

Toxicity of Thiophenes from *Echinops transiliensis* (Asteraceae) against *Aedes aegypti* (Diptera: Culicidae) Larvae

by Hiroshi Nakano^{a)}, Abbas Ali^{d)}, Junaid Ur Rehman^{d)}, Leonid K. Mamonov^{e)}, Charles L. Cantrell^{e)}, and Ikhlas A. Khan^{d)}

^{a)} NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan
(phone: +81-29-8388952; fax: +81-29-8388837; e-mail: nakanohr@affrc.go.jp)

^{b)} NARO Agricultural Research Center for Kyushu Okinawa Region, 496 Izumi, Chikugo 833-0041, Japan

^{c)} USDA-ARS, Natural Products Utilization Research Unit, University, Mississippi 38677, USA

^{d)} The University of Mississippi, National Center for Natural Products Research, University, MS 38677, USA

^{e)} Institute of Plant Biology and Biotechnology, Timiriazeva 45, Almaty 05040, Republic of Kazakhstan

Structure–activity relationships of nine thiophenes, 2,2':5',2''-terthiophene (**1**), 2-chloro-4-[5-(penta-1,3-diyne-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate (**2**), 4-(2,2'-bithiophen-5-yl)but-3-yne-1,2-diyl diacetate (**3**), 4-[5-(penta-1,3-diyne-1-yl)thiophen-2-yl]but-3-yne-1,2-diyl diacetate (**4**), 4-(2,2'-bithiophen-5-yl)-2-hydroxybut-3-yn-1-yl acetate (**5**), 2-hydroxy-4-[5-(penta-1,3-diyne-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate (**6**), 1-hydroxy-4-[5-(penta-1,3-diyne-1-yl)thiophen-2-yl]but-3-yn-2-yl acetate (**7**), 4-(2,2'-bithiophen-5-yl)but-3-yne-1,2-diol (**8**), and 4-[5-(penta-1,3-diyne-1-yl)thiophen-2-yl]but-3-yne-1,2-diol (**9**), isolated from the roots of *Echinops transiliensis*, were studied as larvicides against *Aedes aegypti*. Structural differences among compounds **3**, **5**, and **8** consisted in differing AcO and OH groups attached to C(3'') and C(4''), and resulted in variations in efficacy. Terthiophene **1** showed the highest activity (LC_{50} , 0.16 $\mu\text{g/ml}$) among compounds **1–9**, followed by bithiophene compounds **3** (LC_{50} , 4.22 $\mu\text{g/ml}$), **5** (LC_{50} , 7.45 $\mu\text{g/ml}$), and **8** (LC_{50} , 9.89 $\mu\text{g/ml}$), and monothiophene compounds **9** (LC_{50} , 12.45 $\mu\text{g/ml}$), **2** (LC_{50} , 14.71 $\mu\text{g/ml}$), **4** (LC_{50} , 17.95 $\mu\text{g/ml}$), **6** (LC_{50} , 18.55 $\mu\text{g/ml}$), and **7** (LC_{50} , 19.97 $\mu\text{g/ml}$). These data indicated that *A. aegypti* larvicidal activities of thiophenes increase with increasing number of thiophene rings, and the most important active site in the structure of thiophenes could be the tetrahydro-thiophene moiety. In bithiophenes, **3**, **5**, and **8**, *A. aegypti* larvicidal activity increased with increasing number of AcO groups attached to C(3'') or C(4''), indicating that AcO groups may play an important role in the larvicidal activity.

Introduction. – Mosquitoes transmit pathogens that cause serious human diseases including malaria, Japanese encephalitis, yellow fever, dengue, and filariasis. The urban-adapted *Aedes aegypti* mosquito has become widely distributed across tropical and subtropical latitudes. It emerged from Africa during the slave trade in the 15th through 19th centuries, spread to Asia through commercial exchanges in the 18th and 19th centuries, and has spread globally with the advent of increased travel and trade in the past 50 years [1]. Dengue fever is by far the most rapidly expanding vector borne disease with an estimation of 50–100 million infections occurring annually [2]. Insecticides from various chemical groups are the basic tools used for management of mosquito populations. Due to continuous use of insecticides, mosquitoes have

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE 2014	2. REPORT TYPE	3. DATES COVERED 00-00-2014 to 00-00-2014			
4. TITLE AND SUBTITLE Toxicity of Thiophenes from Echinops transiliensis (Asteraceae) against Aedes aegypti (Diptera: Culicidae) Larvae		5a. CONTRACT NUMBER			
		5b. GRANT NUMBER			
		5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S)		5d. PROJECT NUMBER			
		5e. TASK NUMBER			
		5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.S. Department of Agriculture -Agricultural Research Service,Natural Products Utilization Research Unit,University,MS,38677		8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)			
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified	Same as Report (SAR)	9	

developed resistance against these chemicals, and vector population management has become difficult [3]. The use of synthetic pesticides also poses serious human health and environmental concerns [4]. Hence, natural product-based products have gained special importance as potential new pesticides.

Plants are a rich source of bioactive compounds [5]. Thiophenes, derived from *Echinops* and *Tagetes* species [6][7], are reported to have strong toxicities against insects [8], plants [9], and plant pathogenic fungi [10]. Recently, thiophene compounds **1**, **2**, and **9** (cf. Fig. 1), which exhibit toxicity against the Formosan subterranean termite (*Coptotermes formosanus*), have been isolated from *E. transiliensis* collected from the Republic of Kazakhstan [11][12]. However, phytochemical studies of *E. transiliensis* remained limited. Compound **1** and related thiophenes have been reported to be toxic against mosquito (*A. aegypti* and *A. atropalpus*) larvae [13–15], whereas structure–activity relationships on thiophenes as larvicides have not been systematically studied. In our continuing study of the thiophenes from *Echinops* species [12][16], we isolated

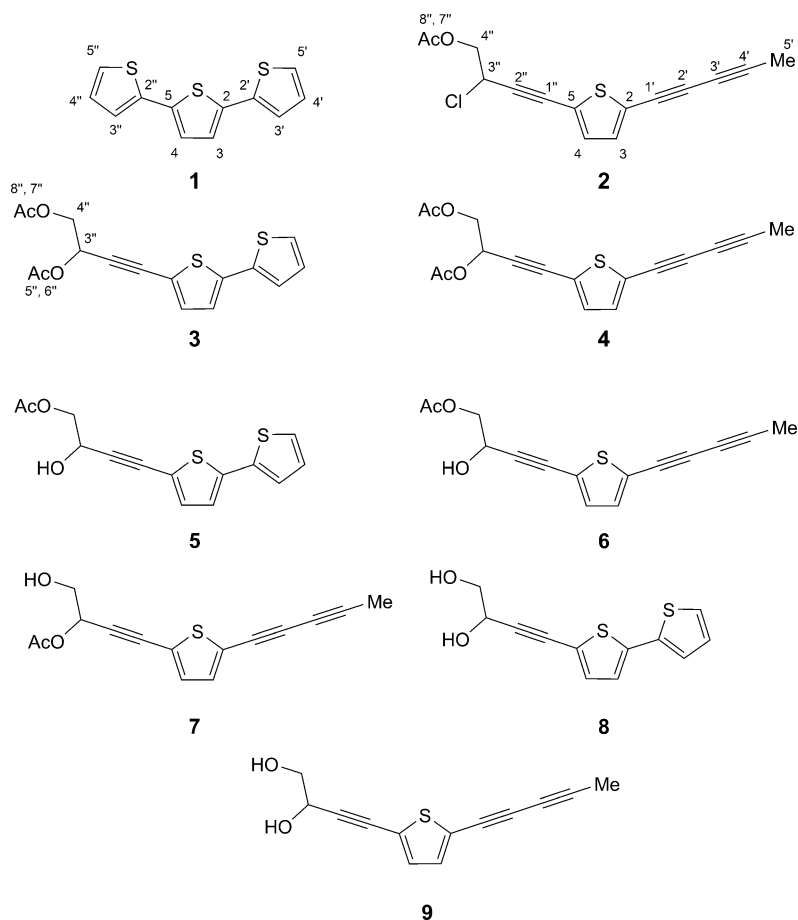


Fig. 1. Structures of compounds 1–9

six known thiophene compounds **3–8** (Fig. 1). Herein, we describe the isolation and structure elucidation of thiophene compounds **3–8**, and the structure–activity relationships of compounds **1–9** as larvicides against *A. aegypti*.

Results and Discussion. – *Isolation and Identification of Compounds 3–8.* To isolate thiophenes from the roots of *E. transiliensis*, the EtOH extract was subjected to normal-phase column chromatography followed by normal-phase HPLC to yield compounds **2–8** (Fig. 2). 2,2':5',2''-terthiophene (**1**) and 4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diol (**9**) have been previously isolated from the EtOH extracts [12]. To further isolate the thiophenes from the roots of *E. transiliensis*, the CH₂Cl₂ extract was subjected to normal-phase column chromatography, followed by normal-phase HPLC to yield compounds **3–9** (Fig. 2). Compounds **1** and **2** have been previously isolated also from the CH₂Cl₂ extracts [11]. The gross structures of compounds **3**, **4**, and **6–8** (Fig. 1) were elucidated by analyses of EI-MS, and ¹H- and ¹³C-NMR data, which were in complete agreement with literature data of 4-(2,2'-bithiophen-5-yl)but-3-yne-1,2-diyl diacetate (**3**) [17], 4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diyl diacetate (**4**) [6], 2-hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate (**6**) [6], 1-hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-2-yl acetate (**7**) [6], and 4-(2,2'-bithiophen-5-yl)but-3-yne-1,2-diol (**8**) [18], respectively. Compound **5** was analyzed by EI-MS, and ¹H- and ¹³C-NMR, and identified as 4-(2,2'-bithiophen-5-yl)-2-hydroxybut-3-yn-1-yl acetate. This is the first report on the presence of compounds **3–8** in *E. transiliensis*.

Structure–Activity Relationships of Compounds 1–9 as Toxicants against A. aegypti Larvae. It has been reported that compound **1** showed toxicity against *A. aegypti* and blackfly (*Simulium vittatum*) larvae, adult nematodes (*Caenorhabditis elegans*) [8], and adult termites (*C. formosanus*) [11], while bithiophenes or monothiophenes have not been systematically studied as toxicants against *A. aegypti* larvae. Furthermore, there are no previous reports on the precise relationship between the number of thiophene rings present and the toxic activity against *A. aegypti* larvae. Compound **1** belongs to the class of compounds designated as terthiophene, compounds **3**, **5**, and **8** are designated as bithiophenes, and compounds **2**, **4**, **6**, **7**, and **9** are monothiophenes (Fig. 1). The structural difference between compounds **3** and **4** consists in differing structural motifs at C(2). At C(2) of **3**, there is a thiophen-2-yl moiety, while C(2) of **4** carries a penta-1,3-diyn-1-yl group. The structural difference between compounds **5** and **6** consists in differing structural motifs at C(2). At C(2) of compound **5**, a thiophen-2-yl is attached, while C(2) of **6** bears a penta-1,3-diyn-1-yl moiety. Similar differences were observed between compounds **8** and **9**: C(2) of compound **8** is attached to a thiophen-2-yl moiety, while that of **9** is attached to a penta-1,3-diyn-1-yl group. Compound **1** showed the highest activity among the compounds tested in this study (Table 1). Based on non-overlapping 95% confidence intervals (CIs), **3**, **5**, and **8** with a thiophen-2-yl substituent at C(2) showed significantly higher activities than compounds **4**, **6**, and **9** with penta-1,3-diyn-1-yl groups at C(2), respectively. This was a clear indicator that larvicidal activities of thiophenes against *A. aegypti* increased with increasing number of thiophene rings, and the most important active site in the structure of thiophenes could be the attached thiophene skeleton. Structural differences between **3**, **5**, and **8** consist in functional groups attached to C(3'') and C(4'') (Fig. 1). AcO groups are

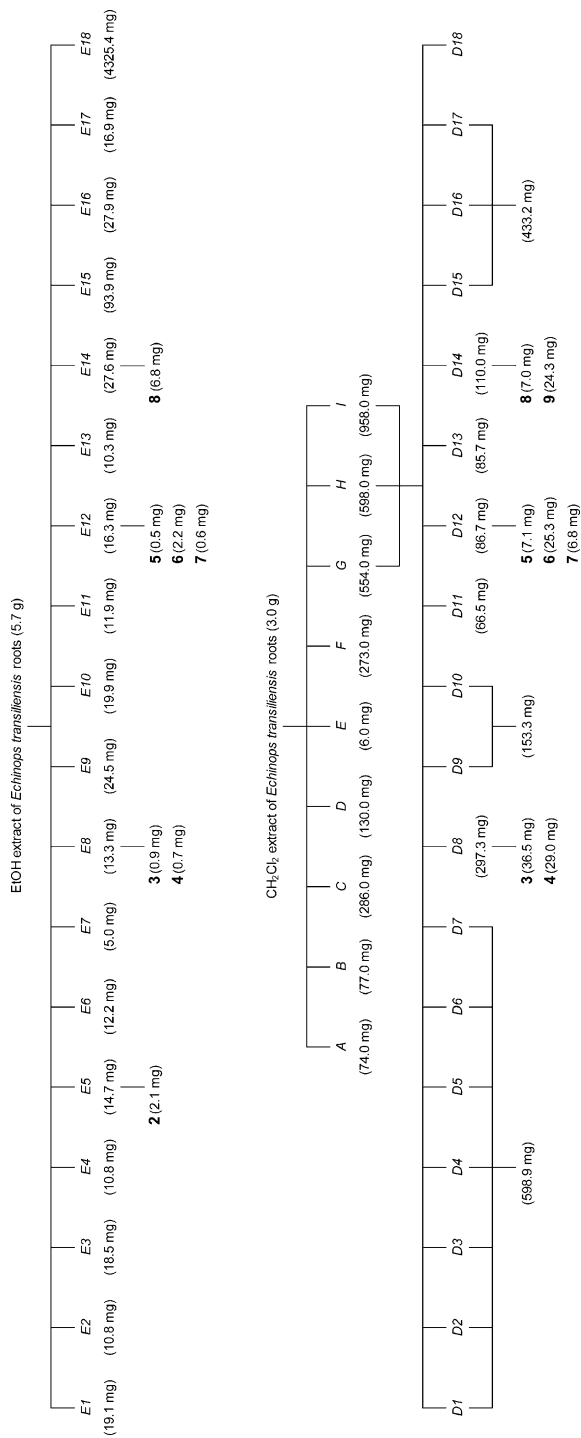


Fig. 2. Schematic representation of the separation of the EtOH and CH₂Cl₂ extracts from the roots from *Echinops transiliensis*

attached to C(3'') and C(4'') of compound **3**. OH and AcO groups are attached to C(3'') and C(4''), respectively, of compound **5**. OH Groups are attached to C(3'') and C(4'') of compound **8**. Compound **3** exhibited the highest activity, followed by **5** and **8** (Table 1). Hence, in bithiophenes, larvicidal activity appears to increase with an increasing number of AcO groups attached to C(3'') or C(4''), indicating that the AcO groups may play important roles in improving the larvicidal activity. In contrast, such a structure–activity relationship was not found among monothiophenes.

Contribution of Compounds 1–9 to Larvicidal Activity of CH₂Cl₂ Extract of A. aegypti. To evaluate the contribution of individual compounds to the larvicidal activity of the CH₂Cl₂ extracts, concentrations of compounds **1–9** in CH₂Cl₂ extracts were studied. Compound **1** had a lower concentration in the extract (1.00 g/kg) than **2** (7.12 g/kg) and higher concentration than other compounds (Table 2). Compound **1** which was *ca.* 92 times more active than compound **2** had *ca.* 1/7 of the concentration of **2**. As a result, contribution indices (concentration of compound in extract/LC₅₀ and concentration of compound in extract/LC₉₀) of compound **1** were the highest among **1–9** (Table 3). Thus, compound **1** might be the most important constituent of the CH₂Cl₂

Table 1. Toxicity of Compounds **1–9** Isolated from *Echinops transiliensis* Roots against *Aedes aegypti* Larvae

Compound	LC ₅₀	95% CI ^{a)}	LC ₉₀	95% CI ^{a)}	χ ²	DF
1	0.16	0.15–0.18	0.25	0.22–0.30	51.69	73
2	14.71	12.74–17.06	25.85	21.55–34.40	50.28	38
3	4.22	3.61–4.89	11.19	9.15–14.73	99.87	73
4	17.95	14.66–22.46	76.58	53.47–131.86	72.70	48
5	7.45	6.53–8.54	16.10	13.42–20.68	98.91	73
6	18.55	16.23–21.25	29.76	25.32–38.67	42.37	73
7	19.97	16.44–24.38	32.01	25.88–49.06	21.88	73
8	9.89	7.82–13.01	29.88	20.77–54.97	46.23	73
9	12.45	11.09–14.02	21.86	18.74–27.32	69.56	48
Permethrin ^{c)}	0.0034	0.0030–0.0038				

^{a)} LC₅₀ and LC₉₀ values are given in µg/ml 95% confidence interval (CI). ^{b)} DF, Degree of freedom. ^{c)} Positive standard; purity, 46.1 and 53.2% for *cis* and *trans*, respectively.

Table 2. Concentration of Compounds **1–9** in Extract and Root

Compound	Weight/extract weight [g/kg]	Weight/root weight [mg/kg]
1	1.00 ± 0.0619 ^{a)}	25.1 ± 1.15
2	7.12 ± 0.373	179 ± 6.52
3	0.501 ± 0.0626	12.6 ± 1.41
4	0.645 ± 0.0490	16.2 ± 0.987
5	0.0337 ± 0.00872	0.843 ± 0.21
6	0.0859 ± 0.00367	2.16 ± 0.0670
7	0.0327 ± 0.00462	0.822 ± 0.115
8	0.293 ± 0.00652	7.37 ± 0.0781
9	0.549 ± 0.00315	1.38 ± 0.0730

^{a)} Means ± SE of results from three extractions.

Table 3. Contribution Index of Compounds 1–9 in *Aedes aegypti* Larvicidal Activity of CH₂Cl₂ Extracts

Compound	Concentration/LC ₅₀ [mg/kg]/[mg/ml]	Concentration/LC ₉₀ [mg/kg]/[mg/ml]
1	6256	4004
2	484	275
3	119	45
4	36	8
5	5	2
6	5	3
7	2	1
8	30	10
9	4	3

extracts in *A. aegypti* larvicidal activity. The CH₂Cl₂ extracts also exhibited high toxicity against *A. aegypti* larvae (LC₅₀, 3.21 mg/l; LC₉₀, 6.81 mg/l). The LC₅₀ value of permethrin was 0.0034 mg/l. In the screening bioassay, permethrin was used at a dose of 0.025 ppm which led to 100% larval mortality.

Results from this study suggest that CH₂Cl₂ extracts or crude compound **1** fraction from CH₂Cl₂ extracts of the roots of *E. transiliensis* could be important tools for *A. aegypti* larval management. Further research is needed to conduct more comprehensive bioassays to explore the ways to effectively use these compounds in mosquito population management programs.

We thank Amber Reichley and Solomon Green III for technical assistance. This study was supported in part by a Deployed War-Fighter Protection Research Program Grant funded by the U.S. Department of Defense through the Armed Forces Pest Management Board, and in part by USDA/ARS grant No. 56-6402-1-612. We thank Dr. James J. Becnel, Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, Gainesville, for supplying *A. aegypti* eggs.

Experimental Part

General. Column chromatography (CC): Biotage Isolera One flash purification system (Biotage, SE-Uppsala). High-performance liquid chromatography (HPLC): Agilent 1100 HPLC system (Agilent, Palo Alto, CA). ¹H- and ¹³C-NMR spectra: Varian Unity Inova AS600 spectrometer (Varian, Palo Alto, CA). EI-MS: Varian CP-3800 gas chromatograph coupled to a Varian Saturn 2000 mass spectrometer (Varian, Palo Alto, CA).

Plant Material. The roots of *Echinops transiliensis* GOLOSK. were collected at the flowering stage on March 12, 2004, on the slopes of mountains near Djandosov village in Zailiysky Alatau, Kazakhstan. A voucher specimen No. 9442/25-1972 was deposited with the Institute of Botany and Phytointroduction herbarium, Almaty, Republic of Kazakhstan.

Isolation of Compounds 2–8 from the EtOH Extracts. Powdered roots (0.5 kg) of *E. transiliensis* were extracted with 3.5 l of EtOH for 24 h at r.t. providing 10.7 g of extracts. A portion of the EtOH extract (5.7 g) was subjected to a normal-phase CC (Flash 25 + M, KP-Sil, 25 × 150 mm, 40 g; Biotage; step gradient (step 1, hexane/acetone from 100:0 to 80:20, 1200 ml; step 2, hexane/acetone from 80:20 to 0:100; 600 ml)) to afford Fr. E1 (19.1 mg), Fr. E2 (10.8 mg), Fr. E3 (18.5 mg), Fr. E4 (10.8 mg), Fr. E5 (14.7 mg), Fr. E6 (12.2 mg), Fr. E7 (5.0 mg), Fr. E8 (13.3 mg), Fr. E9 (24.5 mg), Fr. E10 (19.9 mg), Fr. E11 (11.9 mg), Fr. E12 (16.3 mg), Fr. E13 (10.3 mg), Fr. E14 (27.6 mg), Fr. E15 (93.9 mg), Fr. E16 (27.9 mg), Fr. E17 (16.9 mg), and Fr. E18 (4325.4 mg; MeOH-wash fraction). Previously, 2,2':5',2''-terthiophene (**1**) and compound **9** were isolated from Fr. E3 and Fr. E14, resp. [12]. Fr. E5 was further

separated by a normal-phase CC (*Flash 12 + M, KP-Sil* (12 × 150 mm, 9 g, *Biotage*); hexane/acetone from 100:0 to 90:10, 360 ml) to yield *2-chloro-4-[5-(penta-1,3-diyne-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate* (**2**; 2.1 mg, 0.00079%). *Fr. E8* was further separated by semi-prep. normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 10 × 250 mm; hexane/ⁱPrOH, 99.2:0.8; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield **3** (*t_R* 14.0 min; 0.9 mg, 0.00034%) and compound **4** (*t_R* 14.9 min; 0.7 mg, 0.00026%). *Fr. E12* was further separated by semi-prep. normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 10 × 250 mm; hexane/ⁱPrOH, 97.0:3.0; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield **5** (*t_R* 17.0 min; 0.5 mg, 0.00019%), **6** (*t_R* 18.9 min; 2.2 mg, 0.00083%), and **7** (*t_R* 23.8 min; 0.6 mg, 0.00023%). *Fr. E14* was further separated by semi-prep. normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 10 × 250 mm; hexane/ⁱPrOH, 95.0:5.0; flow rate, 4.7 ml/min; UV detection at 320 nm) to afford compound **8** (*t_R* 22.1 min; 6.8 mg, 0.00255%).

Isolation of Compounds 3–9 from the CH₂Cl₂ Extracts. Powdered roots (0.5 kg) of *E. transiliensis* were extracted with 3.4 l of CH₂Cl₂ for 24 h at r.t. providing 8.2 g of CH₂Cl₂ extracts. A portion of the CH₂Cl₂ extract (3.0 g) was subjected to a normal-phase CC (*Flash 40 + M, KP-Sil*, 40 × 150 mm, 100 g, *Biotage*) using a step gradient (step 1, hexane/AcOEt from 100.0:0.0 to 90.0:10.0, 1101 ml; step 2, hexane/acetone from 90.0:10.0 to 70.0:30.0, 600 ml; step 3, 70.0:30.0 to 0.0:100.0, 600 ml) to furnish *Fr. A* (74.0 mg), *Fr. B* (77.0 mg), *Fr. C* (286.0 mg), *Fr. D* (130.0 mg), *Fr. E* (6.0 mg), *Fr. F* (273.0 mg), *Fr. G* (554.0 mg), *Fr. H* (598.0 mg), and *Fr. I* (958.0 mg; MeOH-wash fraction). Previously, **1** and **2** were isolated from *Fr. D* and *Fr. H*, resp. [11]. *Frs. G, H, and I* were combined and subjected to a normal-phase CC (*Flash 25 + M, KP-Sil*, 25 × 150 mm, 40 g, *Biotage*) using a step gradient (step 1, hexane/acetone from 100:0 to 80:20, 1200 ml; step 2, hexane/acetone from 80:20 to 0:100, 600 ml) to afford *Frs. D1–D7* (598.9 mg), *Fr. D8* (297.3 mg), *Fr. D9* and *D10* (153.3 mg), *Fr. D11* (66.5 mg), *Fr. D12* (86.7 mg), *Fr. D13* (85.7 mg), *Fr. D14* (110.0 mg), *Fr. D15–D17* (433.2 mg), and *Fr. D18* (MeOH-wash fraction). *Fr. D8* was further separated by semi-prep. normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 10 × 250 mm; hexane/ⁱPrOH, 99.2:0.8; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield compounds **3** (*t_R* 15.4 min; 36.5 mg, 0.01995%) and **4** (*t_R* 17.0 min; 29.0 mg, 0.01585%). *Fr. D12* was further separated by semi-prep. normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 10 × 250 mm; hexane/ⁱPrOH, 97.4:2.6; flow rate, 4.7 ml/min; UV detection at 320 nm) to furnish compounds **5** (*t_R* 19.5 min; 7.1 mg, 0.00388%), **6** (*t_R* 21.9 min; 25.3 mg, 0.01328%), and **7** (*t_R* 28.5 min; 6.8 mg, 0.00372%). *Fr. D14* was further separated by semi-prep. normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 10 × 250 mm; hexane/ⁱPrOH, 95.0:5.0; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield compounds **8** (*t_R* 22.1 min; 7.0 mg, 0.00383%) and **9** (*t_R* 29.0 min; 24.3 mg, 0.01328%).

Determination of Amounts of Compounds 1–9 in CH₂Cl₂ Extracts. Powdered roots of *E. transiliensis* (15 g) were extracted with 500 ml of CH₂Cl₂ for 1 d with a *Soxhlet* extractor (3 ×). The weights of extracts were 366.1, 386.1, and 379.7 mg, resp. The CH₂Cl₂ extract was subjected to HPLC (*ZORBAX SB-C18, Agilent*, 4.6 × 250 mm; MeOH/H₂O 75.0:25.0; flow rate, 1.0 ml/min; UV detection at 320 nm) to isolate **1** (*t_R* 29.8 min) and **2** (*t_R* 16.9 min). Also, the CH₂Cl₂ extract was subjected to normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 4.6 × 250 mm; hexane/ⁱPrOH, 99.0:1.0; flow rate, 1.0 ml/min; UV detection at 320 nm) to isolate **3** (*t_R* 6.6 min) and **4** (*t_R* 7.3 min). Similarly, the CH₂Cl₂ extract was subjected to normal-phase HPLC (*Luna 5 μm Silica (2) 100 Å, Phenomenex*, 4.6 × 250 mm; hexane/ⁱPrOH, 94.0:6.0; flow rate, 1.0 ml/min; UV detection at 320 nm) to elute **5** (*t_R* 10.6 min), **6** (*t_R* 11.9 min), **7** (*t_R* 16.2 min), **8** (*t_R* 23.9 min), and **9** (*t_R* 27.5 min). The amounts of compounds **1–9** were calculated from their rel. peak area response values.

4-(2,2'-Bithiophen-5-yl)but-3-yne-1,2-diyl Diacetate (3). Yellow solid. ¹H-NMR (600 MHz, CDCl₃)¹⁾: 2.08 (s, Me(8'')); 2.12 (s, Me(6'')); 4.29 (dd, *J* = 7.2, 11.8, H_a-C(4'')); 4.40 (dd, *J* = 3.6, 11.8, H_b-C(4'')); 5.83 (dd, *J* = 3.6, 7.2, H-C(3'')); 7.00 (m, H-C(3)); 7.00 (m, H-C(4)); 7.12 (d, *J* = 3.8, H-C(4)); 7.15 (dd, *J* = 1.0, 3.6, H-C(3)); 7.22 (dd, *J* = 1.0, 5.1, H-C(5')). ¹³C-NMR (150 MHz, CDCl₃)¹⁾: 20.7 (C(8'')); 20.9 (C(6'')); 62.4 (C(3'')); 64.4 (C(4'')); 80.0 (C(1'')); 87.4 (C(2'')); 119.9 (C(5)); 123.3 (C(3)); 124.5 (C(3)); 125.3 (C(5')); 128.0 (C(4)); 134.1 (C(4)); 136.4 (C(2')); 139.8 (C(2)); 169.7 (C(5'')); 170.4 (C(7')). EI-MS: 334.1 (*M*⁺).

1) Trivial atom numbering for assignments as indicated in *Fig. 1*.

4-[5-(Penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diyl Diacetate (**4**). Yellow solid. ¹H-NMR (600 MHz, CDCl₃)¹: 2.01 (s, Me(5'')); 2.07 (s, Me(8'')); 2.11 (s, Me(6'')); 4.27 (dd, *J*=7.3, 11.9, H_a-C(4'')); 4.38 (dd, *J*=3.6, 11.9, H_b-C(4'')); 5.80 (dd, *J*=3.6, 7.3, H-C(3'')); 7.04 (d, *J*=4.0, H-C(4)); 7.07 (d, *J*=4.0, H-C(3)). ¹³C-NMR (150 MHz, CDCl₃)¹: 4.8 (C(5'')); 20.7 (C(8'')); 20.8 (C(6'')); 62.3 (C(3'')); 64.1 (C(3'')); 64.3 (C(4'')); 66.3 (C(1'')); 79.4 (C(1'')); 79.7 (C(2'')); 83.7 (C(4'')); 87.4 (C(2'')); 123.2 (C(5)); 124.6 (C(2)); 133.0 (C(4)); 133.5 (C(3)); 169.6 (C(5'')); 170.4 (C(7'')). EI-MS: 314.0 (*M*⁺).

4-(2,2'-Bithiophen-5-yl)-2-hydroxybut-3-yn-1-yl Acetate (**5**). Yellow solid. ¹H-NMR (600 MHz, CDCl₃)¹: 2.12 (s, Me(8'')); 4.28 (m, H_a-C(4'')); 4.28 (m, H_b-C(4'')); 4.82 (br. s, H-C(3'')); 6.99 (m, H-C(3)); 6.99 (m, H-C(4)); 7.10 (d, *J*=1.0, H-C(4)); 7.15 (dd, *J*=3.5, 1.0, H-C(3'')); 7.22 (dd, *J*=5.2, 1.0, H-C(5')). ¹³C-NMR (150 MHz, CDCl₃)¹: 20.8 (C(8'')); 61.8 (C(3'')); 67.3 (C(4'')); 79.7 (C(1'')); 90.4 (C(2'')); 120.3 (C(5)); 123.4 (C(3)); 124.5 (C(3'')); 125.2 (C(5'')); 128.0 (C(4'')); 133.7 (C(4)); 136.4 (C(2'')); 139.5 (C(2)); 171.0 (C(7'')). EI-MS: 291.8 (*M*⁺).

2-Hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-1-yl Acetate (**6**). Yellow solid. ¹H-NMR (600 MHz, CDCl₃)¹: 2.01 (s, Me(5'')); 2.11 (s, Me(8'')); 4.26 (m, H_a-C(4'')); 4.26 (m, H_b-C(4'')); 4.79 (br. s, H-C(3'')); 7.01 (d, *J*=3.8, H-C(4)); 7.07 (d, *J*=3.8, H-C(3)). ¹³C-NMR (150 MHz, CDCl₃)¹: 4.8 (C(5'')); 20.8 (C(8'')); 61.7 (C(3'')); 64.1 (C(3'')); 66.3 (C(1'')); 67.1 (C(4'')); 79.0 (C(1'')); 79.6 (C(2'')); 83.6 (C(4'')); 90.6 (C(2'')); 123.6 (C(5)); 124.3 (C(2)); 132.5 (C(4)); 133.5 (C(3)); 170.9 (C(7'')). EI-MS: 271.8 (*M*⁺).

1-Hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-2-yl Acetate (**7**). Yellow solid. ¹H-NMR (600 MHz, CDCl₃)¹: 2.01 (s, Me(5'')); 2.13 (s, Me(6'')); 3.86 (m, H_a-C(4'')); 3.86 (m, H_b-C(4'')); 5.63 (br. s, H-C(3'')); 7.04 (d, *J*=3.8, H-C(4)); 7.07 (d, *J*=3.8, H-C(3)). ¹³C-NMR (150 MHz, CDCl₃)¹: 4.8 (C(5'')); 20.9 (C(6'')); 64.1 (C(3'')); 64.3 (C(4'')); 65.5 (C(3'')); 66.3 (C(1'')); 79.4 (C(1'')); 79.7 (C(2'')); 83.6 (C(4'')); 88.1 (C(2'')); 123.3 (C(5)); 124.5 (C(2)); 132.9 (C(4)); 133.5 (C(3)); 170.0 (C(5'')). EI-MS: 271.8 (*M*⁺).

4-(2,2'-Bithiophen-5-yl)but-3-yne-1,2-diol (**8**). Yellow solid. ¹H-NMR (600 MHz, CDCl₃)¹: 3.76 (dd, *J*=6.7, 10.4, H_a-C(4'')); 3.81 (dd, *J*=3.6, 10.4, H_b-C(4'')); 4.68 (m, H-C(3'')); 6.99 (m, H-C(3)); 6.99 (m, H-C(4)); 7.09 (d, *J*=3.6, H-C(4)); 7.15 (dd, *J*=0.7, 2.7, H-C(3'')); 7.22 (dd, *J*=0.7, 5.1, H-C(5')). ¹³C-NMR (150 MHz, CDCl₃)¹: 63.8 (C(3'')); 66.3 (C(4'')); 79.4 (C(1'')); 91.3 (C(2'')); 120.4 (C(5)); 123.3 (C(3)); 124.4 (C(3'')); 125.2 (C(5'')); 127.9 (C(4'')); 133.6 (C(4)); 136.4 (C(2'')); 139.4 (C(2)).

Compound **3** Derived from Acetylation of **8**. EI-MS: 333.8 (*M*⁺). Methods for acetylation have been described in [19].

Larval Bioassays against Aedes aegypti. *A. aegypti* used in larvicidal bioassays originated from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, United States Department of Agriculture, Agriculture Research Service, Gainesville, Florida. This colony is maintained since 1952 using standard procedures [3]. Eggs were received and stored in our laboratory (Biological Field Station, The University of Mississippi, Abbeville, MS) until needed. Bioassays were conducted as described in [3] to determine the larvicidal activity of compounds **1–9** against 1-d-old *A. aegypti*. Eggs were hatched, and larvae were held in a room maintained at a temp. of 27±2° with 60±10% RH (relative humidity) under ambient fluorescent room light conditions. Five 1-d-old larvae were transferred to individual wells of a 24-well tissue culture plates in a 30–40-μl droplet of H₂O. 50 μl of larval diet of 2% slurry of 3:2 beef liver powder (*Now Foods*, Bloomington, Illinois), Brewer's yeast (*Lewis Laboratories Ltd.*, Westport, CT), and 1 ml of deionized H₂O were added to each well with a *Finnpipette* stepper (*Thermo Fisher*, FI-Vantaa). All the compounds to be tested were diluted with DMSO. After treatment application, the plates were swirled in clockwise and counterclockwise motions, front and back, and side to side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24- and 48-h post treatment. Larvae were deemed dead if they showed no movement in the well after being prodded with a pipette tip. Permethrin (46.1% *cis*/53.2% *trans*, *Chemical Service*, West Chester, PA) was used as positive control. A series of five dosages were used in each treatment to get a range of mortality between 0 and 100%. Treatments were replicated ten times for each compound.

REFERENCES

- [1] L. Mousson, C. Dauga, T. Garrigues, F. Schaffner, M. Vazeille, A.-B. Failloux, *Genet. Res.* **2005**, *86*, 1.
- [2] World Health Organization, 'Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control', World Health Organization, Geneva, 2009.
- [3] J. W. Pridgeon, J. J. Becnel, G. G. Clark, K. J. Linthicum, *J. Med. Entomol.* **2009**, *46*, 335.
- [4] F. E. Dayan, C. L. Cantrell, S. O. Duke, *Bioorg. Med. Chem.* **2009**, *17*, 4022.
- [5] T. Arnason, R. J. Hebda, T. Johns, *Can. J. Bot.* **1981**, *59*, 2189.
- [6] B. M. Abegaz, M. Tadesse, R. Majinda, *Biochem. Syst. Ecol.* **1991**, *19*, 323.
- [7] A. Gila, C. M. Ghersa, S. Perelman, *Biochem. Syst. Ecol.* **2002**, *30*, 1.
- [8] C.-K. Wat, S. K. Prasad, E. A. Graham, S. Partington, T. Arnason, G. H. N. Towers, *Biochem. Syst. Ecol.* **1981**, *9*, 59.
- [9] G. Campbell, J. D. H. Lambert, T. Arnason, G. H. N. Towers, *J. Chem. Ecol.* **1982**, *8*, 961.
- [10] N. Fokialakis, C. L. Cantrell, S. O. Duke, A. L. Skaltsounis, D. E. Wedge, *J. Agric. Food. Chem.* **2006**, *54*, 1651.
- [11] N. Fokialakis, W. L. A. Osbrink, L. K. Mamonov, N. G. Gemejjeva, A. B. Mims, A. L. Skaltsounis, A. R. Lax, C. L. Cantrell, *Pest Manage. Sci.* **2006**, *62*, 832.
- [12] H. Nakano, C. L. Cantrell, L. K. Mamonov, W. L. A. Osbrink, S. A. Ross, *Org. Lett.* **2011**, *13*, 6228.
- [13] T. Arnason, T. Swain, C.-K. Wat, E. A. Graham, S. Partington, G. H. N. Towers, *Biochem. Syst. Ecol.* **1981**, *9*, 63.
- [14] J. T. Arnason, B. J. R. Philogene, C. Berg, A. MacEachern, J. Kaminski, L. C. Leitch, P. Morand, J. Lam, *Phytochemistry* **1986**, *25*, 1609.
- [15] R. J. Marles, R. L. Compadre, C. M. Compadre, C. Soucy-Breau, R. W. Redmond, F. Duval, B. Mehta, P. Morand, J. C. Scaiano, J. T. Arnason, *Pestic. Biochem. Physiol.* **1991**, *41*, 89.
- [16] H. Nakano, C. L. Cantrell, L. K. Mamonov, T. S. Kustova, F. R. Fronczek, S. A. Ross, *Biochem. Syst. Ecol.* **2012**, *45*, 127.
- [17] M. A. Menelaou, F. R. Fronczek, M. A. Hjortso, A. F. Morrison, M. Foroozesh, T. M. Thibodeaux, H. E. Flores, N. H. Fischer, *Spectrosc. Lett.* **1991**, *24*, 1405.
- [18] D.-A. Guo, X.-Y. Liu, L. Qiao, C.-Y. Gao, Z.-C. Lou, *J. Chin. Pharm. Sci.* **1992**, *1*, 82.
- [19] C. L. Cantrell, J. A. Klun, J. Pridgeon, J. Becnel, S. Green III, F. R. Fronczek, *Chem. Biodiversity* **2009**, *6*, 447.

Received August 27, 2013