Iridal-Type Triterpenoids with Ichthyotoxic Activity from *Belamcanda chinensis*

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Ichthyotoxic activity-guided fractionation of the hexane and ether extracts of *Belamcanda chinensis* (Iridaceae) has resulted in the isolation of eleven iridal-type triterpenoids including six new compounds, 3-*O*-tetradecanoyl-16-*O*-acetylisoiridogermanal (**4**), 3-*O*-decanoyl-16-*O*-acetylisoiridogermanal (**5**), belachinal (**7**), anhydrobelachinal (**9**), epianhydrobelachinal (**10**), and isoanhydrobelachinal (**11**). Structures of the new iridals were determined by spectral and chemical methods. The absolute configuration of isoiridogermanal (**1**) at C-16 was determined to be *R* by the modified Mosher's method. Of these compounds, 16-*O*-acetylisoiridogermanal (**3**), **7**, and spiroiridal (**12**) exhibited potent ichthyotoxic activity against killie-fish (*Oryzias latipes*).

Naturally occurring ichthyotoxic substances also often exhibit a variety of biological properties such as insecticide, tumor-promoting, antitumor and antifungal activities, and/or inhibitory effect on activation of Epstein−Barr virus early antigen induced by TPA, etc.[1−4] In a recent study of *Iris germanica* L. (Iridaceae),[5] we reported the characterization of several iridal-type triterpenoids including new iridals, irisgermanicals A, B, and C, as ichthyotoxic components. These active compounds included α-irigermanal, iriflorental, and iripallidal, which were previously demonstrated to have a potent in vivo antiulcerogenic effect on indomethacin- or stress-induced ulceration in rats.[6] These findings prompted further investigation of the iridaceous species *Belamcanda chinensis* (L.) DC, as part of a research program aimed at finding novel biologically active natural products using an ichthyotoxic assay as a primary indicator. The dried rhizomes of *B. chinensis* have been used as a traditional Chinese medicine for disorders of the throat including coughing and pharyngitis.[7] Although belamcandal, a stimulant of the throat membrane, other related iridals[8,9] and many isoflavonoids[10,11,12] have been isolated from the plant, no ichthyotoxic principle has been reported. In a bioassay-directed separation using killie-fish (*Oryzias latipes*; Medaka), hexane and ether extracts of the *B. chinensis* rhizomes afforded a series of iridals including six new compounds. This paper deals with the isolation of these compounds and their characterization by extensive 2D NMR and chemical methods, and the assessment of their ichthyotoxic activity.

Results and Discussion

Fresh rhizomes of *B. chinensis* were extracted with hexane followed by MeOH. The concentrated MeOH extract was partitioned with ether. The active hexane and ether extracts were fractionated separately by an extensive series of chromatographic procedures and afforded eleven iridals (**1**−**5**, **7**−**12**) including six new compounds (**4**, **5**, **7**, **9**−**11**). The known compounds were identified as isoiridogermanal (**1**),[13] iristectorene B (**2**),[14] 16-*O*-acetylisoiridogermanal (**3**),[8] 28-deacetylbelamcandal[8] and (+)-(*6R,10S,11S,14S,26R*)-26-hydroxy-15-methylidenespiroirid-16-enal (**12**),[15] by comparisons of their spectral data with those in the literature.

The electrospray ionization (ESI) MS of **4** gave a $[M + NH_4]^+$ peak at *m*/*z* 744, corresponding to molecular formula $C_{46}H_{78}O_6$. The UV (255 nm) absorption and the ^1H and ^{13}C NMR signals at δ_H 10.16 (1H, s), δ_C 189.7, 162.4, and 133.3 indicated the presence of a fully substituted α,β-unsaturated aldehyde function. The ^1H NMR spectrum also exhibited signals due to three nonconjugated olefinic protons, five vinyl methyl, and two tertiary methyl groups. Two additional signals at δ 0.87 (t, J = 7.5 Hz) and 2.01 (s) were ascribed to a terminal methyl of a fatty acid residue and acetyl methyl, respectively. Acetyl and tetradecanoyl residues were suggested by significant fragment ions at *m*/*z* 667 $[(M - CH_3COOH) + H]^+$ and 211 $[CH_3(CH_2)_{12}CO]^+$ in the ESIMS, and by NMR spectra showing methylene proton and two ester carbonyl carbon resonances. These spectral features, along with ^{13}C NMR data indicating 9 sp^2 and 21 sp^3 carbon signals for the parent alcohol, implied that compound **4** was a diester of isoiridogermanal (**1**). Comparison of the ^1H NMR spectrum of **4** with that of iristectorene B (**2**) (3-*O*-tetradecanoate of **1**)[14] indicated that **4** was the acetyl derivative of **2**. This conclusion was supported by a significant downfield shift of the H-16 signal (δ 3.91 in **2** to δ 4.97 in the ^1H NMR spectrum of **4**). Their structural relationships were substantiated by methanolysis of **4** yielding **1**, **2**, and **3**, in addition to methyl tetradecanoate (GLC). Compound **4** was thus characterized as 3-*O*-tetradecanoyl-16-*O*-acetylisoiridogermanal. This is the first isolation from natural source although **4** was previously reported as derivative of **2**. [14]

Compound **5** showed a pseudo-molecular ion $[M + NH_4]^+$ at *m*/*z* 688 in the ESIMS and its molecular formula was determined to be $C_{42}H_{70}O_6$ by HRESIMS. Although insufficient **5** was available to measure the ^{13}C NMR spectrum, the ^1H NMR was almost superimposable with that of **4** except for methylene protons in a fatty acid residue. Taking the molecular weight, 56 ($CH_2 \times 4$) less than **4**, into consideration, compound **5** was assigned to 3-*O*-decanoyl-16-*O*-acetylisoiridogermanal. Isoiridogermanal (**1**), the parent alcohol of **2**−**5**, was first isolated from *Iris florentina* and *I. pallida,* and its stereostructure, except for the configuration at C-16, was established[13] as shown.

The CD spectra for **1**−**5** are similar (positive Cotton effect at ca. 250 nm), indicating that the absolute configurations at C-10 and C-11 of **4** and **5** are the same as those of **1**. The absolute configuration of **1** at C-16 has been examined by application of the modified Mosher's method[16] using methoxy-(trifluoromethyl)phenylacetic acid (MTPA) esters as follows. Most of the protons of **1**, except for some

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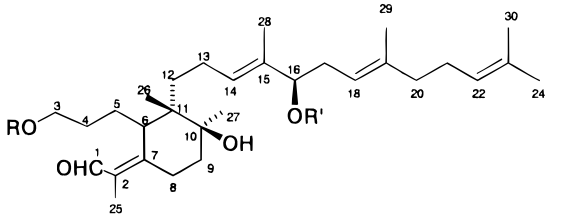
Table 1. ^{1}H NMR Data (δ) of **7** and **9**−**11** (500 MHz in $CDCl_3$)[a]

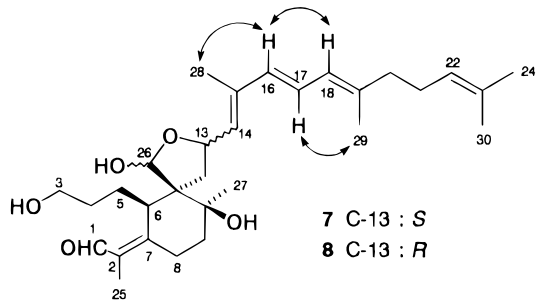
	7	9	10	11
H-1	10.24 [10.16] s	10.30 s	10.34 s	1.83 s
H-3	3.64 m	4.22 dd (5, 12)	4.21 dd (5, 12.5)	4.22 dd (5, 12.5)
	3.58 m	3.55 dt (3, 12)	3.58 dt (3, 12.5)	3.55 dt (3, 12.5)
H-4	1.30−1.25 m	1.60−1.40 m	1.60−1.38 m	1.59−1.35 m
H-5	2.10−2.30 m	2.93 m	2.95 m	2.90 m
H-6	3.64 [3.19] m	3.75 t (6)	3.82 d (10.5)	3.16 d (8.5)
H-8	2.70 m	2.58 m	2.64 dt (5.5, 13.5)	3.23 brt (13.5)
	2.51 m	2.56 m	2.53 brd (13.5)	2.57 brd (13.5)
H-9	1.80−1.65 m	1.84−1.60 m	1.80−1.60 m	1.82−1.39 m
H-12	1.98 dd (8, 13)	1.98 dd (8, 13)	2.10 m	2.04 m
H-13	4.89 [5.06] q (8)	4.89 q (8)	5.16 q (8)	4.89 q (8)
H-14	5.58 [5.35] d (8)	5.59 d (8)	5.44 d (8)	5.63 d (8.5)
H-16	6.16 [6.11] d (15.5)	6.16 d (15.5)	6.11 d (15)	6.18 d (15)
H-17	6.40 [6.43] dd (11, 15.5)	6.40 dd (11, 15.5)	6.40 dd (11, 15)	6.41 dd (11, 15)
H-18	5.88 d (11)	5.87 d (11)	5.86 d (11)	5.87 d (11)
H-20	2.30−2.05 m	2.11 m	2.11 m	2.11 m
H-21	1.49−1.40 m	1.60−1.42 m	1.60−1.46 m	1.60−1.48 m
H-22	5.08 m	5.09 m	5.08 m	5.09 m
H-24	1.68 s	1.68 s	1.68 s	1.68 s
H-25	1.81 s	1.78 s	1.82 s	10.18 s
H-26	5.68 [5.52] s	5.45 s	5.42 s	5.43 s
H-27	1.30 s	1.33 s	1.27 s	1.32 s
H-28	1.78 brs	1.78 brs	1.78 brs	1.78 brs
H-29	1.78 brs	1.78 brs	1.78 brs	1.78 brs
H-30	1.60 s	1.60 s	1.60 s	1.60 s

[a] *J* values (Hz) are in parentheses, and data of the second anomer are found in brackets.

methylene protons, were assigned based on the ^{1}H−^{1}H COSY and TOCSY spectra and comparison with NMR data of derivatives (**2**−**5**). The (*R*) and (*S*)-MTPA esters, **6a** and **6b**, were prepared by treatment of a trityl derivative (**6**) of **1** with (*R*)- and (*S*)-MTPA. The positive and negative $\Delta\delta$ ($\delta_S - \delta_R$) (Hz) values [−33 Hz (14-H), −73.5 Hz (28-CH_3), +15, +30 Hz (17-CH_2), +53 Hz (18-H), +20.5 Hz (29-CH_3)] obtained for the (*R*)- and (*S*)-MTPA esters (**6a** and **6b**) led to assignment of the *R* configuration at C-16. Thus, the full stereostructures of **1** and its mono- (**2** and **3**) and diesters (**4** and **5**) are as shown.



1 : R=R'=H
2 : R=CO(CH$_2$)$_{12}$CH$_3$, R'=H
3 : R=H, R'=Ac
3a : R=R'=Ac
4 : R=CO(CH$_2$)$_{12}$CH$_3$, R'=Ac
5 : R=CO(CH$_2$)$_8$CH$_3$, R'=Ac
6 : R=CPh$_3$, R'=H
6a : R=CPh$_3$, R'=(*R*)-MTPA
6b : R=CPh$_3$, R'=(*S*)-MTPA



7 C-13 : *S*
8 C-13 : *R*

The positive ion FABMS of **7** gave a $[M + Na]^+$ peak at *m*/*z* 509, corresponding to $C_{30}H_{46}O_5$. The UV spectrum

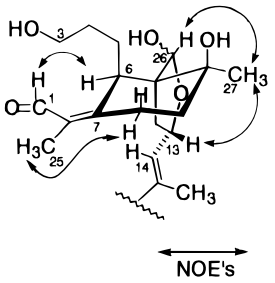


Figure 1. Selective NOE's of **7**.

showed absorption maxima characteristic of the conjugated triene chromophore. The ^{1}H NMR spectrum of **7** exhibited duplicate signals in a ratio of ca. 6:4 for each proton (partly overlapped), as clearly represented by singlets at δ 10.24 (10.16) and 5.68 (5.52) which are attributable to an aldehyde and a hemiacetal proton, respectively. Its ^{1}H (Table 1) and ^{13}C NMR spectra resembled those of the spirobicyclic-iridal [(6*R*,10*S*,11*R*)-26ζ-hydroxy-(13*R*)-oxa-spiroirid-16-enal] (**8**)[5,17] that was isolated as a mixture of two anomers from *Iris pseudacorus* and *I. japonica*. The major differences between the ^{1}H NMR spectra of **7** and **8** were an upfield shift (0.26 ppm) of H-13 and a downfield shift (0.10 ppm) of H-14 in **7** relative to those in **8**, implying **7** to be a 13-epimer of **8**. The structural assignment for **7** was confirmed by the NOESY spectrum. Although the C-27 methyl signal in **8** was reported to show NOE correlations with H-26 and H-14,[5] the corresponding methyl signal in **7** displayed a definite cross-peak with H-13 signal, but not with H-14 (Figure 1). Other NOE's similar to those of **8** were also consistent with the proposed structure **7** having the *S*-configuration at C-13.

Anhydrobelachinal (**9**) was obtained as a white amorphous powder, and the molecular formula was established to be $C_{30}H_{44}O_4$ by HRESIMS. The UV and ^{1}H NMR (Table 1) of **9** revealed some features similar to **7** although the ^{1}H NMR spectrum of **9** showed no duplication of signals.

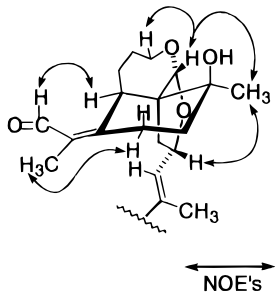


Figure 2. Selective NOE's of **9**.

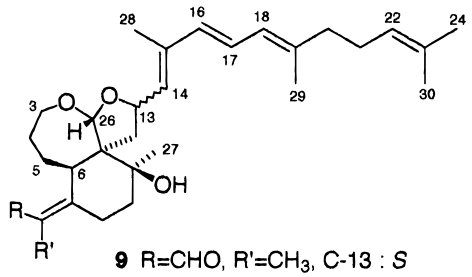
In a comparison of the ^{1}H NMR data for **9** with those of **7**, the C-3 methylene protons of the former resonated at δ 4.22 and 3.55, while those in the latter appeared as essentially equivalent signals near δ 3.64. An upfield shift of the H-26 signal relative to that of **7** was also observed. Taking a molecular weight of 18 mass units less than that of **7** into consideration, these spectral features suggested an acetal structure in **9**. The presence of a seven-membered acetal ring was substantiated by the ^{1}H−^{13}C long-range COSY (J_{CH} = 8 Hz) spectrum showing a three-bond coupling between H-3 and C-26. Additional evidence for the acetal structure was provided by the NOESY spectrum of **9** which displayed an NOE cross-peak between H-3 and H-26. The C-27 methyl signal also showed definite NOE's with H-26 and H-13 (Figure 2). Inspection of the MM2 molecular model calculation of **9** indicated that these NOE interactions were only possible if **9** had the relative configurations at C-26, C-27 and C-13 shown in the formula. The absolute stereochemistry of **9** was concluded to be the same as that of **7** as evidenced by a close similarity of CD spectra between **7** and **9**.

The molecular formulas of compounds **10** and **11** were both indicated by their HRESIMS data to be $C_{30}H_{44}O_4$, which was the same as that of **9**. The UV and ^{1}H NMR spectra of **10** and **11** (Table 1) were also very similar to those of **9**. Significant differences between ^{1}H NMR spectra of **10** and **9** were observed in the chemical shifts of H-13 and H-14 which were at lower and upper fields, respectively, in **10** than the corresponding signals in **9**. The differences of these signals in **9** and **10** were comparable with those observed for **7** and **8**, the epimeric pairs at C-13. Compound **10** was thus concluded to be a C-13 epimer of **9**, which was consistent with the HMBC data.

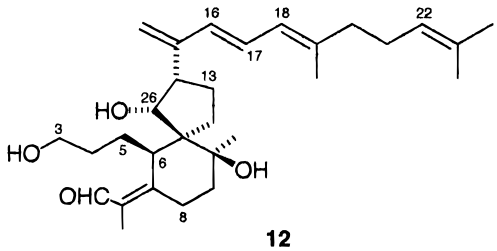
Comparison of the ^{1}H NMR data of **11** and **9** revealed an upfield shift (0.59 ppm) of H-6 and a downfield shift (0.65 ppm) for one of the C-8 methylene proton signals in the former related to the corresponding signals in the latter. This tendency was analogous to that reported for irisgermanical B and iriflorental which are the geometrical isomers at the α,β-unsaturated aldehyde moiety.[5] Structure **11** was confirmed by the NOESY data that included NOE correlations between the H-6 (δ 3.16) and vinyl methyl (δ 1.83) signals and also between the aldehyde (δ 10.18) and H-8 (δ 3.23) protons. The other NOE's similar to those of **9** led us to assign the same stereochemistry at the chiral centers of the ring in **11** as that in **9**.

Structural relationships between belachinal (**7**) and its anhydro analogues (**9**−**11**) were established by treatment of **7** with *p*-toluenesulfonic acid in CH_2Cl_2 yielding **9**, **10**, and **11** as well as some uncharacterized products.

The facile chemical conversion of **7** into anhydro derivatives prompted us to examine the possibility that compounds **9**−**11** might be artifacts produced from **7** or **8**



9 R=CHO, R'=CH_3, C-13 : *S*
10 R=CHO, R'=CH_3, C-13 : *R*
11 R=CH_3, R'=CHO, C-13 : *S*



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during the purification or extraction process. The possibility of their formation when exposed to Si gel was ruled out as when a CH_2Cl_2 solution of **7** with Si gel was left at room temperature for a week, **7** was recovered unchanged, with no trace of **9**, **10**, or **11** detected by HPLC. Furthermore, a lipid fraction obtained by supercritical fluid extraction (SFE)[18] [CO_2 flow rate 12.0 L/min; 22 Mpa; 40 °C with entrainer MeOH (1.0 mL/min)] of fresh *B. chinensis* rhizomes was shown, by HPLC, to contain compounds **9**−**11**, as well as **7** and other iridals. SFE using CO_2 is known as a quick and mild extraction method with relatively little risk of structural alteration of the plant ingredients,[18] and was reported to be applicable to selective extraction of iridals.[20] From these observations, compounds **9**−**11** were considered natural products rather than artifacts.

Among iridals isolated in the present study, compounds **3**, **7**, and spiroiridal (**12**) were highly toxic to killie-fish with median tolerance limit (TL_m)[1] values of 1.6−3.5 μg/mL after 24 h (Table 2). Although most monocyclic iridals are nontoxic to fish,[5] 16-*O*-acetylisoiridogermanal (**3**) showed moderate ichthyotoxic activity. Acetylation of **3** enhanced the activity. Nevertheless, it appears that esterification of the 3-OH of monocyclic and spiro-type iridals with a higher fatty acid, markedly lowers toxicity. Ethers such as **9** and **10** are also much less toxic indicating that the free hydroxyl group at C-3 is important for the toxicity. The TL_m values of the active iridals are comparable to that of buddledin B which was isolated as an ichthyotoxic constituent from *Buddleja davidii* roots[20] and later found to be cytotoxic against P-388 lymphocytic cells.

Experimental Section

General Experimental Procedures. ESIMS were determined on a Micromass Auto Spec OA-Tof spectrometer in the direct inlet mode using a solvent, 50% MeOH−0.1% $AcONH_4$, and FABMS on a VG 70SE spectrometer using 3-nitrobenzyl alcohol as the matrix agent. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR 500 spectrometer, and the chemical shifts are given in δ (ppm) referenced to TMS. Optical rotations were measured on a Jasco DIP-1000 polarimeter. Kieselgel 60 (Merck), Sephadex LH-20 (Pharmacia), and YMC ODS AQ120-S50 (YMC) were used for column chromatography. Analytical HPLC was performed with the following conditions: reversed-phase; Inertsil ODS-2 (4.6 × 250 mm, GL Sciences), 40 °C, solvent system of H_2O-MeOH, normal phase; YMC−Pack

Table 2. Ichthyotoxic Activity of the Iridals Isolated from *B. chinensis*

Compounds	TL_m (μg/mL)[a]
monocyclic Iridals	
isoiridogermanal (**1**)	9.3
16-*O*-acetylisoiridogermanal (**3**)	3.5
3,16-di-*O*-acetylisoiridogermanal (**3a**)	0.8
monocyclic iridal esters	
3-*O*-tetradecanoyl-16-*O*-acetylisoiridogermanal (**4**)	>10
3-*O*-decanoyl-16-*O*-acetylisoiridogermanal (**5**)	>10
iristectorene B (**2**)	>30
spiroiridals	
belachinal (**7**)	2.8
(*6R,10S,11R*)-26ζ-hydroxy-(*13R*)-oxaspiroirid-16-enal (**8**)	5.5
anhydrobelachinal (**9**)	>10
epianhydrobelachinal (**10**)	>10
(+)-(*6R,10S,11S,14S,26R*)-26-hydroxy-15-methylidenespiroirid-16-enal (**12**)	1.6
buddledin B	1.2

[a] TL_m: median tolerance limit after 24 h.

SIL A-003 (4.6 × 250 mm, YMC), solvent system of *n*-hexane−EtOH. In preparative HPLC, a YMC−Pack ODS-A324 column (10 × 300 mm) was used in the reversed-phase mode and Inertsil Sil (10 × 250 mm) was used in the normal phase mode, with a UV detector (254 and 280 nm) or photodiode-array detector.

Plant Material. Rhizomes of *B. chinensis* cultivated at the herbarium of Faculty of Pharmaceutical Sciences, Okayama University, were collected in January, 1995. A voucher specimen (OPH-I03) is kept at the same herbarium.

Extraction and Isolation. Fresh rhizomes (1 kg) of *B. chinensis* were chopped and soaked in hexane (2 L) three times for 24 h each time at room temperature to yield a hexane extract (2.5 g). The residue was further extracted with MeOH (2 L × 3). The concentrated solution was diluted with H_2O and extracted successively with ether and *n*-BuOH to give Et_2O (9.5 g), *n*-BuOH (13 g), and H_2O (36.8 g) extracts. Ichthyotoxic activity was exhibited by the hexane and Et_2O extracts with TL_m values of 4.6 and 4.2 ppm, respectively. The hexane extract was subjected to column chromatography over Si gel (3.8 i.d. × 40 cm) using $CHCl_3$ and $CHCl_3$−acetone. The eluate with $CHCl_3$−acetone (8:2) (800 mg) was further purified by repeated column chromatography over ODS gel (1.1 i.d. × 26 cm) with MeOH−H_2O (93:7) to give **2** (10 mg), **4** (30 mg), and **5** (2 mg). The Et_2O extract (6 g) was chromatographed over Si gel (3.0 i.d. × 58 cm) with $CHCl_3$−acetone (8:2 → 7:3 → 6:4 → 5:5) and acetone−MeOH (1:1) in a stepwise gradient mode. The eluate with $CHCl_3$−acetone (6:4) (316 mg) was purified by a combination of column chromatography over Sephadex LH-20 (1.2 i.d. × 30 cm) with $CHCl_3$−MeOH and preparative HPLC on a RP-18 column (MeOH−H_2O, 17:3) to yield **9** (14 mg) and **10** (17 mg). The $CHCl_3$−acetone (8:2) eluate was divided into three parts (each ca. 400 mg) and fractionated separately by column chromatography over Sephadex LH-20 and preparative HPLC in a way similar to that described above to give additional **9** (49 mg total) and **10** (55 mg), and **11** (18 mg), **12** (68 mg), **1** (209 mg), **7** (51 mg), **3** (76 mg), and 28-deacetylbelamcandal (7 mg).

3-*O*-Tetradecanoyl-16-*O*-acetylisoiridogermanal(4): Glassy solid; $[\alpha]_D$ +25.0° (*c* 1.0, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 255 (4.15) nm; CD (MeOH) $\Delta\epsilon$ (nm) +1.4 (254); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.16 (1H, s, H-1), 5.26 (1H, t, J = 7.0 Hz, H-14), 5.06 (1H, m, H-18), 5.06 (1H, m, H-22), 4.97 (1H, t, J = 7.0 Hz, H-16), 4.01 (2H, t, J = 6.5 Hz, H-3), 3.27 (1H, brd, J = 9.5 Hz, H-6), 2.55 (2H, m, H-8), 2.34, 2.20 (each 1H, m, H-17), 2.27 (2H, t, J = 7.5 Hz, H-2′), 2.01 (3H, s, 16-COCH_3), 1.83 (3H, s, H-25), 1.67 (3H, s, H-24), 1.59 (3H, s, H-29), 1.58 (3H, s, H-30), 1.52 (3H, s, H-28), 1.24 (CH_2, brs), 1.14 (3H, s, H-27), 1.06 (3H, s, H-26), 0.87 (3H, t, J = 7.5 Hz, H-14′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.7 (C-1), 173.9 (C-1′), 170.3 (16-*C*OCH$_3$), 162.4 (C-7), 137.7 (C-19), 133.3 (C-15), 133.3 (C-2), 131.4 (C-23), 127.8 (C-14), 124.1 (C-22), 119.1 (C-18), 78.9 (C-16), 74.9 (C-10), 64.2 (C-3), 44.7 (C-11), 43.2 (C-6), 39.7 (C-20), 37.0 (C-9), 36.6 (C-12), 34.3 (C-2′), 31.9 (C-12′), 31.5 (C-17), 29.67 (CH_2), 29.64 (CH_2), 29.61 (C-7′), 29.46 (C-6′), 29.34 (C-11′), 29.28 (C-5′), 29.17 (C-4′), 28.6 (C-4), 26.7 (C-21), 26.6 (C-5), 26.3 (C-27), 25.7 (C-24), 25.0 (C-3′), 23.8 (C-8), 22.7 (C-13′), 21.8 (C-13), 21.3 (16-CO*C*H$_3$), 17.9 (C-26), 17.7 (C-30), 16.2 (C-29), 14.1 (C-14′), 11.8 (C-28), 11.0 (C-25); ESIMS *m/z* 744 $[M + NH_4]^+$, 667 $[M - CH_3COOH+H]^+$, 649 $[M - CH_3COOH - H_2O + H]^+$, 211 $[CH_3(CH_2)_{12}CO]^+$; HRESIMS *m/z* $[M + NH_4]^+$ 744.6142 (calcd for $C_{46}H_{82}NO_6$, 744.6142).

Methanolysis of 4: A solution of **4** (3 mg) in 0.1% methanolic NaOH (1 mL) was kept at room temperature and the reaction mixture was monitored by reversed-phase HPLC which showed formation of **2** and **3** at an early stage and then of **1** as a final product. Identification of the final product **1** was confirmed after prep. HPLC by ^{1}H NMR data. The liberated fatty acid ester was identified as methyl tetradecanoate by GLC analysis.

3-*O*-Decanoyl-16-*O*-acetylisoiridogermanal (5): Glassy solid; $[\alpha]_D$ +30.0° (*c* 0.39, CH_2Cl_2); UV (CH_3CN) λ_{max} (log ϵ): 255 (4.19) nm; CD (MeOH) $\Delta\epsilon$ (nm) +3.2 (257); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.17 (1H, s, H-1), 5.26 (1H, t, J = 7.5 Hz, H-14), 5.05 (1H, m, H-18), 5.05 (1H, m, H-22), 4.97 (1H, brt, J = 6.5 Hz, H-16), 4.01 (2H, t, J = 6.5 Hz, H-3), 3.27 (1H, brd, J = 10.0 Hz, H-6), 2.56 (2H, m, H-8), 2.33/2.20 (each 1H, m, H-17), 2.27 (2H, t, J = 7.5 Hz, H-2′), 2.01 (3H, s, 16-COCH_3), 1.84 (3H, s, H-25), 1.67 (3H, s, H-24), 1.59 (3H, s, H-29), 1.58 (3H, s, H-30), 1.52 (3H, s, H-28), 1.26 (16H, CH_2, brs), 1.15 (3H, s, H-27), 1.07 (3H, s, H-26), 0.88 (3H, t, J = 7.0 Hz, H-10′); ESIMS *m/z* 688 $[M + NH_4]^+$, 610 $[M - CH_3COOH]^+$, 592 $[M - CH_3COOH - H_2O]^+$; HRESIMS *m/z* $[M + NH_4]^+$ 688.5492 (calcd for $C_{42}H_{74}NO_6$, 688.5516).

Tritylation of 1. Isoiridogermanal (**1**) (11 mg) and triphenyl methyl chloride (20 mg) were dissolved in 2 mL of dried pyridine, and the mixture was allowed to stand for 24 h at room temperature. Preparative TLC [Si gel, $CHCl_3$−MeOH (70:1)] of the crude tritylation product obtained after usual workup afforded 4 mg of 3-*O*-tritylisoiridogermanal (**6**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.12 (1H, s, H-1), 7.41−7.22 (15H, phenyl-H), 5.24 (1H, t, J = 7.0 Hz, H-14), 5.08 (1H, m, H-18), 5.06 (1H, m, H-22), 3.92 (1H, t, J = 7.0 Hz, H-16), 3.24 (1H, brd, J = 9.5 Hz, H-6), 3.03 (1H, m, H-3), 3.01 (1H, m, H-3), 2.55, 2.51 (each 1H, m, H-8), 1.82 (3H, s, H-25), 1.68 (3H, brs, H-24), 1.62 (3H, s, H-29), 1.60 (3H, s, H-30), 1.55 (3H, s, H-28), 1.13 (3H, s, H-26), 1.05 (3H, s, H-27).

(*R*)-MTPA ester 6a: To a solution of 3-*O*-tritylisoiridogermanal (**6**) (9.5 mg) in dry CH_2Cl_2 (1 mL) was added Et_3N (0.6 μL), DMAP (7 mg), and (*R*)-MTPA chloride (0.6 μL) and the mixture was stirred at room temperature for 15 h. The mixture was then purified by preparative TLC [Si gel, $CHCl_3$−MeOH (100:1)] to give the (*R*)-MTPA ester **6a** (12.9 mg) as colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.12 (1H, s, H-1), 7.50−7.22 (20H, phenyl-H), 5.39 (1H, t, J = 7.0 Hz, H-14), 5.32 (1H, m, H-18), 5.06 (1H, m, H-22), 4.92 (1H, t, J = 7.0 Hz, H-16), 3.50 (3H, s, MTPA −OCH$_3$), 3.22 (1H, brd, J = 9.5 Hz, H-6), 3.04 (1H, m, H-3), 3.01 (1H, m, H-3), 2.40 (1H, m, H-17), 2.24 (1H, m, H-17), 2.00 (1H, m, H-21), 1.91 (1H, m, H-13), 1.82 (3H, s, H-25), 1.68 (3H, brs, H-24), 1.55 (3H, s, H-29), 1.54 (3H, s, H-28), 1.12 (3H, s, H-26), 1.05 (3H, s, H-27); ESIMS *m/z* 950 $[M + NH_4]^+$.

(*S*)-MTPA ester 6b: Ester **6b** was prepared in a way similar to that for **6a**. **6b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.10 (1H, s, H-1), 7.48−7.21 (20H, phenyl-H), 5.32 (1H, t, J = 7.0 Hz, H-14), 5.28 (1H, m, H-18), 5.07 (1H, m, H-22), 5.02 (1H, t, J = 7.0 Hz, H-16), 3.53 (3H, s, MTPA −OCH$_3$), 3.20 (1H, brd, J = 9.5 Hz, H-6), 3.04 (1H, m, H-3), 3.00 (1H, m, H-3), 2.46 (1H, m, H-17), 2.27 (1H, m, H-17), 2.03 (1H, m, H-21), 1.97 (1H, m, H-13), 1.82 (3H, s, H-25), 1.63 (3H, brs, H-24), 1.57 (3H, s, H-29), 1.39 (3H, s, H-28), 1.13 (3H, s, H-26), 1.04 (3H, s, H-27); ESIMS *m/z* 950 $[M + NH_4]^+$.

Belachinal (7): Glassy solid; $[\alpha]_D$ +33.0° (*c* 1.0, CH_2Cl_2); UV (EtOH) λ_{max} (log ϵ) 259 (sh) (4.51), 269 (4.54), 280 (4.58), 290 (4.47) nm; CD (CH_3CN) $\Delta\epsilon$ (nm) +5.0 (248), −3.3 (280), −1.3 (290), +0.1 (305); ^{1}H NMR, Table 1; ^{13}C NMR ($CDCl_3$) δ 190.5 (190.1) (C-1), 161.3 (160.7) (C-7), 139.7 (140.1) (C-19), 135.7 (137.2) (C-15), 134.4 (134.3) (C-16), 133.0 (C-2), 131.8

(C-23), 130.8 (C-14), 125.5 (125.7) (C-17), 125.2 (125.1) (C-18), 123.9 (124.0) (C-22), 99.6 (105.3) (C-26), 74.3 (74.2) (C-10), 73.0 (C-13), 62.0 (62.5) (C-3), 61.0 (C-11), 42.6 (46.0) (C-6), 40.2 (39.0) (C-20), 39.0 (39.1) (C-9), 32.0 (CH_2), 30.9 (CH_2), 28.1 (27.7) (C-27), 26.7 (C-21), 25.6 (C-24), 24.0 (23.9) (C-8), 17.8 (C-30), 16.9 (C-29), 12.7 (13.1) (C-28), 11.1 (C-25); FABMS *m/z* 509 $[M + Na]^+$; ESIMS *m/z* 504 $[M + NH_4]^+$; HRESIMS *m/z* $[M + NH_4]^+$ 504.3657 (calcd for $C_{30}H_{50}NO_5$, 504.3689).

Anhydrobelachinal (9): Glassy solid; $[\alpha]_D$ +62.5° (*c* 0.45, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 257 sh (4.21), 268 (4.25), 282 (4.28), 291 (sh) (4.19) nm; CD (MeOH) $\Delta\epsilon$ (nm): +4.9 (249), −0.6 (273), +0.3 (298); ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$) δ 189.9 (C-1), 164.3 (C-7), 139.2 (C-19), 135.3 (C-15), 134.1 (C-16), 133.7 (C-2), 131.7 (C-23), 129.1 (C-14), 125.3 (C-17), 125.0 (C-18), 124.0 (C-22), 110.9 (C-26), 77.9 (C-13), 73.3 (C-10), 70.7 (C-3), 61.2 (C-11), 43.8 (46.4) (C-6), 40.1 (C-20), 39.1 (C-9), 32.0 (CH_2), 31.9 (CH_2), 27.8 (C-27), 26.6 (C-21), 25.7 (C-24), 24.4 (C-8), 17.7 (C-30), 16.8 (C-29), 12.6 (C-28), 10.7 (C-25); EIMS *m/z* 468 $[M]^+$; ESIMS *m/z* 469 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 469.3302 (calcd for $C_{30}H_{45}O_4$, 469.3318).

Epianhydrobelachinal (10): Glassy solid; $[\alpha]_D$ +7.8° (*c* 0.64, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 259 (sh) (4.12), 270 (4.18), 281 (4.23), 290 (sh) (4.11) nm; CD (CH_3CN) $\Delta\epsilon$ (nm) +8.3 (252), −7.9 (280); ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$) δ 190.1 (C-1), 165.0 (C-7), 139.7 (C-19), 137.1 (C-15), 134.2 (C-16), 131.7 (C-2), 129.8 (C-23), 129.2 (C-14), 125.3 (C-17), 125.0 (C-18), 123.9 (C-22), 110.0 (C-26), 75.2 (C-13), 74.2 (C-10), 70.5 (C-3), 60.5 (C-11), 43.8 (45.0) (C-6), 40.1 (C-20), 38.8 (C-9), 32.5 (CH_2), 31.6 (CH_2), 28.3 (C-27), 26.6 (C-21), 25.7 (C-24), 24.4 (C-8), 17.7 (C-30), 16.9 (C-29), 13.2 (C-28), 10.8 (C-25); EIMS *m/z* 468 $[M]^+$; ESIMS *m/z* 469 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 469.3313 (calcd for $C_{30}H_{45}O_4$, 469.3318).

Isoanhydrobelachinal (11): Glassy solid; $[\alpha]_D$ +30.1° (*c* 0.5, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 261 (4.20), 270 (4.23), 280 (4.25), 289 (sh) (4.16) nm; ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$) δ 10.8 (C-1), 164.9 (C-7), 139.3 (C-19), 135.2 (C-15), 134.6 (C-16), 133.8 (C-2), 131.7 (C-23), 128.9 (C-14), 125.2 (C-17), 124.9 (C-18), 123.9 (C-22), 111.1 (C-26), 77.9 (C-13), 73.4 (C-10), 70.7 (C-3), 61.2 (C-11), 50.6 (43.2) (C-6), 40.1 (C-20), 39.8 (C-9), 39.7 (CH_2), 31.9 (CH_2), 32.8 (C-27), 26.6 (C-21), 25.7 (C-24), 17.7 (C-30), 16.8 (C-29), 12.6 (C-28), 190.8 (C-25); EIMS *m/z* 468 $[M]^+$; ESIMS *m/z* 469 $[M + H]^+$; HRESIMS *m/z* $[M + H]^+$ 469.3338 (calcd for $C_{30}H_{45}O_4$, 469.3318).

Chemical Conversion of 7 into 9−11. Compound **7** (5 mg) was treated with *p*-toluenesulfonic acid (1.3 mg) in $CHCl_3$ (2 mL) at room temperature for 5 h. After disappearance of the starting material by HPLC, the reaction mixture was concentrated and subjected to preparative HPLC (YMC−Pack ODS-A324 (10 i.d. x 300 mm), H_2O−MeOH 15:85, 40 °C) to yield **9** (0.8 mg), **10** (1.0 mg) and **11** (0.8 mg) which were identical with naturally occurring compounds by HPLC and ^{1}H NMR spectral comparisons.

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