Three New Thiophene Acetylenes from *Rhaponticum uniflorum* (L.) DC.

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Three new thiophene-acetylenes, 7-chloroarctinone-b (**1**), rhapontiynethiophenes A (**2**) and B (**3**), along with arctinone-b (**4**) were isolated from the MeOH extract of the roots of *Rhaponticum uniflorum* (L.) DC. The structures of the new compounds were elucidated by detailed spectroscopic data analysis and by comparison with known compounds.

Introduction. – Thiophene-acetylenes (=ethynylthiophenes) represent a unique class of natural products that often exhibit a wide variety of biological activities ranging from antitumor [1], antiviral [2], anti-HIV [3], antifungal [4] to insecticidal activities [5][6]. Thiophene-acetylenes are typically found in the plant family Asteraceae [7][8], but they have also been reported in fungi [9].

Rhaponticum uniflorum (L.) DC. (family Asteraceae) is a perennial herbaceous plant widely distributed in the northern part of China. The roots of *R. uniflorum* (commercial name: *Qizhou Loulu*) have been used as folk medicine for the treatment of fever and intoxications [10] in China. Previous phytochemical studies on the title plant have resulted in the isolation of phytoecdysteroids [11], triterpenes [12], diterpenes [13] and thiophene-acetylenes [14–16]. In the course of our search for bioactive secondary metabolites from the Chinese fauna and flora [17–19], we have re-investigated this medicinal plant. Chemical investigation of the AcOEt fraction from the MeOH extract of the roots of the plant led to the isolation of three new thiophene-acetylenes, 7-chloroarctinone-b (**1**), rhapontiynethiophenes A and B (**2** and **3**, resp.), together with arctinone-b (**4**), which has previously been isolated from the same plant [14]. Here, we describe the isolation and structure elucidation of the new compounds **1**–**3**.

Results and Discussion. – The powdered roots of *R. uniflorum* collected from Chifeng City, Inner Mongolia Autonomous Region, China, were extracted exhaustively with MeOH. The MeOH extract was then partitioned between H_2O and AcOEt, and H_2O and BuOH, respectively. The AcOEt-soluble portion was repeatedly subjected to silica-gel and *Sephadex LH-20* column chromatography, followed by reversed-phase (RP) HPLC purification, resulting in the isolation of four thiophene-acetylenes, of which three (7-chloroarctinone-b (**1**), and rhapontiynethiophenes A and B (**2** and **3**, resp.)) were new thiophene-acetylenes (*Figure*). The known compound was identified

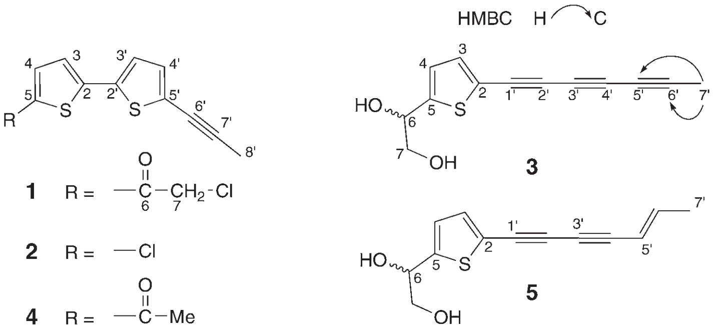

Figure. *Chemical structures of compounds* **1**–**5**[1])

as arctinone-b (**4**) [14] by analysis of its NMR spectra and by comparison with the literature data.

All of the new compounds exhibited very similar spectroscopic properties to co-occurring arctinone-b (**4**). Their UV spectra showed strong absorption maxima around 260 nm and, in the case of **1** and **2**, 371 or 340 nm, respectively, which was indicative for the presence of one or two thiophene rings [20]. The presence of a thiophene ring was further supported by typical IR absorptions at $\tilde{\nu}_{max}$ *ca.* 1400 and *ca.* 800 cm^{-1} [21]. The NMR spectra of **1**–**3** (*Tables 1* and *2*) show strong structural analogies with known thiophene-acetylenes, which possess one or two 2,5-disubstituted thiophene moieties (confirmed by comparison of the ^{1}H- and ^{13}C-NMR data with those of model compounds **4** [14] and **5** [22]). In fact, compounds **1** and **2** like co-occurring **4** possess a common 2,2′-dithiophene-5′-(prop-1-ynyl) partial structure and differ from each other only by the substituents at C(5), while compound **3** differs from the model compound **5**, which was previously obtained from *Leuzea carthamoides* [22], only by the substituent at C(2).

Compound **1**, 7-chloroarctinone-b[2]), was obtained as a yellow powder. The molecular formula, $C_{13}H_9ClOS_2$, consistent with nine degrees of unsaturation, was determined by HR-EI-MS (*m*/*z* 279.9775, M^+; calc. 279.9783). Analysis of 1D- and 2D-(^{1}H,^{1}H-COSY, HMQC, and HMBC) NMR spectra (see *Table 1*) readily allowed us to recognize a typical 2,2′-dithiophene moiety with two substituents at C(5) and C(5′), respectively. Moreover, the characteristic ^{13}C-NMR chemical shifts at δ(C) 92.9 (*s*), 72.5 (*s*), and 4.8 (*q*) indicated the presence of a prop-1-ynyl group in **1**.

The above-mentioned structural features of **1** were strongly reminiscent of those of co-occurring arctinone-b (**4**) [14]. Careful comparison of the NMR data of **1** with those of **4** revealed that the only difference between **1** and **4** exists at C(5), where the Ac function in **4** is replaced by a 7-chloro group in **1**, in agreement with a difference of 34 mass units. This conclusion was further supported by the observation of a downfield ^{1}H-NMR signal at δ(H) 4.55 (*s*, 2 H) in **1**, accompanying the disappearance of the Me

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[1]) Arbitrary numbering, see also in the *Figure*.

[2]) For systematic names, see *Exper. Part.*

Table 1. *^{1}H- and ^{13}C-NMR Data*a)b) *for* **1** *and* **2**

Position1)	**1**		**2**	
	δ(H)	δ(C)c)	δ(H)	δ(C)c)
C(2)	–	146.5 (*s*)	–	135.8 (*s*)
H–C(3)	7.15 (*d*, *J* = 3.9)	124.3 (*d*)	6.90 (*d*, *J* = 3.9)	123.0 (*d*)
H–C(4)	7.67 (*d*, *J* = 3.9)	134.0 (*d*)	6.97 (*d*, *J* = 3.9)	126.9 (*d*)
C(5)	–	138.6 (*s*)	–	129.3 (*s*)
C(6)	–	183.8 (*s*)	–	–
CH$_2$(7)	4.55 (*s*)	45.1 (*t*)	–	–
C(2′)	–	135.5 (*s*)	–	136.5 (*s*)
H–C(3′)	7.16 (*d*, *J* = 3.9)	125.7 (*d*)	6.91 (*d*, *J* = 3.9)	122.7 (*d*)
H–C(4′)	7.04 (*d*, *J* = 3.9)	132.1 (*d*)	6.82 (*d*, *J* = 3.9)	131.7 (*d*)
C(5′)	–	126.0 (*s*)	–	124.0 (*s*)
C(6′)	–	72.5 (*s*)	–	72.6 (*s*)
C(7′)	–	92.9 (*s*)	–	91.5 (*s*)
Me(8′)	2.10 (*s*)	4.8 (*q*)	2.09 (*s*)	4.7 (*q*)

a) *Bruker DRX-400* spectrometer, assignments made by HMQC and HMBC experiments. b) In CDCl$_3$, chemical shift δ [ppm] referred to the solvent signal (δ(H) 7.26, δ(C) 77.0); *J* in Hz. c) Multiplicities by DEPT sequence.

Table 2. *^{1}H- and ^{13}C-NMR Data*a)b) *for* **3** *and ^{13}C-NMR Data*b) *for* **5**

Position1)	**3**		**5** [22]
	δ(H)	δ(C)c)	δ(C)
C(2)	–	121.4 (*s*)	121.9 (*s*)
H–C(3)	7.16 (*d*, *J* = 3.9)	137.1 (*d*)	135.1 (*d*)
H–C(4)	6.78 (*d*, *J* = 3.9)	125.7 (*d*)	125.2 (*d*)
C(5)	–	153.1 (*s*)	151.4 (*s*)
H–C(6)	4.85 (*dd*, *J* = 3.6, 7.5)	72.4 (*d*)	71.9 (*d*)
H$_a$–C(7)	3.68 (*dd*, *J* = 3.6, 11.7)	68.7 (*t*)	68.3 (*t*)
H$_b$–C(7)	3.58 (*dd*, *J* = 7.5, 11.7)		
C(1′)	–	69.8 (*s*)	74.2 (*s*)
C(2′)	–	79.4 (*s*)	78.5 (*s*)
C(3′)	–	59.5 (*s*)	72.5 (*s*)
C(4′)	–	65.5 (*s*)	83.7 (*s*)
C(5′)	–	70.9 (*s*)	110.6 (*d*)
C(6′)	–	81.4 (*s*)	145.4 (*d*)
Me(7′)	1.95 (*s*)	4.5 (*q*)	19.0 (*q*)

a) *Bruker DRX-400* spectrometer, assignments made by HMQC and HMBC experiments. b) In CD$_3$OD, chemical shift δ [ppm] referred to the solvent signal (δ(H) 3.30, δ(C) 49.0); *J* in Hz. c) Multiplicity by DEPT sequence.

signal at δ(H) 2.10 (*s*, 3 H) in **4**. Moreover, the presence of a Cl-atom was clearly indicated by the M^+ peaks at *m*/*z* 279.9775 and 281.9748 with intensities of 1 : 0.44 in the HR-EI-MS. Accordingly, the structure of **1** was determined to be 7-chloroarctinone-b.

Rhapontiynethiophene A (**2**), a yellow oil, had the molecular formula $C_{11}H_7ClS_2$ which is 8 mass units less than for **4**. Comparison of the NMR data of **2** and **4** revealed that the only difference was located at C(5) where the Ac group in **4** was replaced by a Cl-substituent in **2**, while the rest of the structure of **2** was identical to **4**. Consequently, the structure of compound **2** was established as 5-chloro-5′-(prop-1-ynyl)-2,2′-bithiophene.

Rhapontiynethiophene B (**3**), an optically active yellow powder, showed the molecular formula of $C_{13}H_{10}O_2S$, as established by HR-EI-MS (*m/z* 230.0399 (M^+); calc. 230.0402), indicating nine degrees of unsaturation. The typical ^{1}H-NMR signals at δ(H) 7.16 (*d*, $J = 3.9$, H−C(3)) and 6.78 (*d*, $J = 3.9$, H−C(4)), and ^{13}C-NMR signals at δ(C) 59–82 ppm (*Table 2*) indicated that **3** is also a thiophene-acetylene. In addition, the presence of a 1,2-diol partial structure was revealed by the explicit ^{1}H,^{1}H-COSY correlation from H−C(6) to CH_2(7), together with the diagnostic ^{13}C-NMR chemical shifts at δ(C) 72.4 (C(6)) and 68.7 (C(7)). A literature survey showed that the NMR data of **3** (*Table 2*) were almost identical to those of the model compound **5** (1-{5-[(5*E*)-hept-5-ene-1,3-diynyl]thiophen-2-yl}ethane-1,2-diol) [22] with the only exception at the terminus of the side chain at C(2)^1), where the C=C bond in **5** was replaced by a C≡C bond (δ(C) 70.9 and 81.4) in **3**, in agreement with a difference of 2 mass units. Detailed analysis of HMQC and HMBC spectra of **3** allowed the unambiguous assignment of the planar structure of **3**. Especially, HMBC correlations from Me(7′) (δ(H) 1.95) to C(5′) and C(6′) confirmed this conclusion (*Figure*). Rhapontiynethiophene B (**3**) is the 5′,6′-dehydro derivative of **5**.

Compound **3** showed a negative optical rotation ($[\alpha]_D^{22} = -13.1$ ($c = 0.38$, MeOH)), though the related compound **5** was reported to be a racemate. The absolute configuration at C(6) of **3** remains undefined due to the limited amounts of **3** obtained from the plant.

Chlorinated secondary metabolites are rather rare from terrestrial sources. In this context, it cannot be definitively ruled out that compounds **1** and **2** are artifacts formed during the isolation procedure. However, it must be pointed out that several chlorinated thiophene-acetylenes have been previously reported from the family Asteraceae [23–29]. It is noteworthy that several of them were obtained without the use of chlorinated solvents during the extraction and isolation procedures [27–29].

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Experimental Part

General. Column chromatography (CC): commercial silica gel (*Qing Dao Hai Yang Chemical Group Co.*, 100–200 and 200–300 mesh) or *Sephadex LH-20* (*Amersham Biosciences*). TLC: Precoated silica-gel plates (*Yan Tai Zi Fu Chemical Group Co.*, *G60 F-254*) were used for anal. TLC. Reversed-phase (RP) HPLC: *Agilent 1100* series liquid chromatography using a *VWD G1314A* detector at 210 nm. A semi-preparative *ODS-HG-5* (5 μm, 9.4 mm (i.d.) × 25 cm) was employed for the purification. Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *Varian Cary-300-Bio* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna-FT-IR 750* spectometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker DRX-400* (400 MHz for ^{1}H, and 100 MHz for ^{13}C) spectrometer; chemical shift δ in ppm, with the solvent signal in $CDCl_3$ (δ(H) 7.26, δ(C) 77.0) or in CD_3OD (δ(H) 3.30; δ(C) 49.0) as an internal standard, coupling constant J in Hz; assignments supported by HMQC and HMBC experiments. EI-MS and HR-EI-MS: *Carlo-Erba TRIO 2000 VG* and *Kratos MS50* mass spectrometers, in *m*/*z*.

Plant Material. The plants were collected at Chifeng City, Inner Mongolia Autonomous Region, People's Republic of China, in March 2007 and identified as *R. uniflorum* by Associate Prof. *J.-G. Shen* of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (No. 0703P-40) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The powdered dry roots (5 kg) of *R. uniflorum* were exhaustively extracted with MeOH (10 l × 3; 3 d each time) at r.t., and the MeOH extract was concentrated *in vacuo* to give a residue (210 g), which was suspended in H_2O (1000 ml), and then sequentially extracted (3 × 1 l) with AcOEt and BuOH, to afford an AcOEt-soluble extract (40 g) and an BuOH-souble extract (20 g), resp. The AcOEt extract was fractionated by gradient silica-gel column chromatography (0–100% AcOEt in light petroleum ether (PE), successively) yielding three fractions showing interesting green TLC spots after spraying with H_2SO_4 (R_f 0.55 (PE), 0.60 and 0.22 (PE/Me_2CO 7:3), resp.). These fractions were further purified by repeated silica-gel CC (PE/Et_2O, PE/acetone, and $CHCl_3$/MeOH), followed by *Sephadex LH-20* CC (PE/$CHCl_3$/MeOH 2:1:1 and $CHCl_3$/MeOH 1:1), yielding pure compounds **2** (4.5 mg) and **3** (2.0 mg), resp., and a mixture, which was separated into other two pure compounds **1** (22.8 mg) and **4** (8.2 mg) by RP-HPLC (semi-prep. *ODS-HG-5* (5 μm, 9.4 mm (i.d.) × 25 cm), MeOH/H_2O (80%), 2.0 ml/min).

7-Chloroarctinone-b (*=2-Chloro-1-[5′-(prop-1-ynyl)[2,2′-bithiophen]-5-yl]ethanone*; **1**). Yellow powder. UV (MeOH): 371 (4.45), 262 (4.09), 220 (3.92). IR (KBr): 2210, 1640, 1510, 1455, 1420, 1351, 1271, 1250, 1024, 800. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 282 (30, $[M+2]^+$), 280 (72, M^+). HR-EI-MS: 279.9775 (M^+, $C_{13}H_9ClOS_2^+$; calc. 279.9783).

Rhapontiynethiophene A (*=5-Chloro-5′-(prop-1-ynyl)-2,2′-bithiophene*; **2**). Yellow oil. UV (MeOH): 340 (4.40), 260 (4.11), 241 (3.74). IR (KBr): 2211, 1439, 810, 797. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 240 (40, $[M+2]^+$), 238 (100, M^+). HR-EI-MS: 237.9681 (M^+, $C_{11}H_7ClS_2^+$; calc. 237.9678).

Rhapontiynethiophene B (*=(1*R**)-1-[5-(Hepta-1,3,5-triynyl)thiophen-2-yl]ethane-1,2-diol*; **3**). Yellow powder. $[\alpha]_D^{22} = -13.1$ ($c=0.38$, MeOH). UV (MeOH): 269 (4.41), 253 (4.50), 211 (4.44). IR (KBr): 3437, 2214, 2173, 1383, 1086, 1040, 806. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 230 (32, M^+), 199 (100, $[M-CH_2OH]^+$). HR-EI-MS: 230.0399 (M^+, $C_{13}H_{10}O_2S^+$; calc. 230.0402).

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