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Lignans from the rhizomes of *Iris tectorum*

Chun-Lei Zhang [a,b], Yan Wang [b], Yan-Fei Liu [b], Dong Liang [b], Zhi-You Hao [b], Huan Luo [b], Ruo-Yun Chen [b], De-Quan Yu [b,*]

[a] Jiangsu Provincial Key Laboratory for TCM Evaluation and Translational Development, School of TCM, China Pharmaceutical University, Nanjing 211198, People's Republic of China
[b] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

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ABSTRACT

Chemical examination of the ethanol extract of rhizomes of *Iris tectorum* led to the isolation and characterization of three new lignans, (7*R*,7′*R*,8*S*,8′*S*)-5′-methoxy-neo-olivil (1a), (7*S*,7′*S*,8*R*,8′*R*) -5′-methoxy-neo-olivil (1b), (7*S*,7′*R*,8*S*,8′*S*)-neo-olivil (2a), (7*R*,7′*S*,8*R*,8′*R*)-neo-olivil (2b), (7*R*,7′*R*,8*S*,8′*S*,7′′*S*,8′′*S*)-*threo*-neo-olivil-4′-O-8-guaiacylglycerol ether (3), together with six known ones (4–9). Among them, compounds 1 and 2 were found to be racemic mixtures, respectively, which were verified by chiral HPLC analysis, compound 3 was a new sesquineolignan. The structures were elucidated on the basis of extensive spectroscopic analysis. To our knowledge, this is the first report of lignan constituents isolated from *I. tectorum*. All compounds were evaluated for their cytotoxicity against five human tumor cell lines and none of them displayed significant toxicity in tested cell lines at a concentration of 10 μM.

1. Introduction

Iris tectorum Maxim. (Iridaceae) is a perennial herb widely distributed in the gardens. It has been commonly used for the treatment of sore throats [1]. Previous studies of *I. tectorum* reported the occurrence of numerous isoflavonoidsand iridal-type triterpenoids [2,3]. We previously reported several structurally unique iridal-type triterpenoids isolated from *I. tectorum* [4–6]. In our continuing efforts to discover new bioactive compounds from *I. tectorum*, nine lignans (1–9) were isolated from the ethanol extract of rhizomes of the title plant. Lignans present a wide range of biological activities such as cytotoxic [7], antioxidative [8], anti-bacterial [9], immunosuppresive [10], anti-inflammatory [11], anti-HIV [12], etc. We report herein the isolation, structure elucidation, and biological activities of these compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a JASCO P-2000 polarimeter, and UV spectra with a JASCO V-650 spectrophotometer. IR spectra were recorded on a Nicolet 5700 spectrometer by an FT-IR microscope transmission method. NMR measurements were performed on BRUKER AV500-III spectrometers. HRESIMS were obtained using a micromass Autospec-Ultima ETOF spectrometer. Analytical HPLC was conducted on an Agilent 1260 infinity system equipped with a DAD-UV detector. Chiral analysis was performed on a Chiralpak AD-H column (250 × 10 mm, 5 μm). Preparative HPLC was performed using a Shimadazu LC-6 AD instrument with a SPD-20A detector and a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China), Sephadex LH-20 (GE), and ODS (50 μm, YMC, Japan) were used for column chromatography. TLC was carried out with GF254 plates (Qingdao Marine Chemical Factory).

2.2. Plant material

The rhizomes of *I. tectorum* investigated in this study were purchased in November, 2011 from a herbal medicine market in Chengdu, Sichuan Province, People's Republic of China. The plant material was authenticated by Professor Lin Ma, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (ID-S-2469) is deposited at the Herbarium of the Department of Medicinal Plants, the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

2.3. Extraction and isolation

The air-dried powdered rhizomes of *I. tectorum* (20 kg) were extracted three times with 95% EtOH under reflux. The extracts were combined and concentrated under a vacuum to yield a residue (4 kg),

* Corresponding author at: Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 2 Nan Wei Road, Beijing 100050, People's Republic of China.
E-mail address: dqyu@imm.ac.cn (D.-Q. Yu).

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which was suspended in water and partitioned sequentially with EtOAc and *n*-BuOH. The EtOAc extract (2 kg) was chromatographed on a silica gel column and eluted with petroleum ether-acetone (20:1, 3:1, 1:1, and 0:1). The fraction (40 g) that eluted with 1:1 petroleum ether-acetone was subjected to a reversed-phase C_{18} silica gel column (80 × 6 cm), eluted with 10%, 30%, 50%, 70%, and 100% MeOH in H_2O, to afford six fractions (F1–F6). Fraction 2 (124 mg) was purified using Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), followed by preparative HPLC (10% MeCN/H_2O containing 0.05% TFA, 5 mL/min), to afford 8 (3 mg, t_R 41.8 min) and 9 (3 mg, t_R 42.3 min). Fraction 3 (240 mg) was fractionated over silica gel (EtOAC/EtOH/H_2O, 100:2:1) and purified by preparative HPLC (15% MeCN/H_2O, 5 mL/min) to yield 1 (24 mg, t_R 76.6 min), 4 (36 mg, t_R 80.5 min), and 5 (21 mg, t_R 78.2 min). Fraction 4 (185 mg) was submitted to Sephadex LH-20 column and eluted with MeOH to produce four subfractions (F4a–F4d). Of these subfractions, F4b was subjected to further separation by preparative HPLC (16% MeCN/H_2O, 5 mL/min) to obtain 2 (14 mg, t_R 104.7 min), 6 (35 mg, t_R 79.7 min), and 7 (10 mg, t_R 91.3 min). Fraction 6 (130 mg) was purified by preparative HPLC (18% MeCN/H_2O, 5 mL/min) to afford 3 (35 mg, t_R = 180.0 min).

Compound 1: pale yellow oil; $[\alpha]^{20}_D$ + 17.6 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.97) nm; IR ν_{max} 3357, 2934, 1612, 1519, 1463 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) see Table 1; HR-ESIMS *m/z* 429.1535 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_8Na$, 429.1520).

Compounds 2: pale yellow oil; $[\alpha]^{20}_D$ − 9.8 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.98) nm; IR ν_{max} 3328, 2937, 1606, 1518, 1463 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) see Table 1, 1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 2; HR-ESIMS *m/z* 399.1426 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_7Na$, 399.1414).

Table 2
^1H NMR and ^{13}C NMR data of compounds 2, 10, and 11.

Position	2[a]		**10a**, **10b**[b]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1,		131.0		115.2
2	6.89, overlapped	109.1	6.80–6.85, m	99.1
3		146.6		128.8
4		145.3		127.4
5	6.89, overlapped	114.4	6.80–6.85, m	102.5
6	6.89, overlapped	119.5	6.78, d, (8.1)	106.3
7	5.13, d, (9.0)	81.5	5.10, d, (7.9)	76.2
8	2.62, m	51.0	2.56, m	50.5
9	3.40, dd, (10.8, 6.4)	64.0	3.22, dd, (10.4, 5.1)	61.0
	3.19, dd, (10.8, 9.0)		3.16, dd, (10.4,10.0)	
1′		132.1		116.9
2′	7.02, d, (1.8)	109.6	7.10, s	98.9
3′		146.8		129.1
4′		145.8		127.8
5′	6.93, d, (7.8)	114.5	6.95–6.97, m	102.7
6′	6.98, dd, (7.8, 1.8)	119.9	6.95–6.97, m	106.1
7′	4.52, d (9.6)	82.8	4.65, d, (8.6)	77.1
8′	2.29, m	55.0		54.2
9′	3.80, dd, (10.2, 4.2)	63.4	3.71, dd, (11.0, 4.7)	60.5
	3.66, dd, (10.2, 8.4)		3.64, dd, (11.0, 6.1)	
3 − OCH_3	3.88 (3H, s)	56.1	3.85, s	54.9
3′ − OCH_3	3.92 (3H, s)	56.1	3.89, s	54.9

[a] ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of in $CDCl_3$.
[b] ^1H NMR (500 MHz) in CD_3OD, ^{13}C NMR (125 MHz) data in $CDCl_3$.

Compound 3: pale yellow powder; $[\alpha]^{20}_D$ + 18.1 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 204 (5.14) nm; IR ν_{max} 3371, 2938, 1605, 1515, 1463 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) see Table 1; HR-ESIMS *m/z*595.2157 $[M + Na]^+$ (calcd for $C_{30}H_{36}O_{11}Na$,595.2156).

Table 1
^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compounds 1–3 in CD_3OD.

Position	1		2		3	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		135.0		131.8		134.6
2	7.07, d (1.5)	111.2	6.97, d (1.5)	111.4	7.03, overlapped	111.2
3		149.1		148.8		149.2
4		147.4		147.0		147.6
5	6.82, d (8.0)	116.1	6.78, d (8.0)	116.2	6.79, d (8.0)	116.1
6	6.91, dd (8.0, 1.5)	120.5	6.85, d (8.0, 1.5)	120.3	6.88, dd (8.0, 1.5)	120.5
7	4.98, d (8.0)	84.4	5.10, d (8.0)	83.0	4.92, d (8.0)	84.5
8	2.36, m	55.3	2.56, m	50.8	2.31, m	55.3
9	3.73, dd, (11.0, 3.0)	61.8	3.22, dd (10.5, 5.0)	63.9	3.69, (11.0, 3.0)	61.7
	3.64, dd (11.0, 5.0)		3.16, dd (10.5, 10.0)		3.60, (11.0, 5.0)	
1′		134.3		133.8		138.1
2′	6.77, s	104.9	7.11, d (1.5)	111.6	7.10, d, (2.0)	111.7
3′		149.3		149.1		151.8
4′		136.3		147.5		149.1
5′		149.3	6.82, dd (8.0)	115.9	7.05, d (8.0)	118.8
6′	6.77, s	104.9	6.97, dd (8.0, 1.5)	120.6	6.94, dd (8.0, 2.0)	120.3
7′	4.98, d (8.0)	84.6	4.65, d (8.5)	84.1	4.96, d (8.5)	84.1
8′	2.36, m	55.3	2.27, m	55.5	2.31, m	55.4
9′	3.75, dd (11.0, 3.0)	61.9	3.71, dd (11.0, 4.5)	63.3	3.71, dd, (11.0, 3.0)	61.9
	3.66, dd (11.0, 5.0)		3.63, dd (11.0, 6.5)		3.62, dd, (11.0, 5.0)	
1″						133.7
2″					7.03, overlapped	111.7
3″						148.9
4″						147.3
5″					6.75, d (8.0)	115.9
6″					6.86, dd (8.0, 1.5)	120.7
7″					4.89, d (6.0)	74.0
8″					4.30, m	87.2
9″					3.75, dd (12.0, 4.0)	61.9
					3.50, dd (12.0, 5.0)	
3 − OCH_3	3.92, s	56.4	3.85, s	56.4	3.88	56.4
3′ − OCH_3	3.90, s	56.8	3.89, s	56.4	3.90, s	56.6
5′ − OCH_3	3.90, s	56.8				
3″ − OCH_3					3.82, s	56.3