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Isolation and structure determination of triterpenes from *Iris tectorum*

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Abstract

Four iridal-type triterpenoids, two of which were new compounds, have been isolated from rhizomes of *Iris tectorum* Maxim. Their structures were determined by 1D and 2D NMR spectroscopy and ESI-MS spectrometry. The compounds were identified as the iritectols A and B, and the known iridobelamal A and isoiridogermanal. The presence of epoxide and tetrahydrofuran functions are not common in previously isolated iridal-type triterpenoids.

Keywords: *Iris tectorum*; Iridaceae; Rhizome; Traditional Chinese Medicine; Triterpene

1. Introduction

Iris tectorum Maxim. (Iridaceae) is a perennial herb, native to China. Its trivial name in Chinese "蓝蝴蝶" (***Lan Hu Die***) means blue butterfly. It is also known as Japanese Roof Iris in some literature, because it was first observed growing on roofs by a Russian botanist, Carl Maximowicz (1827–1891) (Klingaman, 2005). The rhizome of *I. tectorum* was introduced as a medicine in the first Chinese monograph on herbal medicines "神农本草经" (***Shen Nong Ben Cao Jing***) (also known as "The Divine Farmer's Herb-Root Classic"), which was completed about 200 AD (Tan and Wen, 2001). It was used as a bitter medicine to treat disorders described as "癥瘕结聚" (***Zheng Jia Jie Ju***). These syndromes are similar to modern descriptions of tumours. It has also been commonly used as an anti-helminthic TCM in China (Song et al., 2001). According to the latest edition of the Chinese Pharmacopoeia (Anonymous, 2005), *I. tectorum* is referred to as "川射干" (***Chuan She Gan***), and is used to treat sore throat, to disperse phlegm, and for heat-clearing and detoxifying. It has also been recorded to treat abdominal distension and hepatic cirrhosis (Song et al., 2001) in China. In Japan it is used as an emetic and a laxative (Seki et al., 1994a). A field study and investigation in the present study revealed that the rhizome of *I. tectorum* has been used as traditional folk medicine for the treatment of cancer in Tongren, a small town in Guizhou province, South China (Fang R., 2004, unpublished).

A number of isoflavonoids (Wu and Xu, 1992; Morita et al., 1972), quinones (Seki et al., 1994b) and iridal-type triterpenoids (Krick et al., 1983; Ito et al., 1999) have been isolated form Iridaceous plants. In particular, iridal-type triterpenoids are recognized as characteristic metabolites of the Iridaceae (Miyake et al., 1997). In the present study, two novel triterpenes iritectol A (**1**) and iritectol B (**2**) were isolated from rhizomes of *I. tectorum*, along with two known triterpenes, iridobelamal A (**3**) and isoiridogermanal (**4**), by HPLC.

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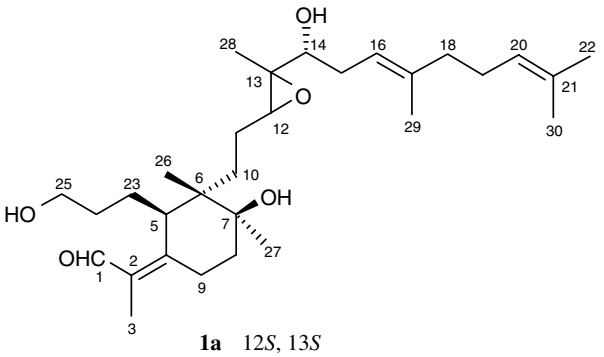
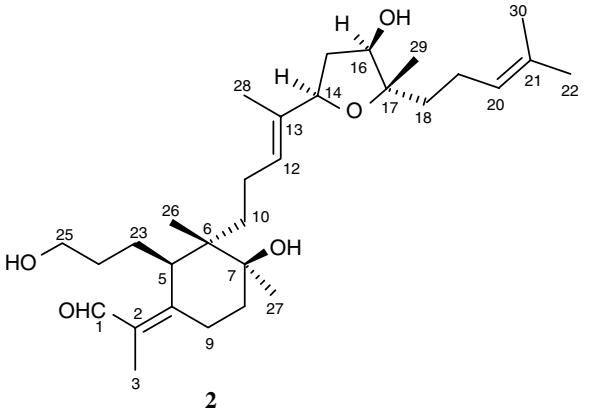


1a 12*S*, 13*S*
1b 12*R*, 13*R*



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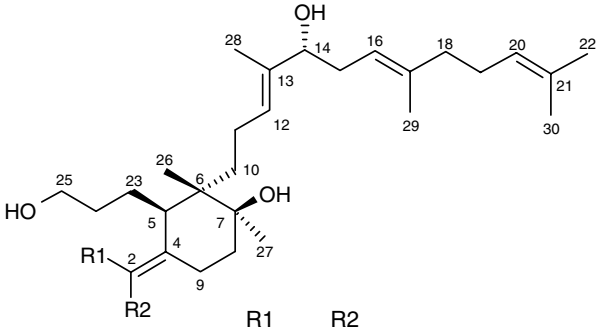

	R1	R2
3	Me	CHO
4	CHO	Me

2. Results and discussion

The ESI-MS spectrum of iritectol A (**1**) showed a peak for $[M+Na]^+$ at *m/z* 513. Its HR-ESI-MS gave a peak at *m/z* 513.359 ($C_{30}H_{50}O_5Na$ requires 513.355). This indicated a likely molecular formula of $C_{30}H_{50}O_5$, with six degrees of unsaturation. The ^{13}C NMR and DEPT spectra revealed that **1** has seven methyl, 10 methylene, six methine and seven quaternary carbons. The following spectral data suggested that **1** had an α-methyl-α,β-unsaturated aldehyde skeleton in the molecule: λ_{max}^{MeCN}nm(log ε): 252.5 (4.1); ν_{max}/cm^{-1}: 1708 [>C=C(*CHO*)–] and 1609 (conjugated >C=C<) (Williams and Fleming, 1995); δ_H 1.84 (3H, *s*, Me), 10.15 (1H, *s*, CHO); δ_C 162.6 (*s*), 133.3 (*s*), 11.0 (*q*), 189.8 (*d*) [>C=C(Me)CHO] (Tables 1 and 2).

In addition, the ^{13}C NMR spectrum showed the presence of two isolated trisubstituted double bonds [δ_C 119.1 (*d*), 138.8 (*s*), 124.0 (*d*) and 131.7 (*s*)], whilst the absence of any other sp^2 carbons hence indicated that **1** must have two ring systems. There was one more oxygen atom in the structure of **1** ($C_{30}H_{50}O_5$) than that of isoiridogermanal (**4**) ($C_{30}H_{50}O_4$) (Takahashi et al., 2000). The signals due to the trisubstituted double bond assigned to C-12, C-13 in the ^{13}C NMR spectrum of **4** [δ_C 125.3 (*d*) and 137.0 (*s*)] were

Table 1
^1H NMR spectral data of **1**, **2**, **3** and **4** (δ, at 500 MHz in $CDCl_3$)

C No.	**1**	**2**	**3**	**4**
1	10.15 *s*	10.16 *s*	10.24 *s*	10.18 *s*
3	1.84 *s*	1.83 *s*	1.80 *s*	1.83 *s*
5	3.22 *br d* ($J = 10.1$ Hz)	3.30 *br d* ($J = 11.0$ Hz)	2.79 *br d* ($J = 9.9$ Hz)	3.31 *br d* ($J = 11.1$ Hz)
8	1.72 *m* 1.87 *m*	1.67 *m* 1.85 *m*	1.62–1.71 *m* 1.79–1.87 *m*	1.65 *m* 1.87 *m*
9	2.57 apparent *br t* ($J = 13.6$ Hz) 2.60 apparent *td* ($J = 13.6$, 4.7 Hz)	2.54 apparent *br t* ($J = 13.5$ Hz) 2.59 apparent *br td* ($J = 13.5$, 4.6 Hz)	3.22 apparent *br d* ($J = 13.9$ Hz) 2.59 apparent *br td* ($J = 13.9$, 2.3 Hz)	2.55 apparent *br t* ($J = 13.8$ Hz) 2.60 apparent *td* ($J = 13.8$, 4.7 Hz)
10	1.29–1.46 *m*	1.17–1.21 *m* 1.28–1.32 *m*	1.13 –1.32 *m*	1.18 *m* 1.32 *m*
11	1.33–1.49 *m*	1.82–1.68 *m* 1.93–1.98 *m*	1.90–1.96 *m* 1.99–2.10 *m*	1.86 *m* 1.96 *m*
12	2.75 *m*	5.32 *t* ($J = 6.9$ Hz)	5.26 *t* ($J = 6.8$ Hz)	5.25 *t* ($J = 7$ Hz)
14	3.26 *dd* ($J = 7.8$, 5.7 Hz)	4.19 apparent *t* ($J = 7.8$ Hz)	3.93 *dd* ($J = 7.5$, 5.1 Hz)	3.92 *dd* ($J = 7.8$, 5.1 Hz)
15	2.18–2.29 *m*	2.35 *ddd* ($J = 13.3$, 7.8, 5.6 Hz) 1.75 *m*	2.13–2.33 *m*	2.22 *m*
16	5.08 *m*	4.03 apparent *t* ($J = 5.6$ Hz)	5.07 *m*	5.07 *m*
18	2.02 *br d* ($J = 6.3$ Hz)	1.38–1.53 *m*	2.02 *br d*	2.02 *m* ($J = 6.5$ Hz)
19	2.07 *m*	2.04 *m*	2.07 *m*	2.07 *m*
20	5.06 *m*	5.10 *t* ($J = 7.0$ Hz)	5.06 *m*	5.06 *m*
22	1.68 *s*	1.68 *s*	1.68 *s*	1.68 *s*
23	1.79 *m* 2.03 *m*	1.78 *m* 2.05 *m*	1.80 *m* 2.10 *m*	1.79 *m* 2.03 *m*
24	1.21–1.28 *m*	1.23–1.29 *m* 1.35–1.39 *m*	1.33–1.41 *m*	1.26 *m* 1.40 *m*
25	3.61 apparent *t* ($J = 6.4$ Hz)	3.61 apparent *t* ($J = 6.4$ Hz)	3.61 apparent *td* ($J = 6.3$, 2.3 Hz)	3.61 apparent *t* ($J = 6.4$ Hz)
26	1.08 *s*	1.08 *s*	1.09 *s*	1.10 *s*
27	1.18 *s*	1.15 *s*	1.16 *s*	1.16 *s*
28	1.20 *s*	1.56 *s*	1.60 *s*	1.55 *s*
29	1.62 *s*	1.23 *s*	1.63 *s*	1.62 *s*
30	1.60 *s*	1.61 *s*	1.60 *s*	1.60 *s*

Table 2
^{13}C NMR spectral data of **1**, **2**, **3** and **4** (δ, at 125 MHz in $CDCl_3$)

C No.	1	2	3	4
1	189.8	190.1	190.7	190.0
2	133.3	133.2	133.1	133.2
3	11.0	11.0	11.9	11.0
4	162.6	163.0	163.5	162.8
5	43.6	43.4	47.3	43.4
6	44.5	44.7	45.2	44.7
7	74.9	75.1	75.2	75.0
8	37.0	37.0	38.0	37.0
9	23.8	23.9	20.0	23.8
10	33.7	36.7	36.9	36.9
11	22.6	21.9	23.0	21.8
12	61.6	125.8	125.7	125.3
13	63.5	135.6	136.9	137.0
14	75.5	80.1	76.8	76.7
15	31.8	39.4	34.3	34.2
16	119.1	77.1	119.9	119.9
17	138.8	84.6	138.9	138.8
18	39.8	38.8	39.8	39.8
19	26.5	22.7	26.5	26.5
20	124.0	124.3	124.1	124.1
21	131.7	131.8	131.7	131.7
22	25.7	25.7	25.7	25.7
23	26.6	26.6	27.1	26.6
24	32.6	32.7	32.0	32.7
25	63.0	63.0	63.2	63.1
26	18.0	17.9	17.8	18.0
27	26.3	26.3	26.4	26.3
28	12.1	11.7	11.9	11.9
29	16.2	20.0	16.3	16.3
30	17.7	17.7	17.7	17.7

replaced by signals at δ_C 61.6 (*d*) and 63.5 (*s*) in that of **1**. These facts indicated the likely presence of an epoxide function in **1**. This proposal was supported by the observation of the ^1H NMR data, where the spectrum of **1** differed from that of **4** by an upfield shift of the olefinic hydrogen signal assigned to H-12 at δ_H 5.25 in the ^1H NMR spectrum of **4** to δ_H 2.75 in that of **1** (Tables 1 and 2), consistent with the signal of an epoxide hydrogen (Williams and Fleming, 1995). Correspondingly, the signal for the C-13 methyl (H-28) was shifted upfield from δ_H 1.55 to δ_H 1.20. Such a structural alteration and differences in relevant chemical shifts are supported by the comparing the spectra of similar pairs of compounds, such as leucanthinin and 3α-hydroxyenhydrin (Quijano et al., 1997), in which similar upfield shifts of ^1H NMR signals were simply due to the epoxidation of a double bond. Except for δ_H 2.75 (1H, *m*), 1.20 (3H, *s*) and 3.26 (1H, *dd*, $J = 7.8$, 5.7 Hz), the spectrum of **1** showed very similar ^1H NMR signals to those in that of **4**. These observations strongly suggested that **1** is likely to be the compound in which the double bond at C-12 in **4** is epoxidised (Fig. 1). This elucidation was also supported by a number of other literature precedents (Quijano et al., 1997; Lenis et al., 1998; Kubota et al., 2003), in which those isolated natural products with epoxide functions exhibited parallel chemical shifts in their NMR spectrum. The above elucidation and the NOESY interpretations support two possible absolute configurations of the epoxide function in **1** but do not allow differentiation between the two possible stereochemical isomers shown as **1a** and **1b** in Fig. 2.

Iritectol B (**2**) was evidently an isomer of **1**. An identical formula $C_{30}H_{50}O_5$ was suggested by the measurement of HR-ESI-MS, which also showed an $[M+Na]^+$ ion peak at *m/z* 513.359 ($C_{30}H_{50}O_5Na$ requires 513.355). Similarly, the ^{13}C NMR spectrum of **2** [δ_C 125.8 (*d*), 135.6 (*s*), 124.3 (*d*) and 131.8 (*s*)] suggested the presence of two isolated trisubstituted double bonds. The existence of a six-membered ring in its structure was suggested by the absence of sp^2 carbons and comparison of its NMR data with those of **4**. By examination of the NMR data of the isolated triterpenoids, it was revealed that both compounds **1** and **2** exhibited NOESY correlations between the aldehyde hydrogen signal H-1 and that of H-5, also between the signals of the vinyl methyl H-3 and one H-9 in their NOESY spectra (Fig. 3), thus the stereochemistry of the 2(4)-double bond is *Z* as in **4** (in contrast with that in **3** which is the *E* isomer of **4**)(Takahashi et al., 2000).

In addition, the formula of **2** ($C_{30}H_{50}O_5$) indicated one more oxygen atom in its structure than that of **4** ($C_{30}H_{50}O_4$) and the ^{13}C NMR spectrum of **2** revealed the absence of signals for a trisubstituted double bond assigned to C-16, C-17 in **4** [δ_C 119.9 (*d*) and 138.8 (*s*)]. Nevertheless, **2** could not be an isomer of **1** (with a 16,17-epoxide, for example) because the anticipated epoxide signals (at δ_C 61.6 and 63.5 in the spectrum of **1**) were not present in the ^{13}C NMR spectrum of **2**. There were, however, signals at δ_C 77.1 (CH from DEPT) and 84.6 (quaternary from DEPT), which are more likely to be due to a cyclic hydroxyether structure. This could secondly be formed via attack by the 14-OH on a formal intermediate 16,17-epoxide, yielding **2**, as shown in Fig. 1.

An estimation made by ChemDraw® Ultra 2004 (CambridgeSoft Corporation, USA) gave values for δ_C of the tetrahydrofuran moiety as 77.3 (C-14), 39.6 (C-15), 77.9 (C-16) and 87.4 (C-17). Similarly, ACD/i-Lab® ACD/CNMR Predictor 8.0 (Advanced Chemistry Development Inc., Canada) calculated those δ_C as 79.7 (C-14), 40.5 (C-15), 77.7 (C-16) and 85.2 (C-17), which are very near to the observed values: 80.1 (C-14), 39.4 (C-15), 77.1 (C-16) and 84.6 (C-17). In addition, there is literature precedent for such a rearrangement: some naturally occurring compounds with a similar type of tetrahydrofuran moiety in their structure had fairly similar ^{13}C NMR signals for carbons 14–17: [δ_C 76.7–82.2 (C-14); δ_C 37.9–40.0 (C-15); δ_C 78.2–78.7 (C-16); δ_C 81.6–83.6 (C-17)] (Fan et al., 2001; Praud et al., 1995; Amico et al., 1989; Kimura et al., 1997). As a result, the observed ^{13}C NMR signals of carbons 14–17 in **2** are very similar to those of a tetrahydrofuran moiety in the literature and strongly suggested the presence of a tetrahydrofuran moiety in **2**. Such a structure is likely to be biosynthesized from **4**, as demonstrated in Fig. 1. This gives extra weight to the proposed stereochemistry of **2**. Its suggested stereostructure with observed

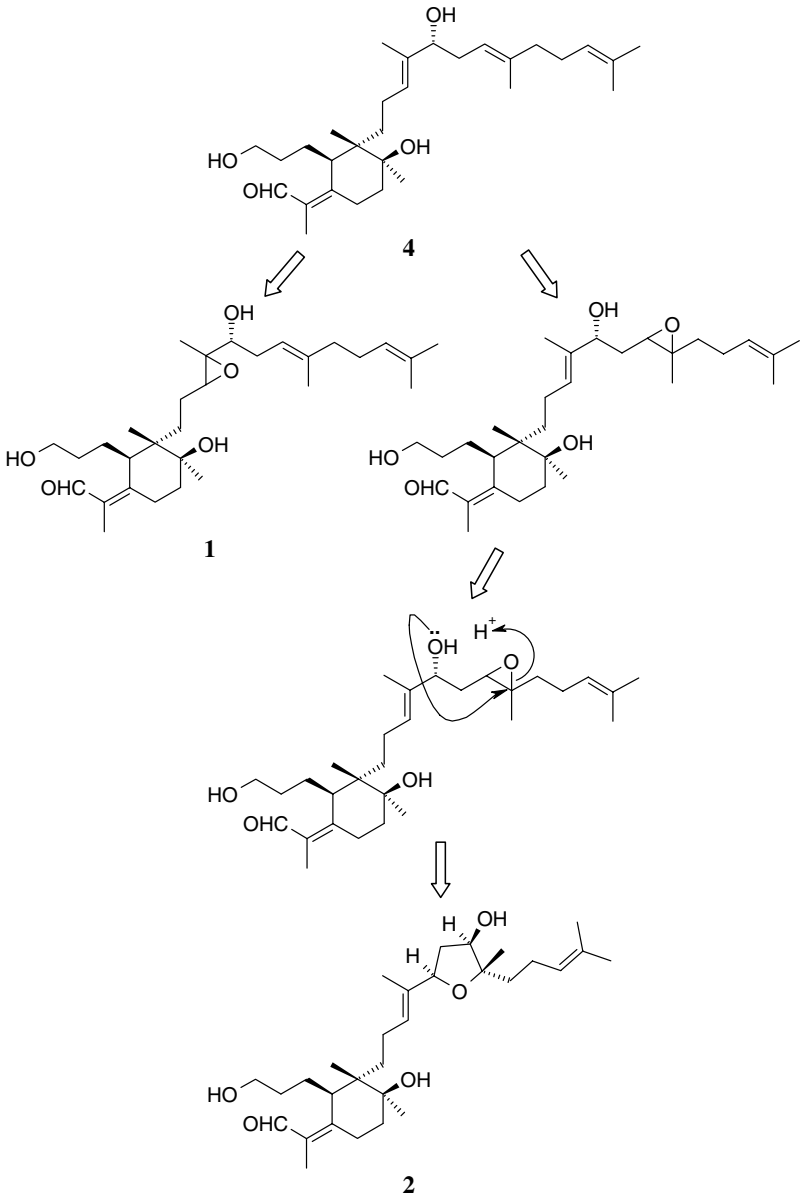

Fig. 1. Proposed biosynthetic route of **1** and **2**.

NOESY correlation signals is shown as Fig. 3. That is also supported by an HMBC experiment (Fig. 4).

Triterpenes **3** and **4** were identified by comparison of their NMR data and other spectral evidence with those of iridobelamal A and isoiridogermanal in the literature (Takahashi et al., 2000; Seki et al., 1994a). Although iridobelamal A (**3**) has been previously isolated from *Belamcanda chinensis*, some ^{1}H NMR data were not shown in the literature, but the overall NMR data of **3** are very similar to those in the literature (Takahashi et al., 2000). The absolute configuration of isoiridogermanal has been well established as that shown in **4** by means of chemical and physical analysis (Ito et al., 1999). Furthermore, the absolute stereochemistry of the related compounds γ-irigermanal and iriflorental, possessing an identical six-membered ring moiety, previously isolated from Iridaceous plants has been unequivocally established by X-ray crystallography (Marner et al., 1982; Miyake et al., 1997). Thereby, from the biosynthetic point of view, the stereochemistry of the two novel triterpenes **1** and **2** are very likely to be those shown in Fig. 1. The presence of epoxide and tetrahydrofuran functions is not common in the previously isolated iridal-type triterpenoids. The results of a cytotoxicity study on the isolated compounds will be published elsewhere.

3. Experimental

3.1. General procedures

^{1}H and ^{13}C NMR spectra were measured on a Bruker DRX500 NMR spectrometer in $CDCl_3$ (500 MHz for ^{1}H

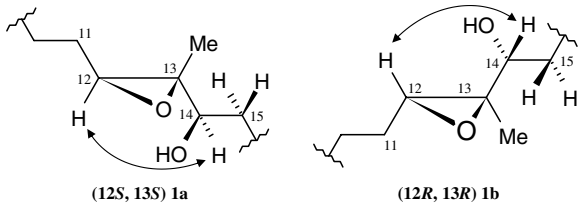

Fig. 2. Explanation of observed NOESY correlation signals on the two possible stereostructures of **1**.

and 125 MHz for ^{13}C), using TMS as internal standard. EIMS was measured on a Jeol AX505W mass spectrometer; ESIMS and HR-ESI-MS measured on a Bruker Apex III FT ion cyclotron resonance mass spectrometer. UV and CD spectra were obtained on a Jasco J720 spectrpolarimeter. Samples were prepared as MeCN solution, and scanned in the region 420–180 nm. IR spectra were measured on a Perkin–Elmer Spectrum One® FT-IR spectrometer with diamond attenuated total reflectance (DATR) technique. Optical rotation was detected on a Perkin–Elmer 141 polarimeter with Sodium D line (λ 589 nm). Samples were prepared as CH_2Cl_2 solution and detected in 10 cm light path cylindrical cell. Hewlett–Packard 1090 HPLC (with diode array detector) and semi-preparative HPLC column: Discovery® HS C18 (250 mm × 10 mm) was used for separation.

3.2. Plant material

The dry rhizomes of *I. tectorum* was purchased as crude TCM form a local herbal shop in Tongren, South China in July 2004. The plant material was authenticated by staff in Royal Botanic Gardens, Kew, and the voucher specimen "TCMK 598" has been reserved in the Kew Herbarium.

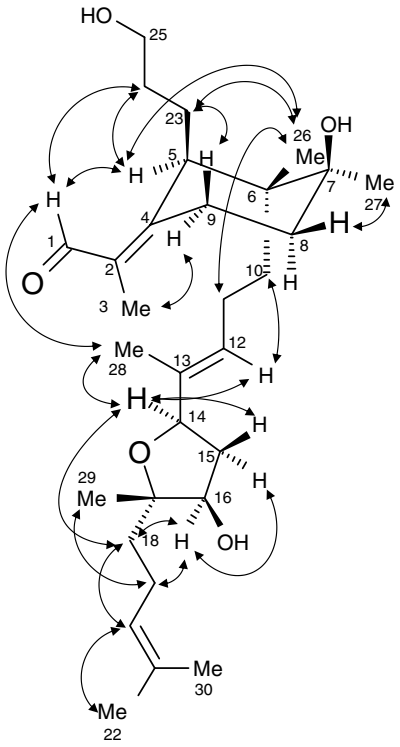

Fig. 3. Observed key NOESY correlation signals on proposed stereostructure of **2**.

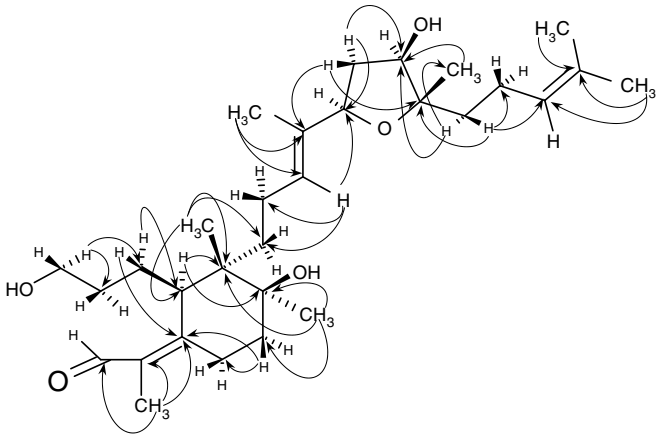

Fig. 4. Observed HMBC (long-range ^{1}H–^{13}C COSY spectra) correlations of **2**.

3.3. Extraction and isolation

The dried powdered rhizomes of *I. tectorum* (2 kg), defatted by supercritical fluid extraction (with liquid CO_2) in Shenzhen Neptunus Bioengineering Co. Ltd., China, was successively extracted for 48 h with $CHCl_3$ by exhaustive Soxhlet extraction yielding 152 g dried extract. The $CHCl_3$ extract was further fractionated by vacuum liquid chromatography (VLC) (15 cm × 16 cm) on silica gel 60 PF_{254} using *n*-hexane, *n*-hexane–$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$–MeOH (1:1) and MeOH successively. This produced six sub-fractions (A–F), among which fraction D (17 g) exhibited the highest cytotoxicity in an in vitro assay. Thereby, this active fraction was purified by using semi-preparative RP-HPLC (Discovery® HS C18 250 mm × 10 mm, flow rate 4 ml min^{-1}) under the following gradient: MeCN–H_2O (3:7–9:1 over 50 min) to yield compounds **1** (11.2 mg), **2** (13.1 mg), **3** (5.6 mg) and **4** (26.8 mg).

3.4. Iritectol A, (2Z)-2-((2R,3S,4S)-4-hydroxy-3-(2-(3-((3E)-1-hydroxy-4,8-dimethylnona-3,7-dienyl)-3-methyloxiran-2-yl)ethyl)-2-(3-hydroxypropyl)-3,4-dimethylcyclohexylidene)-propanal (*1*)

Yellowish glass-like solid, $[\alpha]_D^{23} + 28.02$ (CH_2Cl_2; $c = 0.91$); exhibited a negative CD Cotton effect giving $[\theta]_{252}$ +14,363, $[\theta]_{338}$ −1961; λ_{MeCN}^{max}/nm (log ε): 252.5 (4.10); IR ν_{max}/cm^{-1}: 3394 (OH), 2967, 2931, 2875, 1708 (CHO), 1655 (unconjugated C=C), 1609 (conjugated C=C), 1375, 1182 and 907. Its ESIMS spectrum showed a molecular ion peak at *m/z* 513 $[M+Na]^+$. HR-ESI-MS gave *m/z* 513.359 ($C_{30}H_{50}O_5Na$ requires 513.355). Its ^{1}H and ^{13}C NMR data ($CDCl_3$, 500 MHz) are shown in Tables 1 and 2, respectively.

3.5. Iritectol B, (2Z)-2-((2R,3S,4S)-4-hydroxy-3-((3E)-4-(4-hydroxy-5-methyl-5-(4-methylpent-3-enyl)-tetrahydrofuran-2-yl)pent-3-enyl)-2-(3-hydroxypropyl)-3,4-dimethylcyclohexylidene)-propanal (**2**)

Yellowish glass-like solid, $[\alpha]_D^{23} - 31.06$ (CH_2Cl_2; $c = 0.94$); exhibited a negative CD Cotton effect giving $[\theta]_{254}$ +6556, $[\theta]_{338}$ −1385; UV λ_{MeCN}^{max}/nm (log ε): 252.5 (4.1); IR ν_{max}/cm^{-1}: 3402 (OH), 2968, 2928, 2863, 1713 (CHO), 1650 (unconjugated C=C), 1608 (conjugated C=C), 1375, 1054 and 908. Its ESIMS spectrum showed an ion peak at *m/z* 513 $[M+Na]^+$; the EIMS gave fragment ion peaks at*m/z* (rel. int.) 491 $[M+H]^+$(26), 473 $[M-H_2O+H]^+$ (21), 334 (26), 316 (19), 263 (24), 251 (60), 233 (45), 221 (45), 191 (35), 163 (44), 149 (33), 139 (41), 121 (52), 109 (60), 95 (64), 81 (46), 69 (100), 55 (43) and 41 (33). HR-ESI-MS gave a peak for $[M+Na]^+$ at *m/z* 513.359 ($C_{30}H_{50}O_5Na$ requires 513.355).

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