						31.2,	CH_2	1.27,	m	
30						22.6,	CH_2	1.28,	m	
31						14.1,	CH_3	0.87,	t (6.7)	29, 30

[a] Carbon multiplicities derived from DEPT 135° and 90° experiments. [b] HMBC correlations are from proton(s) stated to the indicated carbon.

to yield echinopsacetylenes A (**1**, 0.3 mg, 0.00016% yield)[3] and B (**2**, 3.2 mg, 0.00174% yield).[4] The roots (0.5 kg) of *E. transiliensis* were also extracted with 3.4 L of EtOH for 24 h at room temperature providing 10.7 g of EtOH extractables. A portion of the EtOH extract (5.7 g) was subjected to normal phase column chromatography followed by normal phase HPLC to yield **1** (1.1 mg, 0.00041% yield) together with the known thiophenes, α-terthienyl (**3**)[5] and 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl) thiophene (**4**).[6]

Echinopsacetylene A (**1**) had the molecular formula, $C_{25}H_{16}OS_4$, established by HRESIMS [*m*/*z* 461.0185-$(M+H)^+$, Δ 4.7 mmu] indicating eighteen degrees of unsaturation. The ^{13}C NMR, DEPT 90° and 135° spectra resolved 25 carbon signals comprising 13 quaternary carbons, 10 methine carbons, 1 methylene carbon, and 1 methyl carbon.

The gross structure of **1** was elucidated by analyses of one- and two-dimensional (1D and 2D) NMR spectra (Table 1, Figure 1). The ^{13}C NMR shifts and DEPT 135° of C-2, C-3, C-4, C-5, C-10, and C-11 revealed the presence of three acetylene groups. The ^{1}H−^{1}H COSY spectra of **1** showed one partial structural unit. The HMBC correlation for H-12 to C-11 and C-10 and their ^{13}C NMR shifts indicated the connection for C-12 to C-11 and C-11 to C-10. The HMBC correlation for H-8 to C-10, the ^{13}C NMR shift of C-9, and the reference to the literature data[6] suggested the connection for C-10 and C-8 through C-9 which is attached to sulfur. The HMBC correlation for H-8 to C-7 indicated the connection of C-8 and C-7. This connection was supported by the *J* (H-8/H-7) value. The HMBC correlations for H-7 to C-5, H-1 to C7, H-1 to C-6, and H-1 to C-5, and the ^{13}C NMR shift of C-6 suggested the connection for C-7 and C-5 through C-6, which is attached to sulfur. Such ^4J, ^5J, ^6J, and ^7J HMBC

(4) Echinopsacetylene B (**2**): yellow solid; $[\alpha]^{24}_D$ −3.6° (*c* 0.213, $CHCl_3$); UV ($CHCl_3$) λ_{max} 320 and 340 nm (ε 24 600 and 22 900); IR (NaCl) ν_{max} 3431, 3008, 2927, 2855, 2232, 2183, 2153, 1741, 1520, 1454, 1377, 1247, 1168, 1092, 1027, 805, and 725 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 515.2676 $(M+Na)^+$ (calcd for $C_{25}H_{16}OS_4$-Na, 515.2596).

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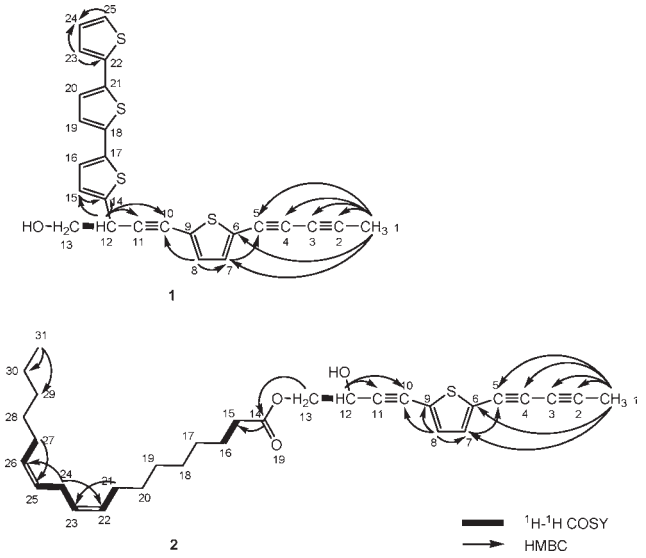

Figure 1. Selected 2D NMR correlations for echinopsacetylenes A (**1**) and B (**2**).

correlations are observed in this partial structure of polyacetylene thiophenes.[6] The HMBC correlation for H-1 to C-4, H-1 to C-3, and H-1 to C-2 and their ^{13}C NMR shifts indicated the connections for C-5 and C-4, C-4 and C-3, C-3 and C-2, and C-2 and C-1. The ^{1}H NMR shift of H-13 and ^{13}C NMR shifts of C-13 suggested that C-13 was connected to a hydroxy group. The HMBC correlation of H-12 to C-14, H-12 to C-15, and H-15 to C-14 and the ^{13}C NMR shift of C-14 indicated the connection for C-12 and C-15 through the quaternary olefinic C-14. The *J* (H-15/H-16) value suggested the connections for C-15 and C-16. The *J* (H-19/H-20) value suggested the connections for C-19 and C-20. The HMBC correlations for H-23 to C-22, H-23 to C-24, and H-25 to C-24 indicated the connection for C-22 to C-25 through C-23 and C-24. These connections were supported by the *J* (H-23/H-24) and *J* (H-24/H-25) values. The remaining structural details were elucidated on the basis of ^{13}C NMR data of isolated α-terthienyl (**3**) (Figure 2) data.[5,7] Thus, the structure of echinopsacetylene A was elucidated to be **1**. The absolute configuration of **1** was not determined due to the limited quantity.

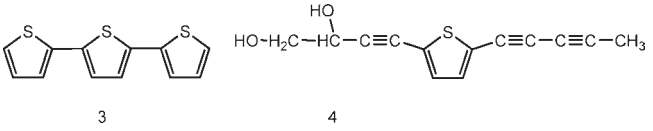

Figure 2. Structure of α-terthienyl (**3**) and 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**4**).

Echinopsacetylene B (**2**) had the molecular formula $C_{31}H_{40}O_3S$, established by HRESIMS [*m*/*z* 515.2676 $(M+Na)^+$, Δ 8.0 mmu], indicating eighteen degrees of unsaturation. The ^{13}C NMR and DEPT 90° and 135° spectra resolved 31 carbon signals comprising 9 quaternary carbons, including 1 carbonyl, 7 methine carbons, 13 methylene carbons, and 2 methyl carbons.

The gross structure of **2** was elucidated by analyses of 1D and 2D NMR spectra (Table 1, Figure 1). The ^{13}C NMR shifts and DEPT 135° of C-2, C-3, C-4, C-5, C-10, and C-11 revealed the presence of three acetylene groups. The ^{1}H−^{1}H COSY spectra of **2** showed five partial structural units. The HMBC correlation for H-12 to C-11 and C-10 and their ^{13}C NMR shifts indicated the connection for C-12 and C-11 and C-11 and C-10. The HMBC correlation for H-8 to C-10, H-8 to C-9 and the ^{13}C NMR shift of C-9 indicated a connection of C-10 and C-8 through C-9 which is attached to sulfur. The HMBC correlation for H-8 to C-7 indicated the connection of C-8 to C-7. This connection was supported by the *J* (H-8/H-7) value and corresponding COSY correlation. The HMBC correlations for H-7 to C-5, H-1 to C7, H-1 to C-6, and H-1 to C-5 and the ^{13}C NMR shift of C-6 indicated the connection for C-7 and C-5 through C-6 which is attached to S. The HMBC correlation for H-1 to C-4, H-1 to C-3, and H-1 to C-2 and their ^{13}C NMR shifts indicated the connections for C-5 and C-4, C-4 and C-3, C-3 and C-2, and C-2 and C-1. The ^{1}H NMR shift of H-12 and ^{13}C NMR shifts of C-12 suggested that C-12 was connected to a hydroxy group. The HMBC correlation for H-13 to C-14 and the ^{1}H NMR shift of H-13 and ^{13}C NMR shifts of C-13 indicated the connection for C-13 and C-14 thorough an oxygen atom. The HMBC correlation for H-15 to C-14 indicated the connection for C-14 and C-15. The HMBC correlation for H-21 to C-23, H-24 to C-22, H-24 to C-26, and H-27 to C-25 suggested the connections for C-22 to C-23 and C-25 to C-26. The HMBC correlation for H-31 to C-29 and H-31 to C-30 and their ^{13}C NMR shifts suggested the connection for C-29 to C-31 through C-30. The remaining structural details were elucidated on the basis of ^{13}C NMR data of authentic linoleic acid data as well as GC-MS spectrometry fragmentation data.[8,9]

Moreover, to confirm the structure of **2**, the GC-FID and GC-MS analyses of methanolysates of **2** by treatment

(7) α-Terthienyl (**3**): ^{13}C NMR data (150 MHz, $CDCl_3$) δC: 137.1, 137.1, 136.2, 136.2, 127.9, 127.9, 124.5, 124.5, 124.3, 124.3, 123.7, and 123.7.

(8) Linoleic acid: ^{13}C NMR data (150 MHz, $CDCl_3$) δC: 180.4, 130.2, 130.0, 128.0, 127.9, 34.1, 31.5, 29.6, 29.3, 29.1, 29.1, 29.0, 27.2, 27.2, 25.6, 24.6, 22.6, and 14.0

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(10) Extracts were dissolved in ethanol solvent. Solutions were pipetted onto Whatman #1 filter paper standardized at a rate of 0.15 mol/g filter paper. The solvent was allowed to evaporate from the filter paper overnight. Treated filter papers were placed in the bottom of glass vials (20 mm diameter × 50 mm) and moistened with water. Glass vials were capped with aluminum foil punctured with pin holes for aeration. Twenty *Coptotermes formosanus* workers (third instar or greater as determined by size) and a single soldier were placed on each treatment. Treatments were replicated four times and held separate from other treatments to prevent vapor contamination. Each replicate originated from a different *C. formosanus* colony to prevent a more sensitive colony from overly biasing the results. Treatments were maintained at ca. 100% RH and 27 °C in the dark. Filter paper receiving water alone served as controls. It was previously determined that the solvent alone had no discernible effect on termite mortality or filter paper removal.

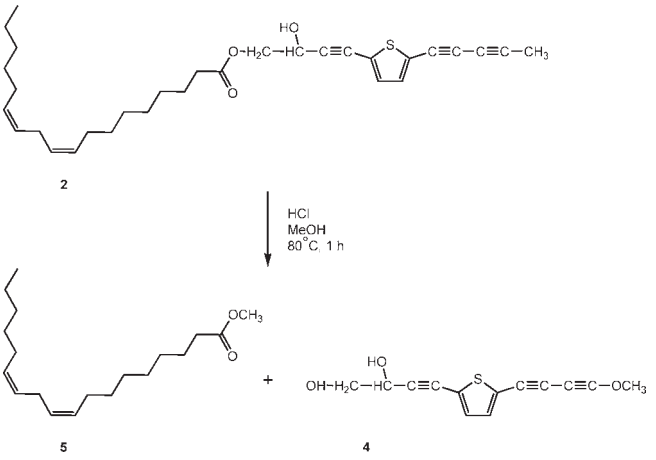

Figure 3. Chemical transformations from echinopsacetylene B (**2**) to methyl linoleate (**5**) and 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**4**).

with 3 N methanolic HCl were conducted (Figure 3). In GC-FID analysis, the retention times of **5** and **4** were the same as those for methyl linoleate and 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene. In GC-MS analysis, the signals of **5** and **4** were the same as those for methyl linoleate and 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene. Thus, the structure of echinopsacetylene B was elucidated to be **2**. The absolute configuration of **2** was not determined due to the limited quantity of **2**.

Echinopsacetylenes A and B (**1** and **2**) are the first natural products possessing an α-terthienyl moiety covalently linked with another thiophene moiety and a thiophene conjugated with a fatty acid moiety, respectively. Echinopsacetylenes A and B (**1** and **2**) might be generated from two molecules of linoleic acid since both 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene and α-terthienyl are derived from linoleic acid via trideca-3,5,7,9,11-pentayn-1-ene. Echinopsacetylene A (**1**) might be generated from the dehydration reaction of α-terthienyl (**3**) and 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**4**). This reaction would likely be controlled by an enzyme because echinopsacetylene A (**1**) is optically active. Echinopsacetylene B (**2**) might be generated from the dehydration reaction of 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**4**) and linoleic acid (**5**).

Table 2. Effects of Echinopsacetylenes A and B (**1** and **2**), α-Terthienyl (**3**), and 2-(3,4-Dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**4**) on the Mortality Rate of Termites (*Coptotermes formosanus*)[a–c]

	mortality (%), days after treatment			
compd	2	4	6	8
1	0.0B	0.0B	13.8B	56.3B
2	0.0B	0.0B	0.0B	0.0C
3	26.3A	67.5A	97.5A	100.0A
4	0.0B	0.0B	0.0B	0.0C
control	0.0B	0.0B	0.0B	0.0C

[a] Concentrations of compounds in the treatments were 0.15 mol/g filter. [b] Values are means of four experiments of twenty workers. [c] Means within a column followed by only the same capital letter do not differ significantly (SNK, $P < 0.05$).

Echinopsacetylene A (**1**) and α-terthienyl (**3**) showed toxicity against the Formosoan subterranean termite (*Coptotermes formosanus*) (Table 2).[10] However, echinopsacetylene B (**2**) and 2-(3,4-dihydroxybut-1-yn-1-yl)-5-(penta-1,3-diyn-1-yl)thiophene (**4**) did not show a toxicity effect. Thus, an α-terthienyl moiety might play an important role in the toxicity effect against the Formosan subterranean termite.

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Supporting Information Available. Detailed experimental section and 1D and 2D NMR data for echinopsacetylenes A and B. This material is available free of charge via the Internet at http://pubs.acs.org.