NPC Natural Product Communications

2010
Vol. 5
No. 0
1 - 3

Chemical Diversity of Iridal-Type Triterpenes in *Iris delavayi* Collected in Yunnan Province of China

Yu Hasegawa,[a] Xun Gong,[b] and Chiaki Kuroda*[a]

[a]*Department of Chemistry and Research Center for Smart Molecules, Rikkyo University, Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan*
[b]*Kunming Institute of Botany, Chinese Academy of Science, Kunming 650204, P. R. China*

chkkuroda@grp.rikkyo.ne.jp

Received: ; Accepted:

From *Iris delavayi* collected in the northwestern Yunnan Province of China, eight iridal-type triterpenoids were isolated, three of which were new. Both 2(7)*Z*- and 2(7)*E*-iridals were isolated in about equal amounts from the sample collected at Laojunshan, while only 2(7)*Z*-iridals were isolated from samples collected in Shangrila area, indicating the presence of chemical diversity in the species.

Keywords: *Iris delavayi*; iridals; triterpene; diversity; structure; Hengduan Mountains

Plants in the area of the Hengduan Mountains, located in the northwestern Yunnan and southwestern Sichuan Provinces, and partly in the eastern Tibet Autonomous Region, China, provide us with interesting materials for studies of plant diversity. To date, we have studied the chemical and genetic diversity of plants in this area focusing on the genus *Ligularia* (Asteracea), and have reported that intra-specific diversity was found in many species [1]. To understand the characteristics of the area, it is essential to study some other plants. The genus *Iris* (Iridaceae) is one of the abundant plants in this area. About 60 species are known in China and about half of them were found in Yunnan/Sichuan area [2]. *Iris* species are a good source of iridals, a minor class of triterpene [3], suggesting that the compounds could be a good chemical index in the diversification study.

In the present study, we focused on the chemical composition of *I. delavayi* Micheli collected at three locations in Yunnan Province. The plants are distributed widely in the area at about 3000-4000 m elevation, making colonies at each population. To the best of our knowledge, the chemical composition of *I. delavayi* has not been reported. Here, we describe that there is diversity in the composition of iridal-type triterpenoid within *I. delavayi* populations of Yunnan Province. Three new compounds were isolated and their structures were determined.

Three samples of *I. delavayi* were collected in Yunnan Province (Table 1 and Fig. 1). Dried roots of each sample were extracted with ethanol, and the extracts were separated by standard procedures such as silica-gel column chromatography and HPLC.

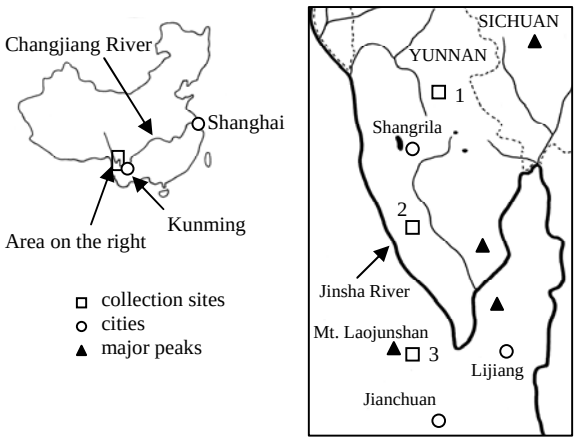
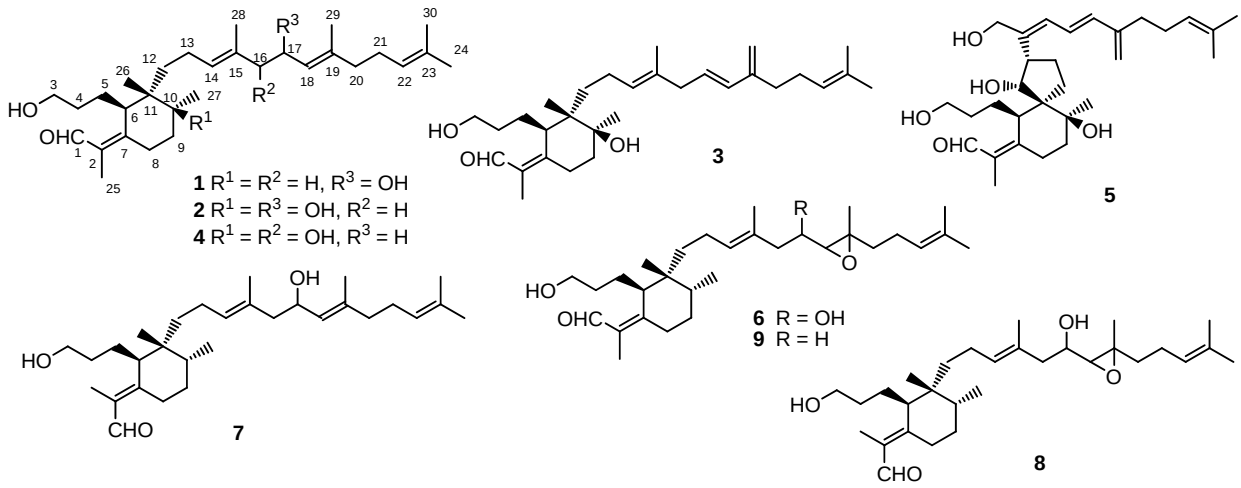


Fig. 1. Locations where samples of *I. delavayi* were collected.

Table 1. Collection locality and isolated compounds of *I. delavayi* samples.

Sample	Location (County)	Isolated compounds
1	Hongshan (Shangrila)	**1-6**
2	Qianhushan (Shangrila)	**1-4, 6**
3	Laojunshan (Jianchuan)	**1, 2, 4, 6-8**



From the samples, eight iridals were isolated. Five of them, **1** [4], **2** [4], **3** [5], **4** (isoiridogermanal) [6, 8], and **5** (28-deacetylbelamcandal) [7, 8], were known, and three compounds, **6**, **7**, and **8**, were new. Structures of the new compounds were determined as follows.

The high-resolution mass spectrum of compound **6** showed a molecular ion peak at *m/z* 474.3703 and its formula was deduced to be $C_{30}H_{50}O_4$. The IR spectrum indicated the presence of hydroxy and conjugated carbonyl groups. The ^{1}H NMR spectrum showed a characteristic singlet due to aldehyde at δ 10.19, together with seven methyl signals (δ 0.80, 0.96, 1.23, 1.54, 1.58, 1.66, and 1.78) and two olefinic triplets (δ 5.04 and 5.11), suggesting that the compound is an iridal derivative (Table 2). ^{13}C NMR signals were very similar to those of **9** [9] except that the signal of C-17 appeared at δ 68.4 (Table 3), as well as low-field shifts of C-16 and C-18 signals. These data suggest the presence of a hydroxy group at C-17. The HMQC spectrum showed that a signal at δ 3.53 (br dt, J = 5.7, 7.5 Hz) was attributable to a hydroxymethine proton at this carbon. The presence of a hydroxy group at C-17 and an epoxide between C-18 and C-19 was confirmed by the HMBC correlation between H_3-29 and C-18, 19, and 20, between H-16 and C-17 and 18, and between H-18 and C-17 and 20 (Fig. 2). The carbon framework was established by the COSY and the other HMBC data shown in Fig. 2. The stereochemistry deduced by the biosynthetic pathway [4] was supported by NOE correlations observed between H_3-27 and H_2-12, between H_3-26 and H_2-5, and between H-14 and H-16. Therefore, the structure of **6** was established as depicted.

The mass spectrum of compound **7** showed a signal at *m/z* 440.3642 ($C_{30}H_{48}O_2$) attributed to $[M - H_2O]^+$. Although the molecular ion peak was not observed, the molecular formula was determined to be $C_{30}H_{50}O_3$ on the basis of the presence of three oxygen atoms detected in the IR and the ^{1}H NMR spectra. Namely, the IR spectrum showed the presence of hydroxy and conjugated carbonyl groups, as in the case of compound **6**. The ^{1}H NMR spectrum showed the presence of an aldehyde (δ 10.18 (s)), seven methyls (δ 0.80, 0.95, 1.58, 1.60, 1.64, 1.66, and 1.78), three olefinic protons (δ 5.06, 5.10, and 5.12), a hydroxymethylene (δ 3.60), and a hydroxymethine (δ 4.40). These signals are characteristic of iridal-type triterpenoids (Table 2). Both ^{1}H and ^{13}C NMR signals were very close to **1** (Tables 2 and 3). A remarkable difference was observed in the chemical shift of the signals due to H-6 and H-8. Namely, a broad doublet due to one of H_2-8 was low-field-shifted (δ 3.33) while the signal of H-6 (δ 2.81 (dd, J = 5.3, 10.3 Hz)) was high-field-shifted, indicating that the compound is a geometrical isomer of **1** with respect to C(2)-C(7) double bond. The COSY and HMBC spectra showed that the carbon connection was the same as for compound **1** (Fig. 3). Thus, the structure of **7** was established as the 2(7)*E* isomer of **1**.

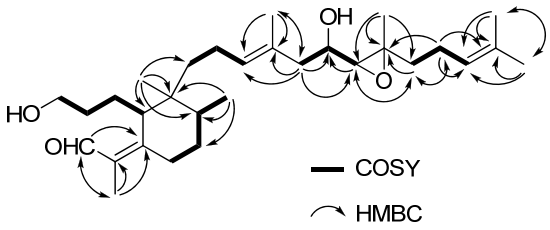


Fig. 2. COSY and HMBC correlation detected for **6**.

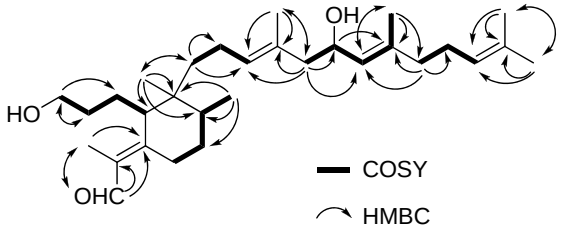


Fig. 3. COSY and HMBC correlation detected for **7**.

Table 2. ^{1}H NMR data of compounds **6**, **7**, and **8** ($CDCl_3$).

carbon number	**6**	**7**	**8**
1	10.19 (s)	1.78 (d, 1.7)	1.78 (d, 1.6)
3	3.61 (br t, 6.8)	3.60 (t, 6.3)	3.60 (t, 6.2)
4	1.30 (m)	1.35 (m)	1.35 (m)
5	1.63 (m)	1.66 (m)	1.68 (m)
6	3.35 (dd, 4.8, 9.3)	2.81 (dd, 5.3, 10.3)	2.80 (dd, 5.4, 10.0)
8	2.63 (br d, 14.3) 2.16 (m)	3.33 (br d, 14.3) 2.12 (m)	3.33 (br d, 14.5) 2.14 (m)
9	1.61/1.35 (m)	1.35/1.62 (m)	1.64/1.34 (m)
10	1.90 (m)	1.89 (m)	1.89 (m)
12	1.16 (m)	1.14 (t-like, 8.5)	1.15 (t, 8.5)
13	1.85 (m)	1.98/2.04 (m)	1.90 (m)
14	5.11 (br t, 6.8)	5.10 (m)	5.13 (br t, 6.7)
16	2.14 (m)	2.08 (d, 6.5)	2.19 (dd, 13.0, 8.1) 2.13 (dd, 13.5, 5.0)
17	3.53 (br dt, 5.7, 7.5)	4.40 (dt, 8.2, 6.5)	3.54 (dt, 8.0, 5.3)
18	2.66 (d, 7.7)	5.12 (m)	2.67 (d, 7.8)
20	1.61/1.40 (m)	1.97 (br t, 7.5)	1.62/1.39 (m)
21	2.02 (m)	2.03 (m)	2.02 (m)
22	5.04 (t sept, 7.0, 1.3)	5.06 (t sept, 6.9, 1.2)	5.04 (br t, 7.1)
24	1.66 (br s)	1.66 (s)	1.66 (s)
25	1.78 (d, 1.0)	10.18 (s)	10.18 (s)
26	0.96 (s)	0.95 (s)	0.95 (s)
27	0.80 (d, 6.8)	0.80 (d, 6.8)	0.81 (d, 6.8)
28	1.54 (br s)	1.60 (s)	1.59 (s)
29	1.23 (s)	1.64 (d, 1.1)	1.24 (s)
30	1.58 (s)	1.58 (s)	1.58 (s)

The molecular formula of **8** was determined to be the same as that of **6** (*m/z* 474.3721, $C_{30}H_{50}O_4$) by HRMS. The ^{1}H and ^{13}C NMR spectra of **8** were very similar to those of **6** except that the signal due to one of H_2-8 was low-field-shifted to δ 3.33, while H-6 was high-field-shifted, suggesting that **8** is an isomer of **6** with respect to C(2)-C(7) double bond. The carbon connection was confirmed by the COSY and HMBC spectra (Fig. 4), establishing the structure as depicted.

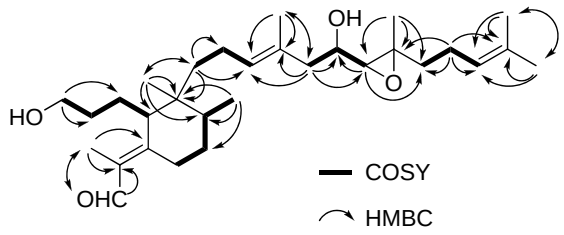


Fig. 4. COSY and HMBC correlation detected for **8**.

Table 3. ^{13}C NMR data of compounds **6**, **7**, and **8** ($CDCl_3$).

carbon number	**6**	**7**	**8**
1	189.9	11.7	11.7
2	133.3	133.0	133.0
3	62.9	63.1	63.1
4	31.7	30.5	30.6
5	24.0	24.4	24.4
6	43.3	47.1	47.2
7	163.2	163.7	163.6
8	27.4	23.7	23.6
9	30.5	31.4	31.4
10	35.7	36.1	36.1
11	40.1	40.7	40.7
12	31.5	31.7	31.7
13	21.3	22.6	22.7
14	128.7	128.9	129.0
15	130.5	131.5	130.3
16	44.2	48.1	44.2
17	68.4	65.8	68.4
18	66.6	127.1	66.7
19	61.4	138.3	61.5
20	38.5	39.5	38.6
21	23.6	26.4	23.6
22	123.4	123.9	123.4
23	132.1	131.6	132.1
24	25.7	25.7	25.7
25	10.8	190.6	190.6
26	24.2	24.1	24.1
27	15.2	15.2	15.2
28	16.2	16.2	16.3
29	17.2	16.6	17.2
30	17.7	17.7	17.7

Although iridal-type triterpenoids were isolated from all three samples, intra-specific diversity in chemical composition of *I. delavayi* was observed (Table 1 and Fig. 5). From sample 1, diol **1** was isolated as the major component, together with compounds **2**-**6**. Sample 2 showed almost parallel chemical composition, except that **5** was absent. Compounds **7** and **8**, geometrical isomers of **1** and **6**, respectively, with respect to the conjugated aldehyde, were isolated from sample 3, while only *Z*-isomer was isolated from samples 1 and 2. In sample 3, *Z*- and *E*-isomers were contained in almost equal amounts for both **1**/**7** and **6**/**8** pairs. This type of *Z*/*E* pairs of geometrical isomers of iridals sometimes occurs in nature [8, 10]. In addition, compounds **3** and **5** were absent in sample 3. These data show that sample 3 is somewhat distant from the other two in terms of chemical composition. Samples 1 and 2 were collected within Shangrila highland, while Mt. Laojunshan where sample 3 was collected is separated by Jinsha River (Fig. 1). In our previous research on the diversity of *Ligularia*, related intra-specific diversity was observed in several species. For example, *L. vellerea* was found widely in the

northwestern Yunnan Province producing furanoeremophilanes, but samples collected in the Shangrila (Zhongdian) area and the Luguhu area were different in the substituents [11]. All compounds isolated from *L. lankongensis* collected at several locations in Yunnan were highly oxygenated bisabolanes but their oxidation pattern was different among samples [12].

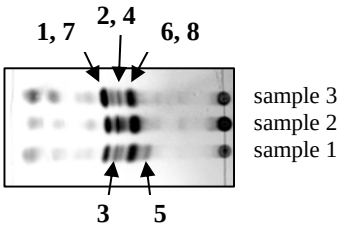


Fig. 5. TLC of the extract of samples 1-3 ($CHCl_3$/MeOH 9:1).

In conclusion, *I. delavayi* in Yunnan Province of China was found to exhibit diversity in the root chemicals. Supporting our previous results on *Ligularia*, it was suggested that the northwestern Yunnan Province is an area where plant diversification is on the way.

Experimental

NMR spectra were recorded on a JEOL ECX-400 (400 MHz for ^{1}H; 100 MHz for ^{13}C) spectrometer with $CDCl_3$ as the solvent and tetramethylsilane as an internal standard. IR spectra were recorded on a JASCO FT/IR-230 spectrometer. Mass spectra (MS) were obtained on a JEOL CMATE II mass spectrometer with EI method in the range of *m*/*z* 250-500. Column chromatography was performed on silica gel (Merck Kieselgel 60 or Wakogel C-200). Analytical TLC was carried out on Merck Kieselgel 60 F_{254} in 0.2 mm thickness using phosphomolybdic acid or *p*-anisaldehyde/AcOH/H_2SO_4 as visualizing agents. HPLC was carried out on a Hitachi L-6200 pump, L-4200H UV detector, and Gasukuro-kogyo Inertsil (20 × 250 mm) ODS column, or Shimadzu LC-20AT pump, SPD-20A UV detector, Nacalai COSMOSIL (10 × 250 mm) ODS column.

Plant Materials: *I. delavayi* was collected at three locations shown in Table 1 and Fig. 1. Each sample was identified by X. G. (author).

Extraction and Purification: The dried roots of each sample were extracted with EtOH at room temperature, and the extract was concentrated under reduced pressure. The resultant oily residue was dissolved in EtOAc and washed with H_2O. The organic phase was dried with Na_2SO_4 and concentrated under reduced pressure to obtain the extract.

The dried root of sample 1 (66 g) was treated as above to obtain the extract (2.17 g), which was subjected to column chromatography (SiO_2 150 g; eluent: hexane-EtOAc 7:3 to 0:10) to give five fractions. The fourth fraction was further separated by column chromatography and HPLC (eluent: MeOH/H_2O 9:1) to afford **1** (18.8 mg), **2** (8.5 mg), **3** (2.6 mg), **4** (2.5 mg), **5** (3.4 mg), and **6** (0.5 mg).

The dried root of sample 2 (57 g) was treated as above to obtain the extract (2.90 g), which was subjected to column chromatography (SiO_2 150 g; eluent: hexane-EtOAc 7:3 to 0:10) to give five fractions. The fourth fraction was further separated by column chromatography and HPLC (eluent: MeOH/H_2O 9:1) to afford **1** (23.6 mg), **2** (17.2 mg), **3** (0.8 mg), **4** (5.2 mg), and **6** (1.5 mg).

The dried root of sample 3 (14 g) was treated as above to obtain the extract (959 mg), which was subjected to column chromatography (SiO_2 100 g; eluent: hexane-EtOAc 7:3 to 0:10) to give five fractions. The third fraction was further separated by column chromatography and HPLC (eluent: MeOH/H_2O 9:1) to afford **1** (8.6 mg) and **7** (6.3 mg). Similar separation of the fourth fraction gave **2** (2.8 mg), **4** (2.8 mg), **6** (2.9 mg), and **8** (2.3 mg).

(6*S*, 10*R*, 11*R*)-18,19-Epoxy-10-deoxy-17-hydroxyiridal (**6**)

An oil. $[\alpha]_D$: +21.3 (*c* 0.22, MeOH). UV (MeOH) λ_{max} 256 nm (log ε 4.1). IR (neat/NaCl): 3400 (br), 1661, 1612 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 2 and 3, respectively. EIMS: *m*/*z* 457 ([M - OH]$^+$), 441, 423, 372, 335, 317, 307. HRMS-EI: *m*/*z* [M$^+$] calcd for $C_{30}H_{50}O_4$: 474.3709; found 474.3703.

(2(7)*E*, 6*S*, 10*R*, 11*R*)- 10-Deoxy-17-hydroxyiridal (**7**)

An oil. $[\alpha]_D$: +21.8 (*c* 0.34, MeOH). UV (MeOH) λ_{max} 254 nm (log ε 4.0). IR (neat/NaCl): 3400 (br), 1662, 1612 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 2 and 3, respectively. EIMS: *m*/*z* 441 ([M - OH]$^+$), 398, 372, 307. HRMS-EI: *m*/*z* [M$^+$-H_2O] calcd for $C_{30}H_{48}O_2$: 440.3654; found 440.3642.

(2(7)*E*, 6*S*, 10*R*, 11*R*)-18,19-Epoxy-10-deoxy-17-hydroxy-iridal (**8**)

An oil. $[\alpha]_D$: +31.6 (*c* 0.19, MeOH). UV (MeOH) λ_{max} 254 nm (log ε 4.0). IR (neat/NaCl): 3400 (br), 1663, 1612 cm^{-1}. ^{1}H and ^{13}C NMR: see Tables 2 and 3, respectively. EIMS: *m*/*z* 441 ([M - H_2O - Me]$^+$), 340, 307, 383. HRMS-EI: *m*/*z* [M$^+$] calcd for $C_{30}H_{50}O_4$: 474.3709; found 474.3721.

Acknowledgements

The authors wish to thank Prof. Xiaojiang Hao and Mrs. Guowen Hu of Kunming Institute of Botany for research coordination. This work was partly supported by a Grant-in-Aid for Scientific Research from JSPS (No. 21404009).

References

[1] (a) Nagano H, Torihata A, Matsushima M, Hanai R, Saito Y, Baba M, Tanio Y, Okamoto Y, Takashima Y, Ichihara M, Gong X, Kuroda C, Tori M. (**2009**) Chemical and genetic study of *Ligularia cyathiceps* in Yunnan Province of China. *Helvetica Chimica*

Acta, ***92***, 2017-2081; (b) Torihata A, Chao X, Kuroda C, Hanai R, Hirota H, Gong X. (**2009**) Chemical and genetic differentiation of *Ligularia hodgsonii* in Japan and China. *Chemistry and Biodiversity*, ***6***, 2184-2191; (c) Tori M, Okamoto Y, Tachikawa K, Mihara K, Watanabe A, Sakaoku M, Takaoka S, Tanaka M, Gong X, Kuroda C, Hattori M, Hanai R. (**2008**) Isolation of new eremophilane-type sesquiterpenoids, subspicatins A-D and subspicatolide from *Ligularia subspicata*, and chemical and genetic diversity of the species. *Tetrahedron*, ***64***, 9136-9142; (e) Nagano, H, Matsushima M, Yamada H, Hanai R, Gong X, Kuroda C. (**2010**) Two new furanoeremophilane sesquiterpenoids from *Ligularia oligonema*. *Natural Product Communications*, **5**, 1-4; (f) Nagano H, Kanda M, Yamada H, Hanai R, Gong X, Kuroda C. (**2010**) Chemical and genetic study of *Ligularia anoleuca* and *L. veitchiana* in Yunnan and Sichuan Provinces of China. *Helvetica Chimica Acta*, ***93***, 1945-1952; and referenced cited therein.

[2] Zhao Y, Noltie HJ, Mathew B. (**2000**) Iridaceae. *Flora of China*, ***24***, 297-312.

[3] (a) Breitmaier E. (**2006**) *Triterpenes*, Wiley-VCH, Weinheim; (b) Jeanicke L, Marner F-J. (**1990**) The irones and their origin. *Pure and Applied Chemistry*, ***62***, 1365-1368; (c) Marner F-J. (**1997**) Iridals and cycloiridals, products of an unusual squalene metabolism in sword lilies (*Iridaceae*). *Current Organic Chemistry*, ***1***, 153-186.

[4] Marner F-J, Longerich I. (**1992**) Isolation and structure determination of new iridals from *Iris sibirica* and *Iris versicolor*. *Liebigs Annalen der Chemie*, 269-272.

[5] Marner F-J, Simic K, Scholz B, Küster B. (**1995**) Isolation and structure determination of a new iridal from *Iris sibirica*. *Journal of Natural Products*, ***58***, 299-301.

[6] Krick W, Marner F-J, Jaenicke L. (**1983**) Isolation and structure determination of the precursors of α- and γ-irone and homologous compounds from *Iris pallida* and *Iris florentina*. *Zeitschrift für Naturforschung*, ***38c***, 179-184.

[7] Abe F, Chen R-F, Yamauchi T. (**1991**) Iridals from *Belamcanda chinensis* and *Iris japonica*. *Phytochemistry*, ***30***, 3379-3382.

[8] Takahashi K, Hoshino Y, Suzuki S, Hano Y, Nomura T. (**2000**) Iridals from *Iris tectorum* and *Belamcanda chinensis*. *Phytochemistry*, ***53***, 925-929.

[9] Bonfils J-P, Marner F-J, Sauvaire Y. (**1998**) An epoxidized iridal from *Iris germanica* var “rococo”. *Phytochemistry*, ***48***, 751-753.

[10] (a) Fang R, Houghton PJ, Luo C, Hylands PJ. (**2007**) Isolation and structure determination of triterpenes from *Iris tectorum*. *Phytochemistry*, ***68***, 1242-1247. (b) Marner F-J, Singab ANB, Al-Azizi MM, El-Emary NA, Schäfer M. (**2002**) Iridal glycosides from *Iris spuria* (Zeal), cultivated in Egypt. *Phytochemistry*, ***60***, 301-307.

[11] Tori M, Nakamizo H, Mihara K, Sato M, Okamoto Y, Nakashima K, Tanaka M, Saito Y, Sono M, Gong X, Shen Y, Hanai R, Kuroda C. (**2008**) Chemical and genetic diversity of *Ligularia vellerea* in Yunnan, China. *Phytochemistry*, ***69***, 1158-1165.

[12] Onuki H, Yamazaki M, Nakamura A, Hanai R, Kuroda C, Gong X, Shen Y, Hirota H. (**2008**) Chemical constituents and diversity of *Ligularia lankongensis* in Yunnan Province of China. *Journal of Natural Products*, ***71***, 520-524.