Polyacetylenes from *Bupleurum longiradiatum*

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Eight new polyacetylenes (**1**−**8**) and six known polyacetylenes were isolated from the entire parts of *Bupleurum longiradiatum*, a poisonous plant. The structures of the new compounds were determined by spectroscopic data interpretation. The absolute configuration of the known compound bupleurotoxin (**9**) was established by the modified Mosher's method. All isolates were also tested for their cytotoxicity against a human leukemia cell line (HL-60).

The genus *Bupleurum* (Apiaceae) includes about 200 species widely distributed in Eurasia and North Africa.[1] Certain species of this genus, such as *Bupleurum chinense*, *B. scorzonerifolium*, and *B. falcatum*, have been used as antiphlogistic, antipyretic, and analgesic agents in traditional folk medicine preparations.[2] However, *B. longiradiatum*, found in northeastern mainland China, is a poisonous species within the *Bupleurum* genus. This species bears a general resemblance to other *Bupleurum* plants, but is not permitted to be used as a herbal medicine due to its toxic properties.[3,4] Previous studies showed the ethyl ether extract of *B. longiradiatum* had strong toxicity against mice, and this toxicity was attributed to its high content of polyacetylenes.[4,5] However, the chemical profile of polyacetylenes in *B. longiradiatum* has not been fully studied yet. Only four polyacetylenes were reported from this plant so far.[4] Moreover, the absolute configurations of the known bupleurotoxin (**9**) and acetylbupleurotoxin (**10**) at the stereogenic carbon at C-14 are not yet established. As part of our interest on *Bupleurum* species,[6,7] we undertook an investigation of a dichloromethane extract of *B. longiradiatum* that led to the isolation of eight new (**1**−**8**) and six known (**9**−**14**) polyacetylenes. We report herein the isolation, structure elucidation, and cytotoxicity of the isolated compounds, along with the determination of the absolute configuration of bupleurotoxin (**9**). Compounds **1**−**5** are the first polyacetylenes containing only a single acetylenic bond isolated from the *Bupleurum* genus.

Results and Discussion

The dichloromethane extract of the whole plant of *B. longiradiatum* was subjected to silica gel, RP-18, and Sephadex LH-20 column chromatographic purification, as well as repeated preparative thin-layer chromatography (TLC) to yield eight new (**1**−**8**) and six known polyacetylenes, namely, bupleurotoxin (**9**),[4] acetylbupleurotoxin (**10**),[4] bupleuronol (**11**),[4] bupleurynol (**12**),[4,8] (2*Z*,9*Z*)-heptadecadiene-4,6-diyn-1-ol (**13**),[9,10] and (2*Z*,9*Z*)-pentadecadiene-4,6-diyn-1-ol (**14**).[11]

Compound **1** exhibited a molecular ion peak $[M]^+$ at *m*/*z* 286.1937 in the HREIMS, corresponding to the molecular formula $C_{19}H_{26}O_2$. The IR spectrum showed ester carbonyl (1741 cm^{-1}), triple-bond (2177 cm^{-1}), and olefinic double-bond (1639 cm^{-1}) absorptions. The UV spectrum showed absorption maxima at 316 and 338 nm, resembling that of a diene-yne-diene polyacetylene.[12] The ^1H NMR data of **1** (Table 1) indicated the presence of four pairs of *trans*-disubstituted double bonds [δ_H 5.83, 6.33 (H-2/H-3, J = 15.2 Hz); δ_H 6.53, 5.79 (H-4/H-5, J = 15.7 Hz); δ_H 5.63, 6.58 (H-8/H-9, J = 15.6 Hz); δ_H 6.11, 5.80 (H-10/H-11, J = 15.0 Hz)], a singlet methyl, and a triplet methyl. The ^{13}C NMR and DEPT spectra of **1** (Table 1) gave 19 carbon resonances due to two methyls, six methylenes, eight methines, and three quaternary carbons, including a triple bond [δ_C 90.6 (C-6) and 93.0 (C-7)] and an acetyl (δ_C 20.9, 170.7). The above data, along with the HMBC correlations from H-4 (δ_H 6.53) to C-2 (δ_C 128.6) and C-6 (δ_C 90.6) and from H-9 (δ_H 6.58) to C-7 (δ_C 93.0) and C-11 (δ_C 138.7) indicated that **1** is a C_{17} ester containing a diene-yne-diene moiety.[12,13] In the $^1H-^1H$ COSY spectrum, the correlation from an oxymethylene proton (δ_H 4.62, H-1) to an olefinic proton (δ_H 5.83, H-2) was observed, indicating that the oxymethylene was linked to the double bond. In addition, the HMBC correlation from H-1 at δ_H 4.62 to the ester carbonyl at δ_C 170.7 suggested that the acetoxy group is connected to the C-1 position. Therefore, compound **1** was determined as (2*E*,4*E*,8*E*,10*E*)-heptadecatetraen-6-yn-1-yl acetate.

Compound **2** gave a molecular formula of $C_{19}H_{28}O_2$, as evidenced by the HREIMS at *m*/*z* 288.2089 $[M]^+$, showing one less unsaturation degree than **1**. The UV spectrum displayed absorption maxima at 267 and 280 nm, indicating the presence of a diene-yne system.[12] The ^1H and ^{13}C NMR spectra (Table 2) revealed the presence of a *cis*-disubstituted double bond [δ_H 5.43, 5.49 (H-9/H-10, J = 10.6 Hz)], two *trans*-disubstituted double bonds [δ_H 5.80, 6.29 (H-2/H-3, J = 15.2 Hz); δ_H 6.50, 5.64 (H-4/H-5, J = 15.4 Hz)], an acetylenic unit [δ_C79.1 (C-6) and 92.1 (C-7)], a deshielded methylene [δ_H 3.09, H-8], an oxymethylene (δ_H 4.62, H-1), and an acetyl (δ_C 20.9 and 170.7). The NMR data of **2** were quite similar to those of **1**, with the major difference being the presence of a deshielded downfield methylene in **2**. The $^1H-^1H$ COSY correlation from this deshielded methylene proton (δ_H 3.09, H-8) to an olefinic proton (δ_H 5.43, H-9), along with the HMBC correlations of H-8 (δ_H 3.09) with C-6 (δ_C 79.1) and C-10 (δ_C 132.2) and of H-9 (δ_H 5.43) with C-7 (δ_C 92.1), clearly established an yne-CH_2-ene moiety in **2**. Therefore, the structure of **2** was established as (2*E*,4*E*,9*Z*)-heptadecatrien-6-yn-1-yl acetate.

Compound **3** was assigned the molecular formula $C_{22}H_{32}O_4$, due to the molecular ion peak at *m*/*z* 360.2309 in the HREIMS. Its UV, IR, and ^1H and ^{13}C NMR spectroscopic data (Table 2) were similar to those of **2**. The main difference between the two compounds was the absence of a triplet methyl and the presence of two additional methylenes (including one oxymethylene), along with an additional acetoxy group signal [δ_H 2.05 (3H, s); δ_C 171.2 (C), 21.0 (CH_3)] in **3**. These data and their mass spectra strongly suggested that **3** is a C_{18} ester. The additional acetoxy was placed at C-18 according to the HMBC correlation from H-18 (δ_H 4.05) to the ester carbonyl (δ_C 171.2). Thus, compound **3** was defined structurally as (2*E*,4*E*,9*Z*)-octadecatrien-6-yne-1,18-diyl diacetate.

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Chart 1

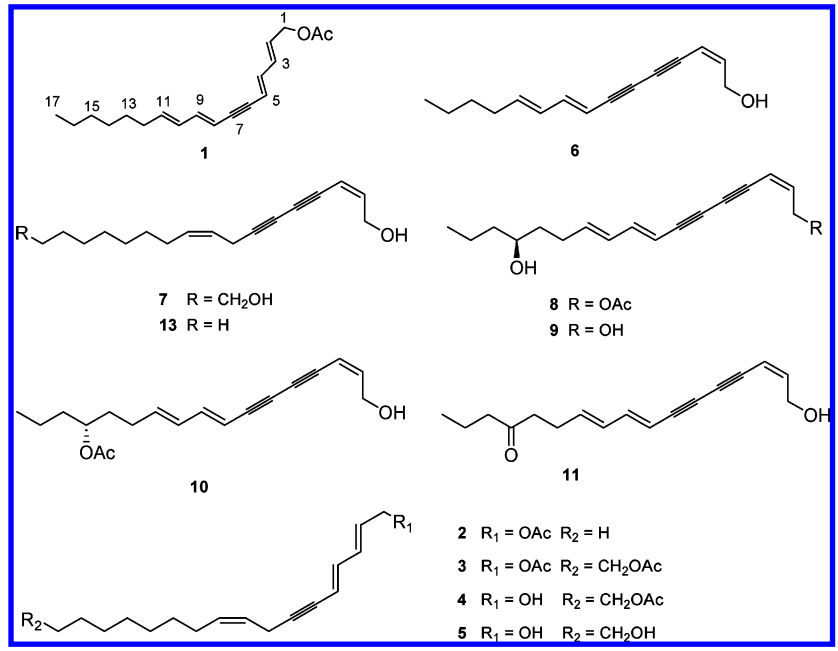

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Compounds **1**, **6**, **7**, and **13** in $CDCl_3$ (δ in ppm, J in Hz in parentheses)

	1		**6**		**7**		**13**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.62 br d (6.5)	64.4 CH_2	4.44 dd (6.4, 1.5)	61.1 CH_2	4.41 dd (6.4, 1.5)	61.1 CH_2	4.62 dd (6.4, 1.5)	61.1 CH_2
2	5.83 (overlapped)	128.6 CH	6.23 dt (11.0, 6.4)	144.9 CH	6.21 dt (11.0, 6.4)	145.0 CH	6.21 dt (11.0, 6.4)	145.0 CH
3	6.33 dd (15.2, 11.0)	133.3 CH	5.69 dd (11.0, 1.5)	109.6 CH	5.60 d (11.0)	109.5 CH	5.60 d (11.0)	109.5 CH
4	6.53 (overlapped)	139.5 CH		77.6 C		70.9 C		70.9 C
5	5.79 dd (15.7, 2.1)	113.0 CH		79.9 C		80.0 C		80.0 C
6		90.6 C		75.1 C		64.5 C		64.5 C
7		93.0 C		83.0 C		84.0 C		84.0 C
8	5.63 dd (15.6, 2.2)	108.6 CH	5.56 d (15.4)	107.0 CH	3.09 br d (6.8)	18.0 CH_2	3.09 br d (6.8)	18.0 CH_2
9	6.58 (overlapped)	142.4 CH	6.71 dd (15.4, 11.0)	145.7 CH	5.41 dt (10.5, 7.3)	122.0 CH	5.41 dt (10.5, 7.2)	122.0 CH
10	6.11 dd (15.0, 10.8)	129.8 CH	6.12 dd (15.2, 11.0)	129.4 CH	5.55 dt (10.5, 7.3)	133.1 CH	5.55 dt (10.5, 7.2)	133.1 CH
11	5.80 (overlapped)	138.7 CH	5.90 dt (15.2, 7.2)	140.5 CH	2.05 q (7.3)	27.1 CH_2	2.05 q (7.2)	27.2 CH_2
12	2.11 q (7.2)	32.8 CH_2	2.14 q (7.2)	32.5 CH_2	1.30 m	29.4 CH_2	1.30 m	29.2 CH_2
13	1.30 m	29.0 CH_2	1.30 m	31.0 CH_2	1.30 m	29.4 CH_2	1.30 m	29.2 CH_2
14	1.40 m	28.8 CH_2	1.30 m	22.2 CH_2	1.30 m	29.1 CH_2	1.30 m	29.1 CH_2
15	1.30 m	31.7 CH_2	0.90 t (7.3)	13.9 CH_3	1.30 m	29.0 CH_2	1.30 m	31.8 CH_2
16	1.30 m	22.6 CH_2			1.30 m	25.7 CH_2	1.30 m	22.6 CH_2
17	0.89 t (7.0)	14.0 CH_3			1.57 quint (7.0)	32.8 CH_2	0.89 t (7.2)	14.1 CH_3
18					3.64 t (6.7)	63.1 CH_2		
OAc-1		170.7 C						
	2.08 s	20.9 CH_3						

Compound **4** was found to possess a molecular formula of $C_{20}H_{30}O_3$, as determined by the molecular ion peak at *m/z* 318.2192 $[M]^+$ in the HREIMS, revealing the compound to be 42 amu less than that of **3**. The ^{1}H and ^{13}C NMR spectroscopic data of **4** indicated its structural similarity to **3**, except for the absence of a 1-*O*-acetyl group in **4** (Table 2). The significant upfield shift of the doublet of H-1 from δ_H 4.60 in **3** to δ_H 4.21 in **4**, together with the loss of 42 amu in **4**, suggested that the latter compound is a deacetylated derivative of **3**. The conclusion was supported further by the HMBC correlation of H-1 (δ_H 4.21) with C-3 (δ_C 130.4) and the ^{1}H−^{1}H COSY correlation from H-1 (δ_H 4.21) to H-2 (δ_H 5.88). Consequently, compound **4** was deduced to be (2*E*,4*E*,9*Z*)-1-hydroxyoctadecatrien-6-yn-18-yl acetate.

The molecular formula of compound **5** was established as $C_{18}H_{28}O_2$ by the HREIMS (*m/z* 276.2094 $[M]^+$), revealing the compound to be 42 amu less than that of **4**. Its ^{1}H and ^{13}C NMR spectra (Table 2) were closely related to those of **3** and **4**, but contained no acetyl group signals. Thus, compound **5** was established as a deacetylated derivative of **4**, namely, (2*E*,4*E*,9*Z*)-octadecatrien-6-yne-1,18-diol.

Compound **6** gave a molecular formula of $C_{15}H_{18}O$ on the basis of the HREIMS (*m/z* 214.1354 $[M]^+$), a loss of 28 amu when compared with bupleurynol (**12**).[4,8] The UV spectrum of **6**, which showed absorption maxima at 249, 264, 277, 294, 313, and 334 nm, was similar to that of **12**, indicating the presence of a diene-diyne-ene chromophore.[4,8,12] Its IR and ^{1}H and ^{13}C NMR spectroscopic data were also similar to those of **12**, except for the loss of two methylenes in compound **6**. On the basis of the NMR and mass data, the structure of **6** was determined as (2*Z*,8*E*,10*E*)-pentadecatriene-4,6-diyn-1-ol.

Compound **7** was found to possess the molecular formula $C_{18}H_{26}O_2$, as inferred from the HREIMS (*m/z* 274.1920 $[M]^+$). The UV spectrum exhibited absorption maxima at 210, 238, 251, 264, and 280 nm, which was typical for an ene-diyne system.[12] In comparison with the NMR data of **13**, the absence of a methyl group at C-17 (δ_H 0.89, δ_C 14.1) and the occurrence of an additional methylene (δ_H 1.57, δ_C 32.8) and an oxymethylene (δ_H 3.64, δ_C 63.1) suggested that **7** is a C_{18} alcohol, and the C-18 position was assigned as a hydroxylmethyl group. Thus, **7** was defined as (2*Z*,9*Z*)-octadecadiene-4,6-diyne-1,18-diol.

Table 2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for Compounds **2**−**5** in $CDCl_3$ (δ in ppm, J in Hz in parentheses)

	2		**3**		**4**		**5**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.62 br d (6.4)	64.4 CH_2	4.60 br d (6.2)	64.4 CH_2	4.21 br d (5.7)	63.1 CH_2	4.21 br d (5.7)	63.1 CH_2
2	5.80 dt (15.2, 6.4)	128.0 CH	5.79 dt (15.3, 6.2)	128.0 CH	5.88 dt (15.2, 5.7)	133.7 CH	5.88 dt (15.2, 5.7)	133.7 CH
3	6.29 dd (15.2, 11.0)	133.3 CH	6.29 dd (15.3, 11.0)	133.2 CH	6.28 dd (15.2, 11.0)	130.4 CH	6.28 dd (15.2, 11.0)	130.4 CH
4	6.50 dd (15.4, 11.0)	139.3 CH	6.50 dd (15.5, 11.0)	139.3 CH	6.52 dd (15.6, 11.0)	139.8 CH	6.52 dd (15.6, 11.0)	139.8 CH
5	5.64 d (15.4)	113.3 CH	5.63 d (15.5)	113.2 CH	5.60 d (15.6)	112.1 CH	5.60 d (15.6)	112.1 CH
6		79.1 C		79.1 C		79.3 C		79.3 C
7		92.1 C		92.0 C		91.5 C		91.5 C
8	3.09 br d (6.5)	18.0 CH_2	3.08 br d (6.6)	18.0 CH_2	3.08 br d (6.8)	18.0 CH_2	3.08 br d (6.7)	18.0 CH_2
9	5.43 dt (10.6, 7.2)	123.6 CH	5.43 dt (10.6, 7.1)	123.7 CH	5.43 dt (10.6, 7.2)	123.8 CH	5.43 dt (10.6, 7.2)	123.7 CH
10	5.49 dt (10.6, 7.2)	132.2 CH	5.48 dt (10.6, 7.1)	132.0 CH	5.49 dt (10.6, 7.2)	132.0 CH	5.49 dt (10.6, 7.2)	132.1 CH
11	2.05 q (7.2)	27.2 CH_2	2.05 (overlapped)	27.1 CH_2	2.08 m	27.1 CH_2	2.05 q (7.2)	27.1 CH_2
12	1.29 m	29.7 CH_2	1.30 m	29.3 CH_2	1.30 m	29.3 CH_2	1.30 m	29.5 CH_2
13	1.29 m	29.2 CH_2	1.30 m	29.2 CH_2	1.30 m	29.1 CH_2	1.30 m	29.3 CH_2
14	1.29 m	28.9 CH_2	1.30 m	29.3 CH_2	1.30 m	29.3 CH_2	1.30 m	29.4 CH_2
15	1.29 m	31.8 CH_2	1.30 m	29.1 CH_2	1.30 m	29.1 CH_2	1.30 m	29.2 CH_2
16	1.37 m	22.6 CH_2	1.30 m	25.9 CH_2	1.30 m	25.8 CH_2	1.30 m	25.7 CH_2
17	0.89 t (7.0)	14.1 CH_3	1.30 m	28.6 CH_2	1.30 m	28.6 CH_2	1.56 m	32.8 CH_2
18			4.05 t (6.8)	64.6 CH_2	4.05 t (6.8)	64.7 CH_2	3.64 t (6.7)	63.1 CH_2
OAc-1		170.7 C		170.7 C				
	2.08 s	20.9 CH_2	2.08 s	20.9 CH_2				
OAc-18				171.2 C		171.3 C		
			2.05 s	21.0 CH_2	2.05 s	21.0 CH_2		

Table 3. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for Compounds **8**−**11** in $CDCl_3$ (δ in ppm, J in Hz in parentheses)

	8		**9**		**10**		**11**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.84 dd (6.5, 1.5)	62.4 CH_2	4.40 dd (6.5, 1.5)	61.0 CH_2	4.42 dd (6.5, 1.5)	61.1 CH_2	4.41 dd (6.5, 1.5)	61.1 CH_2
2	6.14 (overlapped)	139.5 CH	6.22 (11.0, 6.5)	145.2 CH	6.23 dt (11.0, 6.5)	145.3 CH	6.22 dt (11.0, 6.5)	145.2 CH
3	5.76 d (11.0)	111.8 CH	5.66 d (11.0)	109.5 CH	5.66 d (11.0)	109.5 CH	5.66 d (11.0)	109.4 CH
4		77.1 C		77.8 C		77.8 C		77.8 C
5		80.6 C		79.8 C		79.8 C		79.7 C
6		75.3 C		75.3 C		75.4 C		75.5 C
7		83.3 C		82.9 C		82.8 C		82.7 C
8	5.58 d (15.6)	107.5 CH	5.56 d (15.5)	107.5 CH	5.58 d (15.5)	107.7 CH	5.58 d (15.5)	108.0 CH
9	6.71 dd (15.6, 11.0)	145.5 CH	6.69 dd (15.5, 11.0)	145.4 CH	6.69 dd (15.5, 10.8)	145.1 CH	6.66 dd (15.5, 10.8)	145.0 CH
10	6.15 (overlapped)	129.8 CH	6.14 (15.2, 11.0)	129.8 CH	6.12 dd (15.0, 10.8)	129.9 CH	6.12 dd (15.0, 10.8)	130.2 CH
11	5.91 dt (15.2, 7.1)	139.7 CH	5.90 dt (15.2, 7.1)	139.6 CH	5.86 dt (15.0, 7.1)	138.9 CH	5.84 dt (15.0, 7.1)	137.9 CH
12a	2.30 m	29.1 CH_2	2.28 m	29.1 CH_2	2.15 m	28.8 CH_2	2.50 m	26.8 CH_2
12b	2.23 m		2.20 m					
13a	1.58 m	36.0 CH_2	1.56 m	36.0 CH_2	1.65 m	33.3 CH_2	2.50 m	41.5 CH_2
13b	1.53 m		1.51 m					
14	3.62 m	71.0 CH	3.62 m	71.0 CH	4.89 m	73.5 CH		210.0 C
15	1.42 m	39.8 CH_2	1.42 m	39.8 CH_2	1.50 m	36.3 CH_2	2.38 m	44.8 CH_2
16	1.42 m	18.8 CH_2	1.42 m	18.8 CH_2	1.30 m	18.5 CH_2	1.60 m	17.2 CH_2
17	0.93 t (7.0)	14.1 CH_3	0.92 t (7.0)	14.1 CH_3	0.90 t (7.0)	13.9 CH_3	0.90 t (7.0)	13.7 CH_3
OAc-1		170.7 C						
	2.09 s	20.8 CH_3						
OAc-14						170.9 C		
					2.03 s	21.2 CH_3		

Compound **9** was identified as bupleurotoxin on the basis of unequivocal assignments of its 1D- and 2D-NMR spectroscopic data and by comparison with the literature data (including UV, IR, optical rotation).[4] However, the absolute configuration at C-14 of bupleurotoxin is still unknown. In order to complete the structure characterization, the absolute configuration of this compound was determined by application of the modified Mosher ester method.[14] Treatment of **9** with (*R*)-MTPA chloride and (*S*)-MTPA chloride afforded the (*S*)-diester (**9a**) and (*R*)-diester (**9b**), respectively. By analysis of the $\Delta\delta_{H(S-R)}$ values of the protons neighboring the oxygenated methane according to the Mosher model (Figure 1), the assignment of C-14 was the *S* configuration.

Compound **8** gave a molecular formula of $C_{19}H_{24}O_3$, as shown by HREIMS at *m*/*z* 300.1726 $[M]^+$, 42 amu more than that of bupleurotoxin (**9**). The ^{1}H and ^{13}C NMR spectroscopic data of **8** were very similar to those of **9**, with differences being due to the presence of an acetate group [δ_H 2.09 (3H, s); δ_C 170.7 (C), 20.8 (CH_3)] in **8** (Table 3). Considering the significant downfield shift of the H-1 doublet from δ_H 4.40 in **9** to δ_H 4.84 in **8**, the acetate group was presumed to be placed at the C-1 position. This was confirmed by the HMBC correlation from H-1 (δ_H 4.84) to the ester carbonyl (δ_C 170.7). The absolute configuration of **8** was determined to be the same as that of **9**, because the two compounds displayed the same optical sign. Therefore, the structure of **8** was elucidated as (2*Z*,8*E*,10*E*)-14*S*-hydroxyheptadecatriene-4,6-diyn-1-yl acetate.

In a similar way, the other acetylated derivative of bupleurotoxin (**9**) was identified as acetylbupleurotoxin (**10**) by comparing its

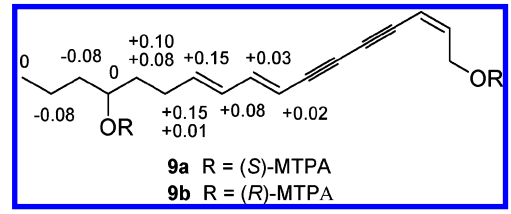

Figure 1. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ obtained for (*S*)- and (*R*)-MTPA esters **9a** and **9b**.

physical and spectroscopic data with the literature values.[4] The opposite specific rotation values for **9** and **10** ($[\alpha]^{17}_D$ +16.3 for **9** versus −13.7 for **10**) were attributed to the opposite absolute configuration at C-14. Therefore, the 14*R* absolute configuration was proposed for **10**.

As far as we know, the ^{13}C NMR spectroscopic data of the known compounds **9**−**11** and **13** have not been reported before in the literature.[4,9,10] Herein, we report the complete ^{1}H NMR and ^{13}C NMR data (Tables 1, 3) on the basis of analysis of their 2D-NMR spectra (COSY, HMQC, and HMBC).

It must be noted that *B. longiradiatum* represents a rich source of polyacetylenes. Natural products of this category have been found only in *B. longiradiatum*,[4] *B. falcatum*,[11] *B. acutifolium*,[8] *B. salicifolium*,[15] and *B. spinosum*[13] of the *Bupleurum* genus, despite the fact that this genus contains more than 200 species. Compounds **1**−**5** are the first polyacetylenes with only a single acetylenic bond isolated from the genus *Bupleurum*. As major compounds of the CH_2Cl_2 extract of *B. longiradiatum*, bupleurotoxin (**9**) and acetylbupleurotoxin (**10**) are claimed to be responsible at least in part for the toxicity of *B. longiradiatum*. However, the isolation of these two compounds has so far been detected only in the title plant. The closest structural variant of bupleurotoxin (**9**) is oenanthotoxin,[16,17] a plant toxin obtained from *Oenanthe fistulosa*, with the difference being the configuration of a single double bond (C-2/C-3), with a *Z* stereochemistry in bupleurotoxin and *E* in oenanthotoxin. Therefore, these polyacetylenes might be viewed as chemotaxonomic markers for *B. longiradiatum*.

Since some polyacetylenes are reported to possess cytotoxic activity against cancer cell lines,[18] all the isolates were tested for their cytotoxicity against a human leukemia cell line (HL-60). Only compounds **8** and **9** were found to be cytotoxic, with IC_{50} values of 9.4 and 4.9 μM, respectively. Since all the remaining isolates were inactive ((IC_{50} >10 μM)), a hydroxy group on the side chain appears to enhance the cytotoxicity of these polyacetylenes. Compounds **1**, **2**, **6**, and **10**−**14** exhibited IC_{50} values in the range 11−18 μM.

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a Perkin-Elmer 341 polarimeter. UV spectra were obtained by a Shimadzu UV-2550 UV−vis spectrophotometer. IR spectra were recorded on a Bruker Vector 22 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer in $CDCl_3$ with TMS as internal standard. EIMS and HREIMS were acquired on a Thermo DSQ II and a Finnigan MAT 95 mass spectrometer, respectively. Materials for column chromatography were silica gel (100−200 mesh; Huiyou Silical Gel Development Co. Ltd., Yantai, People's Republic of China), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-gel ODS-A (50 μm; YMC, Milford, MA). Preparative TLC (0.4−0.5 mm) was conducted with silica gel precoated glass plates GF_{254} (Yantai). Compounds were visualized by exposure to UV light at 254 nm.

Plant Material. The whole plant of *B. longiradiatum* was collected from Mao'ershan Town, Shangzhi City, Heilongjiang Province, People's Republic of China, in September 2008, and identified by Prof. Hanming Zhang, Department of Pharmacognosy, Second Military Medical University. A voucher specimen (20081002) is kept in the Herbarium of Second Military Medical University, Shanghai.

Extraction and Isolation. The air-dried and powdered sample of *B. longiradiatum* (2.0 kg) was extracted in a Soxhlet apparatus sequentially with CH_2Cl_2 (5 L) and ethanol (5 L). The CH_2Cl_2 extract (60 g) was separated into five fractions (A−E) by column chromatography on silica gel using gradient mixtures of hexane−EtOAc (100−0%). Fraction A was further chromatographed on silica gel with hexane−EtOAc (20:1) to give seven subfractions (FA.1−FA.7). Fraction A.2 was purified by preparative TLC (hexane−EtOAc, 19:1), yielding compounds **1** (22 mg, R_f = 0.35) and **2** (4 mg, R_f = 0.28). Fraction B was chromatographed using reversed-phase MPLC in a gradient system of H_2O−MeOH (50%−100%) to give nine subfractions (FB.1−FB.9). Subfraction FB.3 was further purified by preparative TLC (hexane−EtOAc, 10:1) to give **6** (8 mg, R_f = 0.28) and **14** (12 mg, R_f = 0.20). Subfraction FB.6 was purified on a silica gel column ($CHCl_3$−MeOH, 30:1) to afford **12** (46 mg) and **13** (5 mg). Subfraction FB.9 was separated by silica gel chromatography, eluting with hexane−EtOAc (8:1), and then by Sephadex LH-20 chromatography eluting with $CHCl_3$−MeOH (1:1), to give **5** (24 mg). Fraction C was subjected to column chromatography on silica gel (hexane−EtOAc, 6:1) and Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) to give **4** (7 mg), **8** (13 mg), **10** (27 mg), and **11** (8 mg). Repeated column chromatography of fraction D over silica gel (hexane−EtOAc, 2:1; $CHCl_3$−MeOH, 15:1) gave **7** (5 mg) and **9** (47 mg). Fraction E was chromatographed on a silica gel column eluting with hexane−EtOAc (1:1), followed by a Sephadex LH-20 column (MeOH) to give **5** (6 mg).

(2*E*,4*E*,8*E*,10*E*)-Heptadecatetraen-6-yn-1-yl acetate (1): colorless oil; UV (MeOH) λ_{max} (log ε) 338 (4.04), 316 (4.01) nm; IR (KBr) ν_{max} 2929, 2858, 2177, 1741, 1639, 1456, 1367, 1234, 1047, 985 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; EIMS 70 eV *m*/*z* 286 $[M]^+$ (15), 244 (8), 226 (10), 175 (10), 169 (19), 159 (38), 156 (34), 155 (100), 145 (34), 144 (31), 141 (27), 133 (29), 129 (36), 117 (36), 115 (94), 107 (27), 91 (64), 79 (24), 77 (11); HREIMS *m*/*z* 286.1937 $[M]^+$ (calcd for $C_{19}H_{26}O_2$, 286.1941).

(2*E*,4*E*,9*Z*)-Heptadecatrien-6-yn-1-yl acetate (2): colorless oil; UV (MeOH) λ_{max} (log ε) 280 (4.07), 267 (3.88) nm; IR (KBr) ν_{max} 2925, 2854, 2212, 1743, 1630, 1457, 1378, 1232, 1047, 980 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; EIMS 70 eV *m*/*z* 288 $[M]^+$ (5), 246 (3), 157 (28), 144 (22), 143 (100), 129 (52), 128 (35), 117 (22), 91 (10), 79 (16), 77 (12); HREIMS *m*/*z* 288.2089 $[M]^+$ (calcd for $C_{19}H_{28}O_2$, 288.2088).

(2*E*,4*E*,9*Z*)-Octadecatrien-6-yne-1,18-diyl diacetate (3): colorless oil; UV (MeOH) λ_{max} (log ε) 280 (4.05), 267 (3.91) nm; IR (KBr) ν_{max} 2930, 2856, 2189, 1740, 1638, 1437, 1367, 1242, 1043, 980 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; EIMS 70 eV *m*/*z* 360 $[M]^+$ (2), 318, (15), 300 (26), 276 (6), 157 (33), 155 (35), 143(100), 129 (84), 128 (52),117 (38), 115 (34), 91 (60); HREIMS *m*/*z* 360.2309 $[M]^+$ (calcd for $C_{22}H_{32}O_4$, 360.2317).

(2*E*,4*E*,9*Z*)-1-Hydroxyoctadecatrien-6-yn-18-yl acetate (4): colorless oil; UV (MeOH) λ_{max} (log ε) 280 (4.02), 267 (3.88) nm; IR (KBr) ν_{max} 3429, 2927, 2856, 2212, 1737, 1638, 1463, 1367, 1242, 1043, 980 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; EIMS 70 eV *m*/*z* 318 $[M]^+$ (3), 300 (9), 276 (10), 157 (6), 155 (30), 143(83), 129 (88), 128 (59), 115 (52), 91 (100), 79 (59), 77 (30); HREIMS *m*/*z* 318.2192 $[M]^+$ (calcd for $C_{20}H_{30}O_3$, 318.2189).

(2*E*,4*E*,9*Z*)-Octadecatrien-6-yne-1,18-diol (5): colorless oil; UV (MeOH) λ_{max} (log ε) 280 (3.95), 267 (3.86) nm; IR (KBr) ν_{max} 3289, 3148, 2919, 2852, 2206, 1641, 1467, 1415, 983 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; EIMS 70 eV *m*/*z* 276 $[M]^+$ (11), 143(46), 129 (55), 128 (44), 117 (81), 115 (36), 91 (100), 79 (53), 77 (27); HREIMS *m*/*z* 276.2094 $[M]^+$ (calcd for $C_{18}H_{28}O_2$, 276.2098).

(2*Z*,8*E*,10*E*)-Pentadecatriene-4,6-diyn-1-ol (6): colorless oil; UV (MeOH) λ_{max} (log ε) 334 (3.90), 313 (4.02), 294 (3.91), 277 (3.50) 264 (3.88), 249 (3.92) nm; IR (KBr) ν_{max} 3400, 2956, 2871, 2198, 1642, 1463, 1380, 1182, 1080, 980 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; EIMS 70 eV *m*/*z* 214 $[M]^+$ (81), 171 (14), 157 (22), 152 (17), 142 (13), 141 (41), 128 (100), 127 (40), 117 (18), 115 (74), 85 (36), 85 (36), 77 (38), 71 (56); HREIMS *m*/*z* 214.1354 $[M]^+$ (calcd for $C_{15}H_{18}O$, 214.1351).

(2*Z*,9*Z*)-Octadecadiene-4,6-diyne-1,18-diol (7): colorless oil; UV (MeOH) λ_{max} (log ε) 280 (3.74), 264 (3.94), 251 (3.87), 238 (3.53), 210 (4.03) nm; IR (KBr) ν_{max} 3347, 3022, 2927, 2854, 2233, 1685, 1637, 1457, 1290, 1029, 732 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; EIMS 70 eV *m*/*z* 274 $[M]^+$ (15), 173 (16), 159 (59), 145 (43), 141 (60), 131 (41), 129 (66), 128 (59), 117 (59), 115 (73), 106 (43), 91 (100), 77 (29), 68 (58), 55 (38); HREIMS *m*/*z* 274.1920 $[M]^+$ (calcd for $C_{18}H_{26}O_2$, 274.1908).

(2*Z*,8*E*,10*E*)-14*S*-Hydroxyheptadecatriene-4,6-diyn-1-yl acetate (8): colorless oil; $[\alpha]^{17}_D$ +14.3 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 334 (3.92), 314 (4.05), 295 (3.91), 277 (3.67), 265 (3.90), 249 (3.96) nm; IR (KBr) ν_{max} 3427, 2931, 2871, 2202, 1740, 1636, 1630, 1440, 1371, 1232, 1022, 980 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; EIMS 70 eV *m*/*z* 300 $[M]^+$ (5), 256 (20), 240 (32), 211 (12), 197 (19), 179 (30), 169 (35), 153 (83), 141 (71), 128 (72), 115 (100), 91 (30); HREIMS *m*/*z* 300.1726 $[M]^+$ (calcd for $C_{19}H_{24}O_3$, 300.17242).

MTPA Esters of Bupleurotoxin (9). Bupleurotoxin (**9**, 1.0 mg) was dissolved in 0.5 mL of dry pyridine and treated with (*R*)-MTPA chloride

(10 μL) and *N*,*N*-dimethylaminopyridine (DMAP, a spatula tip), then maintained at room temperature under stirring overnight. After removal of the solvent, the reaction mixture was purified by preparative TLC (hexane−EtOAc, 5:1, $R_f = 0.35$), affording the (*S*)-MTPA diester **9a** in a pure state. Using (*S*)-MTPA chloride, the same procedure afforded the (*R*)-MTPA diester **9b** in the same yield.

Bupleurotoxin 1-*O*-14-*O*-(*S*)-MTPA diester (9a): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 and 7.34 (MTPA phenyl protons), 6.62 (H-9, dd, $J = 15.5$, 11.0 Hz), 6.07 (H-2, dt, $J = 11.0$, 6.5 Hz), 6.03 (H-10, dd, $J = 15.0$, 10.8 Hz), 5.85 (H-11, $J = 15.0$, 7.0 Hz), 5.76 (H-3, d, $J = 11.0$ Hz), 5.52 (H-8, d, $J = 15.5$ Hz), 5.04 (H-14, m), 5.01 (H_2-1, d, $J = 6.5$ Hz), 3.49 (MTPA OCH_3, s), 2.12 (H-12a, overlapped), 1.95 (H-12b, overlapped), 1.72 (H-13a, m), 1.65 (H-13b, m), 1.48 (H_2-15, m), 1.48 (H_2-16, m), 0.81 (H_3-17, d, $J = 7.0$ Hz); EIMS *m*/*z* 690 $[M]^+$.

Bupleurotoxin 1-*O*-14-*O*-(*R*)-MTPA diester (9b): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 and 7.34 (MTPA phenyl protons), 6.59 (H-9, dd, $J = 15.5$, 11.0 Hz), 6.07 (H-2, dt, $J = 11.0$, 6.5 Hz), 5.95 (H-10, dd, $J = 15.0$, 10.8 Hz), 5.77 (H-3, d, $J = 11.0$ Hz), 5.70 (H-11, $J = 15.0$, 7.0 Hz), 5.49 (H-8, d, $J = 15.5$ Hz), 5.04 (H-14, m), 5.01 (H_2-1, d, $J = 6.5$ Hz), 3.49 (MTPA OCH_3, s), 1.97 (H-12a, overlapped), 1.94 (H-12b, overlapped), 1.61 (H-13a, m), 1.57 (H-13b, m), 1.49 (H_2-15, m), 1.49 (H_2-16, m), 0.81 (H_3-17, d, $J = 7.0$ Hz); EIMS *m*/*z* 690 $[M]^+$.

Cytotoxicity Assay. Growth inhibiton of all isolates (compounds **1**−**14**) against HL-60 (human leukemia) cells was tested using an established colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay protocol.[19] Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 4000−5000 cells per well and allowed to adhere for 24 h before drug addition. Each cancer cell line was exposed to the tested compounds with concentrations of 0.01, 0.1, 1, 10, and 100 μg/mL. After 3 days in culture, attached cells were incubated with MTT (5 mg/mL) and solubilized in DMSO 4 h later. The optical densities (OD) were read on an enzyme-labeled detector (Denley MK-2) at a wavelength of 570 nm. The inhibitory rate of cell proliferation was calculated by the following formula: Growth inhibition (%) = $(OD_{control} - OD_{treated})/OD_{control} \times 100\%$. Doxorubicin was used as positive control and exhibited an IC_{50} value of 0.09 μM.

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Supporting Information Available: 1D and 2D NMR spectra of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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