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Polycycloiridals A–D, Four Iridal-Type Triterpenoids with an α-Terpineol Moiety from *Iris tectorum*

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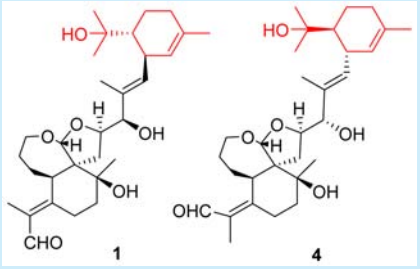
Supporting Information

ABSTRACT: Polycycloiridals A–D, four novel iridals with an unprecedented α-terpineol moiety resulting from cyclization of the homofarnesylside chain, were isolated from the ethanol extract of rhizomes of *Iris tectorum*. Their structures were elucidated on the basis of comprehensive spectroscopic analysis. The absolute configuration of **1** was determined by the modified Mosher's method and comparison of experimental and calculated electronic circular dichroism (ECD) spectrum. A possible biosynthetic pathway was postulated.



Iridal-type triterpenoids are generally recognized as characteristic metabolites of the plant family Iridaceae.[1] A common feature of these compounds is a multisubstituted cyclohexane ring with a homofarnesyl side chain.[2] These substances display a wide range of biological properties such as cytotoxicity,[3] ichthyotoxicity,[4] antiplasmodial activity,[5] and PKC activation.[6] Their unique structures and diverse biological activities make them attractive targets for chemical synthesis and biomimetic synthesis.[7] As a representative plant of the Iridaceae family, *Iris tectorum* Maxim., is well-known for containing structurally diverse iridals.[8] In our continued research on the discovery of structurally unique iridals,[9] four novel iridals in trace amounts, polycycloiridals A–D with an unprecedented α-terpineol moiety resulting from cyclization of the homofarnesyl side chain, were isolated from the ethanol extract of rhizomes of *I. tectorum*. Iridal-type triterpenoids could be divided into three classes: monocycloiridals, bicycloiridals, and spirioiridals in previous studies.[1] Polycycloiridals A–D belong to none of the three classes mentioned above and represent the first examples of iridals with a cyclic homofarnesyl side chain and only 30 C atoms. Therefore, we propose here a new structural class, the polycycloiridals, to distinguish them from previously reported iridals. In this paper, we report the isolation and structural elucidation of the novel iridals, along with the evaluation of their hepatoprotective activities against D-galactosamine-induced HL-7702 cell damage.

Polycycloiridal A (**1**, Figure 1) was obtained as a colorless gum. Its molecular formula was established to be $C_{30}H_{46}O_6$ by HRESIMS at m/z 525.3184 $[M + Na]^+$, indicating eight degrees of unsaturation. The UV (250 nm) absorption and IR (1711, 1614 cm^{-1}) absorption suggested the presence of an α,β-unsaturated aldehyde group.[10] The ^1H NMR spectrum

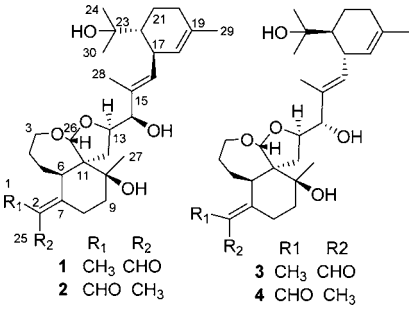


Figure 1. Structures of conpounds **1**–**4**.

exhibited a characteristic singlet due to an aldehyde group at δ_H 10.19, two olefinic proton signals at δ_H 5.48, 5.04, an oxygen-bearing methylene signal at δ_H 4.16, 3.58, and two oxygenated methine signals at δ_H 5.42, 3.88, together with six methyl signals (δ_H 1.83, 1.73, 1.59, 1.24, 1.19, 1.19). These signals are characteristic of iridals (Table 1).[10] The ^{13}C NMR spectrum confirmed the presence of 30 carbon resonances composed of one carbonyl, six olefinic, and 23 aliphatic carbons including six oxygenated ones. A notable feature in the ^{13}C NMR spectrum of **1** was the observation of a signal of a doubly oxygenated tertiary carbon at δ_C 109.6, suggesting the presence of an acetal structure. Further comparison of the NMR data with those of the known analogue epianhydrobelachinal revealed that they share a common 6/5/7 tricyclic ring system.[4] In addition, the ^{13}C NMR spectrum showed the presence of five other oxygenated carbon signals at δ_C 79.8, 78.0, 74.2, 72.4, and

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Table 1. ^{1}H and ^{13}C NMR Data of 1 and 3[a]

	1		3	
no.	δ_H	δ_C	δ_H	δ_C
1	1.83, s	10.8	1.76, s	10.7
2		129.2		129.1
3	4.16, dt (12.0, 2.4)	70.1	4.14, dt (12.0, 1.8)	69.6
	3.58, td (12.0, 2.4)		3.57, td (12.0, 2.4)	
4	1.76, m	32.2	1.73, m	31.4
	1.72, m			
5	2.93, m	31.4	2.92, m	31.0
	1.37, m		1.36, m	
6	3.11, d (10.2)	48.9	3.06, d (10.2)	48.6
7		165.6		165.9
8	3.23, m	20.4	3.20, m	20.4
	2.60, m		2.58, m	
9	1.67, m	39.2	1.68, m	39.3
10		74.2		74.5
11		60.2		59.8
12	1.53, m	38.4	1.60, m	35.7
	1.27, m		1.04, m	
13	4.37, m	79.8	4.45, m	79.7
14	3.88, d (5.4)	78.0	4.34, brs	74.0
15		132.0		130.9
16	5.48, d (10.2)	127.5	5.56, d, (10.2)	125.3
17	3.19, m	35.2	3.13, m	35.1
18	5.04, d (4.8)	122.8	5.06, d (4.8)	122.9
19		134.0		133.8
20	2.01, brd (5.4)	31.3	1.97, brd (6.0)	31.9
21	1.77, m	19.3	1.76, m	19.2
	1.68, m		1.58, m	
22	1.57, m	47.7	1.56, m	47.6
23		72.4	1.06, s	72.5
24	1.19, s	28.8	10.17, s	28.5
25	10.19, s	190.8	5.40, s	190.9
26	5.42, s	109.6	1.26, s	109.8
27	1.24, s	27.8	1.65, s	27.5
28	1.73, s	13.3	1.59, s	13.9
29	1.59, s	23.3	1.00, s	23.3
30	1.19, s	28.2	1.06, s	27.5

[a] ^{1}H NMR data (δ) were measured in $CDCl_3$ at 600 MHz; ^{13}C NMR data (δ) were measured in $CDCl_3$ at 150 MHz.

70.1, of which three were assignable to C-3, C-10, and C-13, indicating the existence of two hydroxyl groups at the homofarnesyl side chain. HMBC correlations from Me-24/Me-30 to a quaternary carbon at δ_C 72.4, together with $^{1}H-^{1}H$ COSY correlation from H-13 to a proton signal at δ_H at 3.88, which corresponded to the carbon signal at δ_C 78.0, confirmed the location of the two hydroxys at C-23 and C-14, respectively. Furthermore, the two isolated double bonds were located at C-15/C-16 and C-18/C-19 by detailed analysis of the 2D NMR spectrum (Figure 2). The aldehyde group, three double bonds, and three rings accounted for 7 degrees of unsaturation; therefore, there should be one more ring to achieve its degrees of unsaturation. The formation of an α-terpineol ring was established by HMBC correlations observed from H-16, H-17, and H-18 to C-22 and from H-22 to C-17. NOESY correlations between the aldehyde hydrogen signal and one proton of H-8 and the vinyl methyl and H-6 suggested that the geometric configuration of double bond between C-2 and C-7 was *E*. The geometry of the double bond $\Delta^{15(16)}$ was deduced to be *E* from the NOESY crosspeak of H-14/H-16 (Figure 3). The

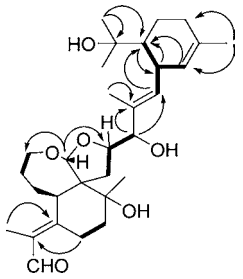

Figure 2. Selected HMBC(→), and $^{1}H-^{1}H$ COSY (−) correlations for 1.

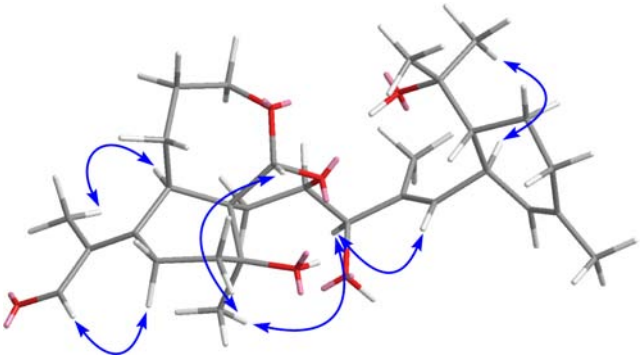

Figure 3. Selected NOESY (↔) correlations for 1.

configuration of the typical six-membered iridal ring system has been determined by X-ray analysis and chemical degradation.[11] From biosynthetic considerations, the absolute configurations of C-6, C-10, and C-11 were determined to be (6*R*,10*S*,11*R*) as in all other iridals reported so far.[12] The absolute configuration of C-14 was determined to be *R* by the modified Mosher's method (Figure 4).[13] The NOESY

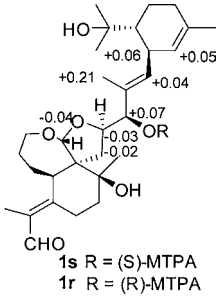

Figure 4. Values of $\Delta\delta_S - \Delta\delta_R$ of the MTPA esters of 1.

crosspeaks of H-27/H-26, H-27/H-14 as well as the absence of NOESY correlation between H-27 and H-13 suggested the configurations of 26*R* and 13*R*, respectively (Figure 3).[4] The C-30 methyl signal exhibited a strong NOE correlation with H-17 but no correlation with H-16. Consequently, C-16 and C-23 were deduced to be in opposite axial directions, which is in accordance with the energy requirement. Therefore, the relative configuration of the α-terpineol moiety was established as (a: 17*S*,22*R*) or (b: 17*R*,22*S*).

Our repeated attempts to obtain suitable crystals for X-ray diffraction were unsuccessful, and so the absolute configuration of the α-terpineol moiety was established by comparison of the experimental and theoretical ECD spectrum predicted using the time-dependent density functional theory (TDDFT) at the

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B3LYP/6-311G** level. The ECD spectrum of **1** showed an intense negative Cotton effect at 211 nm as well as weakly negative Cotton effects at 250 and 324 nm. According to reports in the literature, Cotton effects at 250 and 324 nm originated from the α,β-unsaturated aldehyde group.[14] Therefore, the Cotton effect at 211 nm was a contribution from the two isolated double bonds, and the sign might be associated with the configuration of the α-terpineol ring. Detailed analysis of the molecular orbitals (MO) involved in transitions at these wavelengths confirmed this notion. It showed that the negative Cotton effect around 210 nm resulted from the $\pi \rightarrow \pi^*$ electronic transition of the two isolated double bonds from MO137 to MO139 (see Figure S4, Supporting Information). The overall pattern of calculated ECD spectrum for (17*S*,22*R*)-stereoisomer was in accord with the experimental data of **1**. It should be noted that the experimental Cotton effect around 250 nm of compound **1** is not correlated with the calculated data well. However, the negative Cotton effect at 210 nm matched well with the experimental data, while the Cotton effect of the (17*R*,22*S*)-stereoisomer showed the opposite sign (Figure 5). Thus, the structure of polycycloiridal A was determined as depicted.

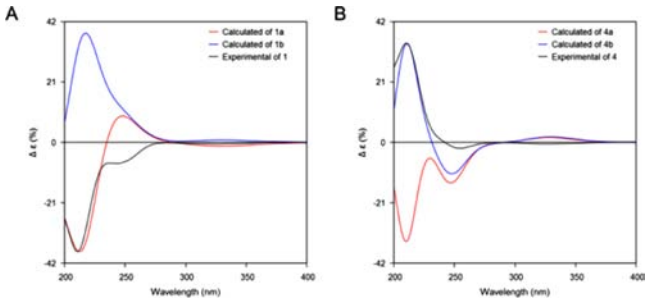


Figure 5. Experimental ECD spectrum of **1** and **4** and calculated ECD spectrum of **1a**, **1b**, **4a**, and **4b** in MeOH.

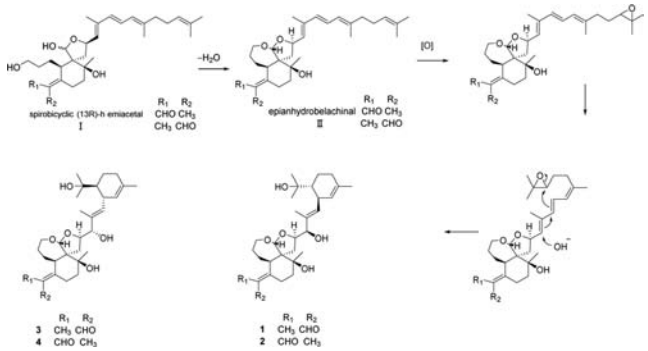
Polycycloiridal B (**2**) exhibited the same molecular formula $C_{30}H_{46}O_6$ as **1**, as established by HRESIMS at *m*/*z* 525.3172 $[M + Na]^+$. The NMR spectroscopic data of **2** were very similar to those of **1**. The major differences were a downfield shift ($\Delta\delta$ 0.64) of H-6 and an upfield shift ($\Delta\delta$ 0.60) of one H-8 in **2** relative to those for **1** in the ^{1}H NMR spectrum and a further upfield shift ($\Delta\delta$ 4.1) of C-6 and an downfield shift ($\Delta\delta$ 3.9) of C-8 in the ^{13}C NMR spectrum as compared with the corresponding signals of **1**, suggesting that **2** is a geometric isomer of **1** at the α,β-unsaturated aldehyde moiety. The presence of NOESY correlations between the aldehyde hydrogen proton and H-6 and the vinyl methyl and one of H-8 further supported this conclusion.

Polycycloiridal C (**3**) gave the same molecular formula, $C_{30}H_{46}O_6$, as **1** from its HRESIMS (*m*/*z* 525.3174 $[M + Na]^+$). Its ^{1}H NMR spectroscopic data were similar to those of **1**. The main differences between **3** and **1** were the chemical shift and splitting pattern of H-14 in **3** (δ_H 4.34, brs) compared to those of H-14 in **1** (δ_H 3.88, d, $J_{13/14}$= 5.4), thus indicating that they possess distinct C-14 configurations, a remarkable difference in chemical shift of C-14 between the two compounds further supported the above conclusion. Compound **3** did not form MTPA esters when treated with MTPA chloride, presumably due to steric hindrance. Additionally, other differences were observed in the chemical shifts of the Me-24 and Me-30 resonances [δ_H 1.08 (Me-24), 1.01 (Me-30) for **3** vs 1.19 (Me-24), 1.19 (Me-30) for **1**] between the two compounds in the ^{1}H NMR spectrum, indicating that the two compounds might have different configuration at the α-terpineol moiety. The relative configuration of the α-terpineol moiety in **3** was shown to be identical to that in **1** by analysis of the NOESY data. The absolute stereochemistry of **3** was concluded to be the same as that of **4**, which was evident from a close similarity of ECD spectrum between **3** and **4**.

Polycycloiridal D (**4**) was deduced to have the same molecular formula of $C_{30}H_{46}O_6$ as **3** from its HRESIMS (*m*/*z* 525.3173 $[M + Na]^+$). Its ^{1}H and ^{13}C NMR spectroscopic data were similar to those of **3** except for a little difference in chemical shifts of H-6, H-8, C-6, and C-8. The differences of these signals for **4** and **3** were comparable with those observed for **2** and **1**. Therefore, compound **4** was concluded to be a geometrical isomer of **3** at the α,β-unsaturated aldehyde moiety. Comparison of theoretically calculated and experimental ECD curves (Figure 5) permitted the assignment of the absolute configuration of α-terpineol moiety in **4** as (17*R*,22*S*), which was contrary to that in **1** and **2**. MO analysis at 210 nm suggested a resemblant electronic transition pattern for **4** and **1**. However, the optical rotatory strength of the 10th excited state of **4** was 86.1516, while that of the corresponding ninth excited state of **1** was −136.7562. Thus, they possessed different signs of Cotton effects at 210 nm.

The biogenetic precursor of **1**−**4** could be plausibly traced back to spirobicyclic(13*R*)-hemiacetal and its geometrical isomer (I) at the α,β-unsaturated aldehyde moiety,[4,15] which further underwent a series of reactions including intramolecular dehydration, epoxidation, and nucleophilic addition reaction to form **1**−**4** (Scheme 1).

Scheme 1. Plausible Biogenetic Pathway of 1−4



Compounds **1**−**4** were tested for their hepatoprotective activities against D-galactosamine-induced toxicity in HL-7702 cells using the hepatoprotective activity drug bicyclol as the positive control. They exhibited pronounced hepatoprotective activities at a concentration of 10 μM (see Table S5 in the Supporting Information). *I. tectorum* has also been used as a traditional folk medicine for the treatment of hepatic cirrhosis in China.[16] Whether these trace components are responsible for the activity needs further exploration.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02982.

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Experimental procedures and UV, IR, CD, MS, and NMR spectra for **1**−**4**; related original ECD calculation data for **1** and **4** (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Marner, F. J. *Curr. Org. Chem.* **1997**, *1*, 153−186.
(2) Jaenicke, L.; Marner, F. J. *Pure Appl. Chem.* **1990**, *62*, 1365−1368.
(3) (a) Bonfils, J. P.; Pinguet, F.; Culine, S.; Sauvaire, Y. *Planta Med.* **2001**, *67*, 79−81. (b) Fang, R.; Houghton, P. J.; Hylands, P. J. *J. Ethnopharmacol.* **2008**, *118*, 257−263.
(4) Ito, H.; Onoue, S.; Miyake, Y.; Yohida, T. *J. Nat. Prod.* **1999**, *62*, 89−93.
(5) Benoit-Vical, F.; Imbert, C.; Bonfils, J. P.; Sauvaire, Y. *Phytochemistry* **2003**, *62*, 747−751.
(6) (a) Takahashi, K.; Hano, Y.; Suganuma, M.; Okabe, S.; Nomura, T. *J. Nat. Prod.* **1999**, *62*, 291−293. (b) Takahashi, K.; Suzuki, S.; Hano, Y.; Nomura, T. *Biol. Pharm. Bull.* **2002**, *25*, 432−436.
(7) (a) Corbu, A.; Aquino, M.; Pratap, T. V.; Retailleau, P.; Arseniyadis, S. *Org. Lett.* **2008**, *10*, 1787−1790. (b) Corbu, A.; Gauron, G.; Castro, J. M.; Dakir, M.; Arseniyadis, S. *Org. Lett.* **2007**, *9*, 4745−4748. (c) Takanashi, N.; Tamura, K.; Suzuki, T.; Nakazaki, A.; Kobayashi, S. *Tetrahedron Lett.* **2015**, *56*, 327−330. (d) Marner, F. J.; Kasel, T. *J. Nat. Prod.* **1995**, *58*, 319−323.
(8) (a) Takahashfi, K.; Hoshino, Y.; Suzuki, S.; Hano, Y.; Nomura, T. *Phytochemistry* **2000**, *53*, 925−929. (b) Seki, K.; Tomihari, T.; Haga, K.; Kaneko, R. *Phytochemistry* **1994**, *36*, 425−431. (c) Seki, K.; Tomihari, T.; Haga, K.; Kaneko, R. *Phytochemistry* **1994**, *37*, 807−815.
(9) (a) Zhang, C. L.; Wang, Y.; Liu, Y. F.; Ni, G.; Liang, D.; Luo, H.; Song, X. Y.; Zhang, W. Q.; Chen, R. Y.; Chen, N. H.; Yu, D. Q. *J. Nat. Prod.* **2014**, *77*, 411−415. (b) Zhang, C. L.; Wang, Y.; Liu, Y. F.; Liang, D.; Hao, Z. Y.; Luo, H.; Zhang, Q. J.; Shi, G. R.; Chen, R. Y.; Cao, Z. Y.; Yu, D. Q. *Tetrahedron* **2015**, *71*, 5579−5583.
(10) Fang, R.; Houghton, P. J.; Luo, C.; Hylands, P. J. *Phytochemistry* **2007**, *68*, 1242−1247.
(11) (a) Marner, F. J.; Krick, W.; Gellrich, B.; Jaenicke, L.; Winter, W. *J. Org. Chem.* **1982**, *47*, 2531−2536. (b) Lamshöft, M.; Schmickler, H.; Marner, F. J. *Eur. J. Org. Chem.* **2003**, *2003*, 727−733.
(12) (a) Song, Z. J.; Xu, X. M.; Deng, W. L.; Peng, S. L.; Ding, L. S.; Xu, H. H. *Org. Lett.* **2011**, *13*, 462−465. (b) Bonfils, J. P.; Marner, F. J.; Sauvaire, Y. *Phytochemistry* **1998**, *48*, 751−753. (c) Taillet, L.; Bonfils, J. P.; Marner, F. J.; Sauvaire, Y. *Phytochemistry* **1999**, *52*, 1597−1600.
(13) Su, B. N.; Park, E. J.; Mbwambo, Z. H.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2002**, *65*, 1278−1282.
(14) Miyake, Y.; Ito, H.; Yoshida, T. *Can. J. Chem.* **1997**, *75*, 734−741.
(15) Marner, F. J.; Hanisch, B. *Helv. Chim. Acta* **2001**, *84*, 933−938.
(16) Song, L. R.; Hong, X.; Ding, X. L.; Zai, Y. Z. *Modern dictionary on Traditional Chinese Medicine*; People's Medical Publishing House: Beijing, 2001; pp 1254−1255.

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