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Authors: Xikang Zheng, Xiaoqing Zheng, Chen Zhang, Qingying Zhang, Yong Jiang, Pengfei Tu

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Communication

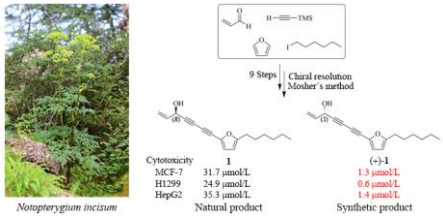
Cytotoxic polyacetylenes isolated from the roots and rhizomes of *Notopterygium incisum*

Xikang Zheng, Xiaoqing Zheng, Chen Zhang, Qingying Zhang, Yong Jiang, Pengfei Tu*

State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

* Corresponding author.
E-mail address: pengfeitu@vip.163.com (P. Tu).

Graphical Abstract



A new polyacetylene, notopolyenol A, isolated from *Notopterygium incisum* was identified by spectroscopic technique and chemical method, and its synthetic enantiomer displayed significant cytotoxicity against MCF-7, H1299, and HepG2 cancer cells with IC_{50} values ranging from 0.6μmol/L to 1.4 μmol/L.

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ABSTRACT

Phytochemical investigation on the roots and rhizomes of *Notopterygium incisum* led to the isolation of a new polyacetylene, notopolyenol A (**1**), along with thirteen known analogues (**2**–**14**). Their structures were elucidated by extensive analyses of NMR and HRMS data, and the absolute configuration of **1** was unambiguously determined as 3*R* by comparison of its retention time and ECD curve with those of synthetic enantiomers (–)-**1** and (+)-**1**, whose absolute configurations were established by using the modified Mosher's method. Subsequent activity screening revealed that (3*S*)-**1** exhibited the most significant cytotoxicity against MCF-7, H1299, and HepG2 cancer cells with IC_{50} values of 1.3 μmol/L, 0.6 μmol/L and 1.4 μmol/L, respectively.

Polyacetylenes, characterized by possessing conjugated carbon-carbon triple bonds in structural skeleton, are a kind of secondary metabolites distributed widely in organisms, comprising plants [1], marine organisms [2,3], microorganisms [4], and animals [5]. This class of compounds has aroused great interest of medicinal chemists and pharmaceutical industries due to its broad variety of biological properties, such as anti-inflammatory [6], cytotoxic [7,8], and immunosuppressive [9] activities. During our ongoing program aimed at searching for bioactive constituents from *Notopterygium incisum*, a traditional Chinese herb used for treatment of inflammation-related diseases [10], a sub-fraction of its 95% aq. EtOH was found to exhibit potent cytotoxicity against different cancer cells. Subsequent chemical investigation led to the isolation of 14 polyacetylenes, including a new compound, notopolyenol A (**1**) (Fig. 1).

Notopolyenol A (**1**) was isolated as a colorless oil with the molecular formula of $C_{17}H_{20}O_2$ determined by HREIMS at *m*/*z* 256.1456 [M^+] (calcd. for $C_{17}H_{20}O_2$: 256.1458), corresponding to eight indices of hydrogen deficiency. The ^{1}H NMR spectrum (Table 1) displayed characteristic signals for a terminal vinyl group [δ_H 6.00 (ddd, 1H, *J* = 17.1, 10.2, 5.3 Hz), 5.53 (dt, 1H, *J* = 17.1, 1.2 Hz), and 5.31 (dt, 1H, *J* = 10.2, 1.2 Hz)], an aliphatic chain [δ_H 2.63 (t, 2H, *J* = 7.6 Hz), 1.65 (quintet, 2H, *J* = 7.5 Hz), 1.32 (m, 6H), and 0.91 (t, 3H, *J* = 6.9 Hz)], and two coupled furan protons [δ_H 6.68 (d, 1H, *J* = 3.3 Hz) and 6.01 (d, 1H, *J* = 3.3 Hz)]. Inspection of the ^{13}C NMR data (Table 1) in combination with the HSQC correlations classified 17 carbons into two conjugated acetylenic bonds (δ_C 83.8, 78.0, 70.9, and 69.6), two trisubstituted double bonds (δ_C 134.4, 119.9 and 159.9, 106.7), a monosubstituted double bond (δ_C 117.5 and 136.0), an oxygenated sp^3 methine (δ_C 63.9), five sp^3 methenes (δ_C 31.6, 28.9, 28.6, 27.9, and 22.7), and one methyl (δ_C 14.2). The aforementioned

information suggested that **1** was a derivative of falcarindiol (**5**) [11] with the presence of a furan ring consisted of C-8 (δ_C 134.4), C-9 (δ_H 6.68, δ_C 119.9), C-10 (δ_H 6.01, δ_C 106.7), and C-11 (δ_C 159.9), which accounted for the one remaining indice of hydrogen deficiency. The HMBC correlations of H-9/C-8 and C-11, H-10/C-8 and C-11, and H_2-12/C-10 and C-11 and the NOESY correlation of H-10/H_2-12 (Fig. S3 in Supporting information) supported the above deduction. Thus, the 2D structure of **1** was defined as shown.

To establish the absolute configuration of **1**, the modified Mosher's method was carried out. However, attempts to prepare two Mosher's esters of **1** failed due to the limitation of its amount (1.1 mg). Therefore, the total synthesis of racemic (±)-**1** should be conducted, and its retrosynthetic analysis is depicted in Scheme 1. (±)-**1** could be constructed by Sonogashira reaction [12] from diyne **15** and iodofuran **16**. Disconnection at the conjugated acetylenic bond of **15** would give two alkynes **17** and **18** as intermediates for Cadiot-Chodkiewicz coupling [13], while the former subunit could be further disassembled into aldehyde **21** and TMS-protected acetylene **18** as substrates of 1,2-addition. Fragment **16** would be prepared by substitution reaction from furan **19** and alkyl iodide **20**.

The synthesis of (±)-**1** is summarized in Scheme 2. 1,2-Addition of acrolein (**21**) with trimethylsilylacetylene (**18**) in the presence of *n*-BuLi followed by protection of the resulting hydroxyl in **22** with *t*-butyldiphenylsilyl (TBDPS) group afforded **23** as a silyl ether. Treatment of **23** with *N*-bromosuccinic imide (NBS) and catalytic amount of $AgNO_3$ gave brominated alkyne **17**, which was coupled with **18** subsequently *via* Cadiot-Chodkiewicz reaction [13] to furnish the conjugated diyne **24**. The terminal TMS group of **24** was then selectively removed in K_2CO_3/MeOH to obtain **15**. The coupling reaction of **15** with **16**, which was prepared by alkylation of furan (**19**) with 1-iodohexane (**20**) using *n*-BuLi followed by iodination, under Sonogashira reaction condition [12] yielded **26** smoothly. Finally, deprotection of the TBDPS group of **26** by tetra-*n*-butylammonium fluoride (TBFA) provided (±)-**1** in 20.6% overall yield for 7 steps from **21**.

HPLC chiral resolution of (±)-**1** afforded two enantiomers, (−)-**1** at 8.7 min and (+)-**1** at 11.4 min, respectively. Both enantiomers were subjected to esterification with (*R*)- and (*S*)-α-methoxyphenylacetic acid (MPA) to obtain the corresponding esters, and analyses of their $\Delta\delta^{RS}$ ($\delta_R - \delta_S$) values led to the assignment of 3*R* configuration for (−)-**1** and 3*S* configuration for (+)-**1** (Fig. S1 in Supporting information). By comparison of the HPLC chromatogram and ECD cruve of **1** with those of the synthetic products (Figs.S4 and S5 in Supporting information), the absolute configuration of **1** was unambiguously defined as 3*R*.

A plausible biogenetic pathway to **1** is proposed with falcarindiol (**5**), a known co-isolated compound with 3*R* and 8*S* configuration determined by the modified Mosher's method (Fig. S2 in Supporting information), as the precursor (Scheme S1 in Supporting information). Allylic oxidation of **5** resulted in the generation of carbonyl group at C-11 followed by nucleophilically attacked by hydroxyl group at C-8. Then the removal of H_2O induced the electrons transfer and formation of a furan ring, and finally converted to **1**.

All isolated compounds **1**−**14,** as well as the synthetic product (+)-**1,** were evaluated for their cytotoxicity against three cancer cell lines, MCF-7, H1299, and HepG2, using the sulforhodamine B (SRB) assay [14], and their IC_{50} values are presented in Table 2. Of these, the synthetic compound (+)-**1** exhibited the most potent cytotoxic activity against the three cancer cell lines with IC_{50} values ranging from 0.6 μmol/L to 1.4 μmol/L, at least 24-fold lower than those of its enantiomer **1**, indicating the importance of 3*S* configuration for the cytotoxic effect. Panaxydiol-type polyacetylenes (**2**−**4**), with IC_{50} values of 10.7−24.9 μmol/L, displayed stronger inhibitory effects on the test cancer cells than those of most of falcarindiol-type polyacetylenes (**5**−**12**) and their reduction products (**13** and **14**), suggesting that the conjugated system enlarged by 8*E*-double bond may play a positive role in their cytotoxicity.

[a] $IC_{50} > 100$ μmol/L.

[b] Positive control.

[c] Values presented in nmol/L.

In summary, a new polyacetylene (**1**), together with thirteen analogues (**2**−**14**), was isolated from the roots and rhizomes of *N. incisum*. Its absolute configuration was determined as 3*R* by applying the modified Mosher's method to synthetic enantiomers (−)-**1** and (+)-**1** followed by comparing their HPLC retention times and ECD spectra. Interestingly, the synthetic product (+)-**1**, the enantiomer of **1**, displayed the most significant cytotoxic activity against three cancer cell lines (MCF-7, H1299, and HepG2).

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References

[1] K. Xu, Z. Feng, J. Jiang, et al., Chin. Chem. Lett. 28 (2017) 597−601.
[2] M. Kladi, C. Vagias, P. Papazafiri, et al., J. Nat. Prod. 72 (2009) 190−193.
[3] E.J. Mejia, L.B. Magranet, N.J. de Voogd, et al., J. Nat. Prod. 76 (2013) 425−432.
[4] J. Chen, W. Lin, C. Liao, et al., J. Nat. Prod. 70 (2007) 989−992.
[5] G. Fabrias, M. Barrot, F. Camps, Insect Biochem. Molec. Biol. 25 (1995) 655−660.
[6] M.C. Yang, H.C. Kwon, Y. Kim, et al., J. Nat. Prod. 73 (2010) 801−805.
[7] S. Sun, G. Du, L. Qi, et al., J. Ethnopharmacol. 132 (2010) 280−285.
[8] H.R. Jin, J. Zhao, Z. Zhang, et al., Cell Death Dis. 3 (2012) e376.

[9] S. Mitsui, K. Torii, H. Fukui, et al., J. Pharmacol. Exp. Ther. 333 (2010) 954−960.
[10] Editorial Committee of Chinese Materia Medica of State Administration of Traditional Chinese Medicine, Chinese Materia Medica, Shanghai Scientific & Technical Publishers, Shanghai, 1999, p. 992.
[11] D. Lechner, M. Stavri, M. Oluwatuyi, et al., Phytochemistry 65 (2004) 331−335.
[12] K. Ma, Y. Miao, X. Gao, et al., Chin. Chem. Lett. 28 (2017) 1035−1038.
[13] B.V. Subba Reddy, R. Nageshwar Rao, B. Kumaraswamy, J.S.Yadav, Tetrahedron Lett. 55 (2014) 4590−4592.
[14] V. Vichai, K. Kirtikara, Nat. Protoc. 1 (2006) 1112−1116.

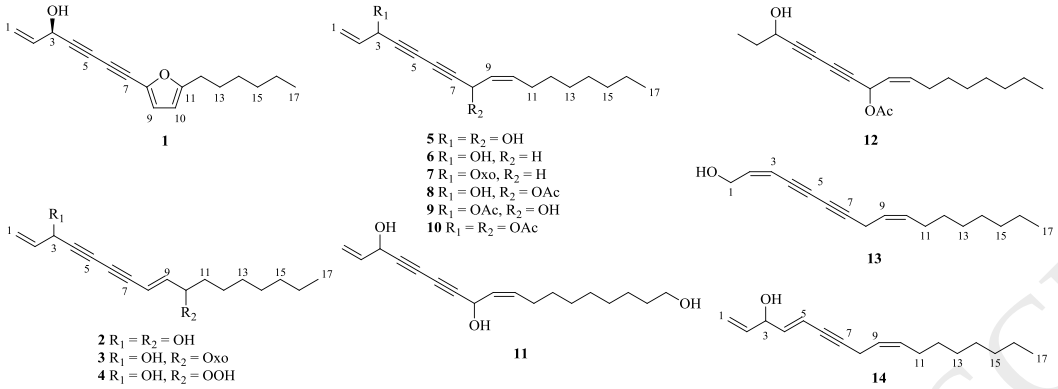
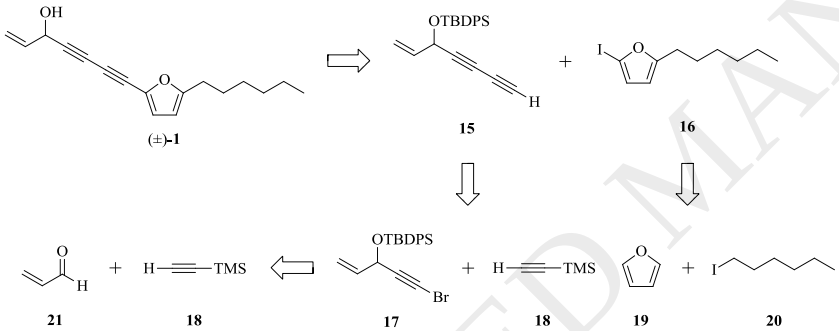
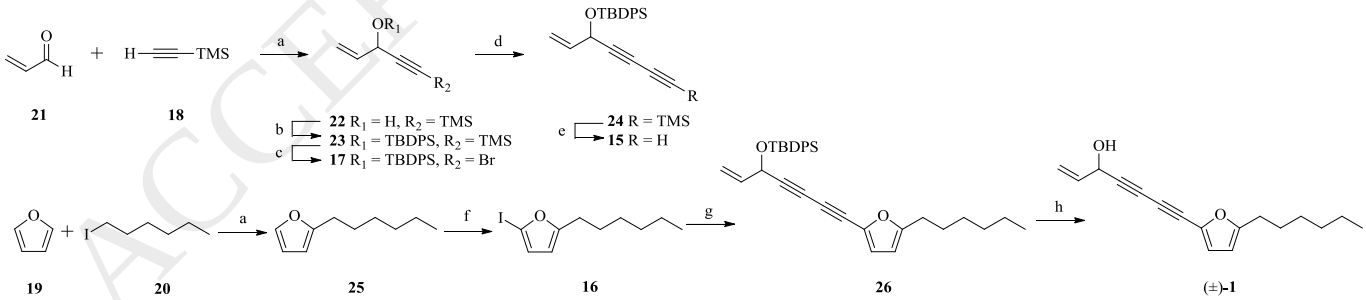


Fig. 1. Structures of compounds **1**−**14**.



Scheme 1. Retrosynthetic analysis of compound (±)-**1**.



Scheme 2. Synthesis of compound (±)-**1**. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C to r.t., 87% for **22**, 70% for **25**; (b) TBDPSCl, Et_3N, DMAP, DCM, 0 °C to r.t., 90%; (c) NBS, $AgNO_3$, Me_2CO, r.t. 88%; (d) **18**, CuCl, *n*-$BuNH_2$, $NH_2OH{\cdot}HCl$, DCM, 0 °C, 63%; (e) K_2CO_3, MeOH, r.t., 82%; (f) I_2, *n*-BuLi, THF, −78 °C to 0 °C, 70%; (g) **15**, CuI, PPh_3, $(PPh_3)Pd_2Cl_2$, Et_3N, 60 °C, 69%; (h) TBAF, DCM, r.t., 84%.

Table 1

^1H (400 MHz) and ^{13}C (100 MHz) NMR data of compound **1** (δ in ppm) in $CDCl_3$.

Position	δ_H (mult., *J* in Hz)	δ_C, type
1a	5.53 (dt, 17.1, 1.2)	117.5, CH_2
1b	5.31 (dt, 10.2, 1.2)	
2	6.00 (ddd, 17.1, 10.2, 5.3)	136.0, CH
3	5.05 (t, 5.9)	63.9, CH
4		83.8[a], C
5		78.0[a], C
6		70.9[a], C
7		69.6[a], C
8		134.4, C
9	6.68 (d, 3.3)	119.9, CH
10	6.01 (d, 3.3)	106.7, CH
11		159.9, C
12	2.63 (t, 7.6)	28.6, CH_2
13	1.65 (quintet, 7.5)	27.9, CH_2
14	1.32 (m)	28.9, CH_2
15	1.32 (m)	31.6, CH_2
16	1.32 (m)	22.7, CH_2
17	0.91 (t, 6.9)	14.2, CH_3
3-OH	1.95 (d, 6.7)	

[a] Assignments may be interchangeable.

Table 2

Cytotoxic effects of compounds **1**–**14** and (+)-**1** against three cancer cell lines.

Compound	IC_{50} (μmol/L)		
	MCF-7	H1299	HepG2
1	31.7 ± 1.3	24.9 ± 0.9	35.3 ± 0.5
(+)-**1**	1.3 ± 0.6	0.6 ± 0.2	1.4 ± 0.7
2	13.5 ± 1.9	12.8 ± 0.9	24.9 ± 0.6
3	15.1 ± 1.9	12.1 ± 0.9	15.1 ± 1.3
4	7.3 ± 0.4	10.7 ± 0.8	19.2 ± 2.2
5	29.4 ± 1.0	22.1 ± 0.9	23.6 ± 2.0
6	43.1 ± 0.1	30.8 ± 0.1	45.2 ± 0.2
7	–[a]	–[a]	–[a]
8	19.0 ± 0.9	16.4 ± 0.7	15.9 ± 0.7
9	29.6 ± 1.9	21.3 ± 1.9	11.7 ± 1.2

10	67.8 ± 2.3	37.6 ± 1.3	22.7 ± 0.2
11	–[a]	–[a]	29.7 ± 2.7
12	45.6 ± 1.5	14.6 ± 0.8	20.8 ± 1.2
13	66.7 ± 1.2	36.0 ± 1.6	47.6 ± 1.9
14	85.7 ± 0.4	31.9 ± 0.2	54.2 ± 1.6
Taxol[b]	2.2 ± 0.3[c]	1.8 ± 0.8[c]	2.0 ± 0.7[c]