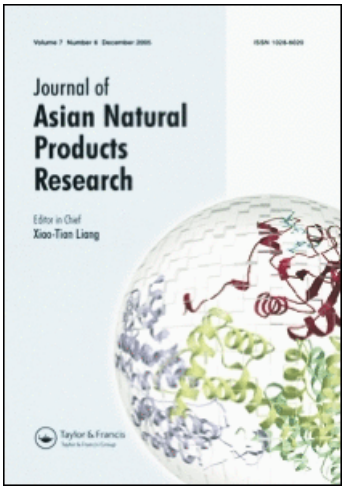
This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two novel thiophenes from *Echinops grijissi* Hance

Peng Zhang[a]; Wen-Rong Jin[a]; Qiang Shi[a]; Hua He[a]; Zhong-Jun Ma[a]; Hai-Bin Qu[a]
[a] College of Pharmaceutical Sciences, Zhejiang University, Institute of Pharmaceutical Informatics, Hangzhou, Zhejiang, China

To cite this Article Zhang, Peng , Jin, Wen-Rong , Shi, Qiang , He, Hua , Ma, Zhong-Jun and Qu, Hai-Bin(2008) 'Two novel thiophenes from *Echinops grijissi* Hance', Journal of Asian Natural Products Research, 10: 10, 977 — 981
To link to this Article: DOI: 10.1080/10286020802240467
URL: http://dx.doi.org/10.1080/10286020802240467

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research
Vol. 10, No. 10, October 2008, 977–981

Taylor & Francis
Taylor & Francis Group

Two novel thiophenes from *Echinops grijissi* Hance

Peng Zhang, Wen-Rong Jin, Qiang Shi, Hua He, Zhong-Jun Ma* and Hai-Bin Qu

College of Pharmaceutical Sciences, Zhejiang University, Institute of Pharmaceutical Informatics, Hangzhou, Zhejiang, China

(*Received 25 February 2008; final version received 23 May 2008*)

Two novel thiophenes, grijisyne A (**1**), and grijisone A (**2**), were isolated from the crude ethanolic extract of the roots of *Echinops grijissi* Hance. Their structures were determined by spectral methods, especially 2D NMR spectra. All the isolated compounds were tested for their anti-tumor activities against three human tumor cell lines, HL-60, K562, and MCF-7.

Keywords: *Echinops grijissi* Hance; grijisyne A; grijisone A; thiophenes; cytotoxicity

Downloaded At: 18:52 22 January 2011

1. Introduction

The genus *Echinops* belongs to the family Compositae and comprises over 120 species, of which 17 are known to occur in China. *Echinops grijissi* Hance is mainly distributed in the southeastern part of China [1]. The roots of *E. grijissi* (commercial Chinese name: Yuzhou Loulu) are listed in *Chinese Pharmacopeia* and used to clear heat, expel miasma, and stimulate milk secretion for a long history [2]. Previous chemical investigation on the roots of *E. grijissi* demonstrated the presence of essential oil [3] and thiophenes [4–8], which have been proven to possess anti-tumor [9,10], insecticide [11,12], anti-virus [9,13,14], and anti-inflammatory activities [15]. Our previous research showed that the CH_2Cl_2 extract possessed medium cytotoxic activity [16]. The present study on the constituents of the crude ethanolic extract of the roots of *E. grijissi* led to the isolation of the two new compounds **1** and **2** (Figure 1). This paper describes the isolation and structure elucidation of two new thiophenes, together with their anti-tumor activities against different human tumor cell lines.

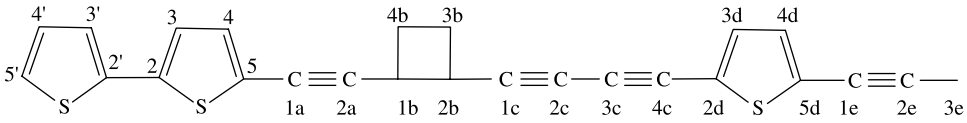
2. Results and discussion

Compound **1** was obtained as green crystals. Its molecular formula $C_{25}H_{16}S_3$ was determined by the pseudomolecular-ion peaks at *m/z* 412 ($[M]^+$) in the EIMS and 412.0429 ($[M]^+$) in the HREIMS. The structure of **1**, grijisyne A, was established by analysis of ^1H and ^{13}C NMR, HMQC, HMBC and ^1H–^1H COSY spectral data (Table 1 and Figure 2).

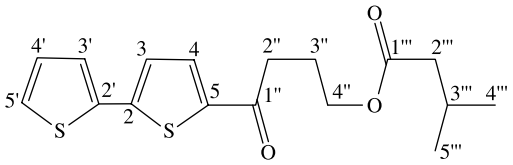
The UV absorption maxima of **1** at 250.6 and 340.4 nm showed the presence of a bithiophene group [4]. The ^1H NMR spectrum of **1** showed characteristic signals [8] of 5-substituted 2,2′-bithiophene protons at δ 7.23 (1H, dd, $J = 4.4$, 0.9 Hz), 7.16(1H, dd, $J = 3.2$, 0.9 Hz), 7.02 (1H, dd, $J = 4.4$, 3.2 Hz), 7.04 (1H, d, $J = 3.6$ Hz), and 7.02 (1H, d, $J = 3.6$ Hz). Additionally, the proton signals that appeared at δ 3.32 (1H, m), 3.34 (1H, m), 2.31 (1H, m), 2.29 (1H, m), 2.16 (1H, m), and 2.14 (1H, m) indicated the presence of cyclobutyl [17], which was confirmed by ^1H–^1H COSY experiment. The signal at δ 2.05 (3H, s) indicated the protons due to the methyl group. The ^{13}C-NMR spectrum of **1** exhibited 25 carbon

*Corresponding author. Email: mazj@zju.edu.cn

ISSN 1028-6020 print/ISSN 1477-2213 online
© 2008 Taylor & Francis
DOI: 10.1080/10286020802240467
http://www.informaworld.com



1



2

Figure 1. Structures of compounds **1** and **2**.

signals, of which eight bithiophene carbons appeared at δ 138.1, 136.7, 132.3, 127.8, 124.7, 123.2, 124.0, and 121.9, four thiophene carbons appeared at δ 133.3, 130.6, 126.6, 122.0, along with eight ynyl carbons at δ 95.4, 91.6, 87.1, 78.0, 76.3, 72.4, 70.0, 67.4, four cyclobutyl carbons at δ 33.4, 33.3, 26.9, 26.4 and a methyl carbon at δ 4.6. In the

Table 1. ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectral data of compounds **1** and **2**.

	1			**2**	
Position	$\delta_{(H)}$	$\delta_{(C)}$	Position	$\delta_{(H)}$	$\delta_{(C)}$
2		138.1	2		145.5
3	7.02 (d, $J = 3.6$ Hz)	123.2	3	7.03 (d, $J = 3.8$ Hz)	128.2
4	7.04 (d, $J = 3.6$ Hz)	132.3	4	7.14 (d, $J = 3.8$ Hz)	132.2
5		121.9	5		141.9
2′		136.7	2′		136.2
3′	7.16 (dd, $J = 3.2$, 0.9 Hz)	124.0	3′	7.18 (dd, $J = 3.1$,0.9 Hz)	125.6
4′	7.02 (dd, $J = 4.4$, 3.2 Hz)	127.8	4′	7.02 (dd, $J = 5.2$, 3.1 Hz)	126.4
5′	7.23 (dd, $J = 4.4$, 0.9 Hz)	124.7	5′	7.25 (dd, $J = 5.2$, 0.9 Hz)	124.1
1a		76.3	1″		191.6
2a		95.4	2″	2.99 (t, $J = 3.2$ Hz)	35.2
1b	3.34 (m)	33.4[A]	3″	2.15 (m)	22.1
2b	3.32 (m)	33.3[A]	4″	4.19 (t, $J = 3.2$ Hz)	63.2
3b	2.29 (m, H_a), 2.16 (m, H_b)	26.9[B]	1‴		173.0
4b	2.31 (m, H_a), 2.14 (m, H_b)	26.4[B]	2‴	2.20 (d, $J = 2.8$ Hz)	43.3
1c		87.1	3‴	2.14 (m)	25.6
2c		67.4	4‴	0.97 (d, $J = 6.4$ Hz)	22.3
3c		78.0	5‴	0.97 (d, $J = 6.4$ Hz)	23.6
4c		70.0			
2d		122.0			
3d	7.10 (d, $J = 4.0$ Hz)	133.3			
4d	6.92 (d, $J = 4.0$ Hz)	130.6			
5d		126.6			
1e		72.4			
2e		91.6			
3e	2.05 (s)	4.6			

A, B: the signals can be exchanged.

Downloaded At: 18:52 22 January 2011

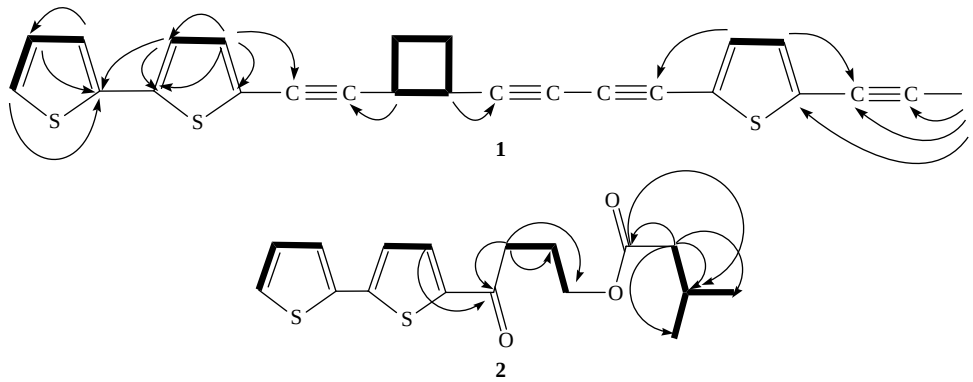


Figure 2. $^{1}H-^{1}H$ COSY (▬) and key HMBC (H → C) correlations of **1** and **2**.

Downloaded At: 18:52 22 January 2011

HMBC experiment (Figure 2), the long-range correlation between H-4 (7.04) and C-1a (76.3) indicated that C-1a was connected with the bithiophene at C-5. In addition, the long-range correlations between H-1b (δ 3.34) and C-2a (δ 95.4), H-2b (δ 3.32) and C-1c (δ 87.1) indicated the linkage of C-1b with C-2a, as well as C-1c with C-2b. The long-range correlation between H-3e (δ 2.05) and C-5d (δ 126.6), as well as H-4d (δ 6.92) and C-1e (δ 72.4) indicated that C-1e was connected with the C-5d. From the above data, the structure of **1** was elucidated as 5-[2-[4-(5-propyneylthiophen-2-yl)buta-1,3-diynyl]cyclobutaneyl]ethynyl]-2,2′-bithiophene.

Compound **2** was obtained as green powder. Its molecular formula $C_{17}H_{20}S_2O_3$ was determined by the pseudomolecular ion peaks at *m/z* 336 ($[M]^+$) in the EIMS and 336.0838 ($[M]^+$) in the HREIMS. The structure of **2**, grijisone A, was established based on analysis of ^{1}H and ^{13}C NMR, HMQC, HMBC, and $^{1}H-^{1}H$ COSY spectral data (Table 1 and Figure 2).

The UV absorption maxima of **2** at 375, 262, and 220 nm showed the presence of a bithiophene group. Furthermore, the IR spectrum showed the presence of a 2-monosubstituted thiophene (839, 717 cm^{-1}) and a 2,2′-bithiophene moiety (801 cm^{-1}; [4]), which was further confirmed by ^{1}H NMR signals at δ 7.25 (1H, dd, $J = 5.2$, 0.9 Hz), 7.18 (1H, dd, $J = 3.1$, 0.9 Hz), 7.14 (1H, d, $J = 3.8$ Hz), 7.03 (1H, d, $J = 3.8$ Hz), and 7.02 (1H, dd, $J = 5.2$, 3.1 Hz). Besides, the ^{1}H NMR spectrum of compound **2** exhibited signals of two CH_3 groups at δ 0.97 (3H, s, 4‴-CH_3) and 0.97 (3H, s, 5‴-CH_3), four CH_2 groups at δ 4.19 (2H, t, 4″-CH_2), 2.99 (2H, t, 2″-CH_2), 2.20 (2H, d, 2‴-CH_2), and 2.15 (2H, m, 3″-CH_2), as well as one CH group at δ 2.14 (1H, m, 3‴-CH). IR absorption bands at 1729 and 1655 cm^{-1} and ^{13}C NMR signals at δ 191.6, 173.0 indicated that **2** has two carbonyl groups (including one ester carbonyl group, C-1‴), The ^{13}C NMR spectrum showed 17 carbon-signals, attributed to 8 bithiophene C-atoms, 1 methenyl, 4 methylenes, 2 methyl, and 2 carbonyl groups. Analysis of the $^{1}H-^{1}H$ COSY and HMQC spectral data of **2** led to the identification of two partial structures: **a** (—CH_2—$CH(CH_3)$—CH_3) and **b** (—CH2—CH2—CH2—). The HMBC correlations between H-3″ and C-1″, and H-3‴ and C-1‴ suggested the presence of buta-1-onyl and isovaleroyloxy. Based on HMBC correlations from H-4 to C-1″, H-2″ to C-5, H-4″ to C-1‴, buta-1-onyl and isovaleroyloxy were located at positions C-5 and C-4″, respectively. Thus, on the basis of the above conclusions, the structure of compound **2** was determined as 5-[(4-isovaleroyloxy) buta-1-onyl]-2,2′-bithiophene.

Compounds **1** and **2** were tested for their anti-tumor activities against different human tumor cell lines. The IC_{50} values are

summarized in Table 2. Compound **2** exhibited moderate anti-tumor activities against HL60, K562, and MCF-7. However, compound **1** was active only to HL60 and K562, and the IC_{50} values were higher than those of compound **2**.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yamaco hot-stage (Japan) and are uncorrected. UV spectra were performed on a HITACHI U-3010 spectrometer (Japan). IR spectra were obtained by using a Bruker Vector 22 FTIR (Germen) spectrophotometer. NMR spectra were recorded on a Bruker Biospin AMX (Switzerland) using TMS as an internal standard. HREIMS data were performed on a JEOL JMS SX-102A mass spectrometer (Japan). Preparative HPLC separations were performed on an Agilent 1100 (American) with column Zorbax SB-C_{18} (21.2 mm × 250 mm, 10 μm, American). Silica gel for chromatography was produced by Qingdao Ocean Chemical Group Co. (Qingdao, China).

3.2 Plant material

The roots of *E. grijissi* were collected in Bozhou, north of Anhui province, China, in June 2006. The plant material was identified by the authors, and a voucher specimen (EGH060703) has been deposited in the herbarium of the Institute of Pharmaceutical Informatics, College of Pharmaceutical Sciences of Zhejiang University.

3.3 Extraction and isolation

The air-dried roots (14.3 kg) were split into pieces, and extracted with 95% ethanol for three times, each for 3 h. The extract was concentrated to give a brown residue (434.5 g), which was suspended in distilled water (4 l) and then partitioned with dichloromethane (3 × 2 l) and *n*-butanol (3 × 2 l) successively. The dichloromethane fraction (132.2 g) was subjected to column chromatography over silica gel (5 × 50 cm, 300–400 mesh, 1.0 kg), eluted with petroleum ether (60–90°C)/EtOAc to obtain fractions A–J (1:0, 200:1, 100:1, 50:1, 30:1, 20:1, 10:1, 5:1, 1:1, 0:1, each 3 l). fractions C and D (total 35.0 g) were combined according to the TLC analysis and separated on a silica gel column (5 × 50 cm, 300–400 mesh, 300 g) eluted with petroleum ether/EtOAc (100:1, 50:1, 20:1, 10:1, each 1 l) to give subfractions. Then subfraction CD-1 was separated by preparative HPLC using acetonitrile/H_2O (90:10) as elution to obtain compound **1** (5.2 mg, $t_R = 22.6$ min). Fraction E (5 g) was separated by preparative HPLC using acetonitrile/H_2O (85:15) as elution to yield compound **2** (4.8 mg, $t_R = 13.5$ min).

3.3.1 Compound *1*

Yellow powder ($CDCl_3$), mp 134.2–135.0°C. UV (MeOH) λ_{max} (log ε): 340.4 (4.2), 250.6(3.5) nm. IR (KBr pellet) ν_{max} 2192, 796, 836, 674 cm^{-1}. For the ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data see Table 1. HREIMS: *m/z* 412.0429 $[M]^+$ (calcd for $C_{25}H_{16}S_3$, 412.0414). EIMS: *m/z* 413.0 (100), 414.0 (28.5), 415.1 (12.9), and 166 (5.6).

Table 2. IC_{50} values for cytotoxicities of compounds **1** and **2** against different cell lines ('–' means IC_{50} 50 μg/ml).

	IC_{50}			
Cell lines	**1** (μg/ml)	**2** (μg/ml)	Platinol (μg/ml)	Adriamycin (μg/ml)
HL60	21.2	19.6	2.3	
K562	35.2	18.9		2.6
MCF-7	–	28.7		2.1

Downloaded At: 18:52 22 January 2011

3.3.2 Compound **2**

Yellow powder ($CDCl_3$), mp 62.3–62.7°C. UV (MeOH) λ_{max} (log ε): 375 (4.3), 262 (3.2), and 220 (3.6) nm. IR (KBr pellet) ν_{max} 1729, 1655, 839, 801, 717 cm^{-1}. For the ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data see Table 1. HREIMS: *m/z* 336.0838 $[M]^+$ (calcd for $C_{17}H_{20}O_3S_2$, 336.0854). EIMS: *m/z* 336.1 (100), 337.1 (19.8), 338.0 (10.4), and 166 (4.3).

3.4 Determination of cytotoxicity

The cytotoxicities of compounds **1** and **2** were tested against different human tumor cell lines. The cytotoxic activity of the isolated thiophenes against HL-60 and K562, evaluated by a modification of the sulforhodamine B assay [18], and MCF-7, were assessed by using the method described in the literature [19]. Compound **2** exhibited moderate antitumor activity against HL60, K562, and MCF-7 with IC_{50} values of 19.6, 18.9, and 28.7 μg/ml, respectively. Compound **1** was active only to HL60 and K562 with IC_{50} values of 21.1 and 25.2 μg/ml, respectively.

Downloaded At: 18:52 22 January 2011

Acknowledgements

The authors express their gratitude to Mr Chen, Department of Chemistry, for the NMR measurements. The research was supported by Program for New Century Excellent Talents in University (NCET-06-0515).

References

[1] C. Shih, *Flora Sinica*. (Scientific Press, Beijing, 1987).
[2] National Pharmacopoeia Committee, *Pharmacopoeia of PRC*. (Chemical Industry Press, Beijing, 2005).
[3] D.A. Guo, Z.C. Lou, and Z.A. Liu, *Chin. J. Chin. Mater. Med.* **19**, 100 (1994).
[4] D.A. Guo, Y.J. Cui, Z.C. Lou, C.Y. Gao, and L.R. Huang, *Chin. Trad. Herbal Med.* **23**, 3 (1992).
[5] D.A. Guo, Z.C. Lou, C.Y. Gao, and D. Wang, *Chin. Trad. Herbal. Med.* **23**, 512 (1992).
[6] K. Koike, Z.H. Jia, T. Nikaido, Y. Liu, Y.Y. Zhao, and D.A. Guo, *Org. Lett.* **1**, 197 (1999).
[7] Y.L. Lin, R.L. Huang, and Y.H. Kuo, *J. Chen. Chin. Pharm.* **51**, 201 (1999).
[8] Y. Liu, M. Ye, H.Z. Guo, and Y.Y. Zhao, *J. Asian Nat. Prod. Res.* **4**, 75 (2002).
[9] J.D.H. Lambert, G. Campbell, J. Arnason, and W. Majak, *Can. J. Plant Sci.* **71**, 215 (1991).
[10] R.J. Marles, J.B. Hudson, E.A. Graham, and J.T. Arnason, *Photochem. Photobiol.* **56**, 479 (1992).
[11] M. Nivsarkar, G.P. Kumar, and M. Laloraya, *Arch. Insect. Biochem. Physiol.* **16**, 249 (1991).
[12] A. Sharma and H.C. Goel, *Indian J. Exp. Biol.* **32**, 745 (1994).
[13] J.B. Hudson, L. Harris, A. Teeple, and G.H. Towers, *Antivir. Res.* **20**, 33 (1993).
[14] J.B. Hudson, E.A. Graham, and G.H. Towers, *Planta Med.* **60**, 329 (1994).
[15] C.C. Lin, C.H. Lin, and H.F. Chiu, *Am. J. Chin. Med.* **20**, 127 (1992).
[16] W.R. Jin, S. Qiang, C.T. Hong, Y.Y. Cheng, Z.J. Ma, and H.B. Qu, *Phytomedicine*, (unpublished).
[17] A. Selva, A. Arnone, R. Mondelli, V. Sprio, L. Ceraulo, S. Petruso, S. Plescia, and L. Lamartina, *Phytochemistry* **17**, 2097 (1978).
[18] Z.P. Chen, A. Malapetsa, and A. McQuillan, *Mol. Pharmacol.* **52**, 815 (1997).
[19] J. Carmichael, W.G. DeGraff, A.F. Gazadar, J.D. Minna, and J.B. Mitchell, *Cancer. Res.* **47**, 943 (1987).