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NEW PISCICIDAL TRITERPENES FROM *IRIS GERMANICA*

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Three new piscicidal triterpenens named irisgermanicals A, B and C along with seven known iridal-type triterpenes including iripallidal and iriflorental were isolated from the bark of *Iris germanica* rhizome, upon bioassay-guided fractionation using the Medaka (killie-fish; *Oryzias latipes*), and their bicyclic structures were elucidated based on the spectral analyses. Irisgermanicals B and C were characterized as geometrical iriflorental and iripallidal isomers, respectively, concerning the α,β-unsaturated aldehyde moiety. The piscicidal activity was observed for bicyclic iridals, among which iriflorental exhibited most potent activity.

KEY WORDS *Iris germanica*; Iridaceae; piscicidal activity; iridal; irisgermanical

Some piscicidal substances have been found to show the various biological activities as exemplified the by insecticidal, aphrodisiac, and anti-tumor promoting activities of rotenone,1) potent tumor-promoting activity2) of phorbol-type diterpenoid,3) and antifungal activitiy of 5-*O*-methyl embelin.4) In a continuing study of the biologically active substances in plants using piscicidal activity to the Medaka (killie-fish; *Oryzias latipes*) as a primary screening, the MeOH extract of the bark of *Iris germanica* rhizome was shown to exhibit a potent activity. Although many isoflavonoids5) and iridals, mono- and bicyclic triterpenoids6) have been isolated from this plant, the biological activity of these constituents is little known. We have thus investigated to clarify the active constituents in the extract, and isolated eight active triterpenoids including three new compounds named irisgermanicals A (**7**), B (**8**), and C (**10**). This communication deals with the characterization of these piscicidal constituents.

The EtOAc-soluble portion of the MeOH extract of the bark of *I. germanica* rhizome was subjected to bioassay-guided fractionation by column chromatography over silicic acid ($CHCl_3$-acetone) followed by preparative HPLC (Superspher RP-18, MeOH-H_2O) to give irisgermanicals A (6.9×10^{-4} % from dried bark), B (2.2×10^{-3} %), and C (2.9×10^{-3} %), together with seven known iridal-type triterpenoids which were identified with α- (**1**) and γ-irigermanal (**2**), isoiridogermanal (**3**),6) 16-*O*-acetyl-isoirigermanal (**4**),7) α-dehydroirigermanal (**5**),8) iriflorental (**6**), and iripallidal (**9**),6) by comparisons of their spectral data with those reported.

Irisgermanical A (**7**), a glassy solid, $[\alpha]_D$ +46.2° (CH_2Cl_2), showed the $(M+H)^+$ ion peak at *m/z* 471 corresponding to the molecular formula $C_{31}H_{50}O_3$, in FAB-MS. The ^{13}C-NMR spectrum showed nine sp^2 and twenty-two sp^3 carbon resonances. The presence of an α,β-unsaturated aldehyde function in the molecule was indicated by its UV [λ_{max} (EtOH) 237 nm (log ε 4.43)] and NMR spectra [δ_H 10.20 (1H, s), δ_C 190.0, 162.7 and 133.3]. The ^1H-NMR spectrum further disclosed the presence of a secondary methyl [δ 0.84 (d, J=7.5 Hz)], six tertiary methyl groups [δ 0.63, 0.85, 1.10, 1.16, 1.69 and 1.84 (3H each, s)] and an exomethylene group [δ 4.49 and 4.72 (1H each, br s)]. The signals attributable to three olefinic protons [δ 5.20 (dd, J=6.5, 7.5 Hz), 5,64 (dd, J=10, 15.5 Hz), and 5.94 (d, J=15.5 Hz)] were also observed. These spectral features suggested that **7** is an analog of bicyclic iridal of γ-type. Actually, the ^1H-NMR spectrum of **7** was closely similar to that of iriflorental (**6**), except for the lack of an oxygen-bearing methylene group and presence of an extra tertiary methyl group in the former. The structure **7** for irisgermanical A

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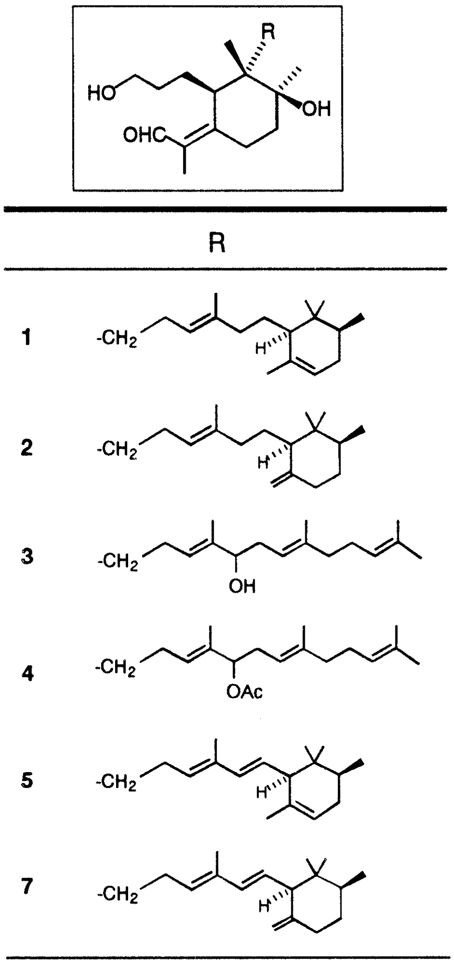
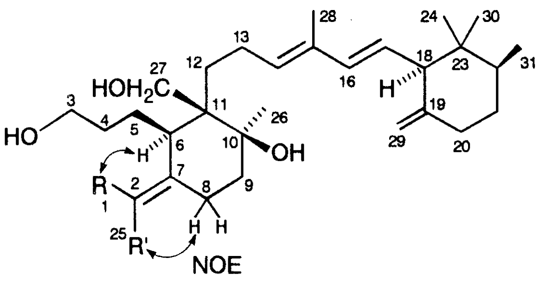
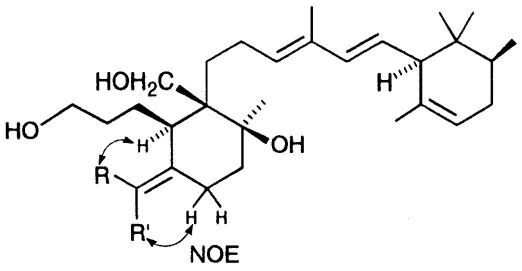
was consistent with the ^{13}C-NMR data upon comparison with those of **6**[6] (Table 1). The CD spectrum of **7** [Δε +8.6 (231 nm), -4.7 (251 nm)] was also similar to that of **6**,[6] indicating the same absolute configurations at chiral centers in **7** as those of **6**.





6 : R = CHO; R' = CH_3
6a : R = CH_2OH; R' = CH_3
8 : R = CH_3; R' = CHO
8a : R = CH_3; R' = CH_2OH



9 : R = CHO; R' = CH_3
9a : R = CH_2OH; R' = CH_3
10 : R = CH_3; R' = CHO
10a : R = CH_3; R' = CH_2OH

Table1. ^{13}C-NMR Data for **6**, **7**, **8**, **9** and **10** (126 MHz, $CDCl_3$)

	6	7	8	9	10		6	7	8	9	10
C-1	189.9	190.0	12.0	190.1	12.0	C-24	14.2	14.3	14.3	14.8	14.7
C-2	133.2	133.3	133.0	133.3	133.0	C-25	11.0	11.1	190.8	11.1	190.9
C-3	62.6	63.1	62.8	62.6	62.8	C-26	26.5	26.4	26.6	26.5	26.5
C-6	42.5	43.5	46.6	42.7	46.6	C-27	68.3	18.0	68.2	68.5	68.3
C-7	162.2	162.7	162.7	162.5	162.8	C-28	16.1	16.2	16.1	15.7	15.6
C-10	76.2	75.1	76.4	76.3	76.4	C-29	107.9	107.9	107.8	23.2	22.6
C-11	46.8	44.8	47.3	46.8	47.3	C-30	12.6	12.7	12.7	12.6	12.7
C-14	126.7	126.5	126.7	128.6	128.6	C-31	27.1	26.7	27.6	26.6	26.6
C-15	134.2	134.1	134.1	134.2	134.0	CH_2	21.6	22.4	20.0	21.7	20.0
C-16	136.7	136.9	136.7	134.6	134.5		23.9	23.9	22.6	24.0	23.2
C-17	129.6	130.1	129.8	129.3	129.4		27.7	27.8	27.7	27.2	27.6
C-18	58.2	58.3	58.2	56.4	56.3		32.2	32.3	31.8	32.0	31.8
C-19	150.5	150.7	150.5	137.3	137.2		32.2	32.8	32.1	32.2	31.9
C-20	42.2	42.3	42.2	121.7	121.7		36.5	37.1	35.5	35.6	35.5
C-22	38.7	38.8	38.7	38.2	38.2		37.2	37.1	38.2	37.2	38.2
C-23	35.5	36.6	36.5	35.7	35.6						

Irisgermanicals B (**8**), $[\alpha]_D$ +69.8° (CH_2Cl_2), $C_{31}H_{50}O_4$, [FAB-MS *m/z* 509 $(M+Na)^+$] and C (**10**), $[\alpha]_D$ +37.4° (CH_2Cl_2), $C_{31}H_{50}O_4$, [FAB-MS *m/z* 487 $(M+H)^+$, 509 $(M+Na)^+$] showed ^{1}H9)- and ^{13}C-NMR (Table 1) spectra closely similar to those of iriflorental (**6**) and iripallidal (**9**), respectively. The distinguishing features in the ^{1}H-NMR spectra of these compounds were the upfield shift ($\Delta\delta$ 0.6 ppm) of the H-6 signal and low field shift ($\Delta\delta$ 0.7 ppm) of one of the C-8 methylene proton signals in **8** and **10** relative to the corresponding signals of **6** and **9**. Although the definite NOEs between the aldehyde proton (δ 10.22 for **6** and **9**) and H-6 proton (δ 3.59 for **6** and 3.7 for **9**), and also between vinyl methyl and H-8 proton (δ 2.54 for **6** and **9**), respectively, were observed in the NOESY spectra of **6** and **9**, the aldehyde proton and vinyl methyl signals of **8** showed NOEs with H-8 and H-6, respectively. Irisgermanical C (**10**) also disclosed the NOEs, as detected in **8** (see formulas). Upon reduction of the aldehyde group with $NaBH_4$, **8** and **10** gave the dihydro-derivatives [**8a** and **10a**; *m/z* 511 $(M+Na)^+$], which showed the CD spectra [$\Delta\varepsilon$ -0.3 (230 nm) for **8a** and $\Delta\varepsilon$ -4.6 (230 nm) for **10a**] similar to those of the corresponding dihydro-derivatives from **6** and **9**. These data clearly indicate that **8** and **10** are isomers of **6** and **9**, differing only in the geometry of the double bond conjugated with aldehyde group. Although more than forty iridals have been isolated from *Iris* spp. in the past decade,6,7,10) the analogs having the geometry shown in **8** and **10** are the first examples, which might be of interest from the view point of biosynthesis of iridals.

Among the iridals isolated in the present study, the most potent piscicidal activity was observed for iriflorental (**6**) with 0.1 μg/ml of median tolerance limit (TLm)2) value after 24 h, and the other known bicyclic iridals showed the activity of TLm (24h) 0.5-0.9 μg/ml. The TLm value of irisgermanical A was 0.8 μg/ml, while those of irisgermanicals B and C were 3 μg/ml. The monocyclic iridals (**3** and **4**) were inactive. The other biological activities of these piscicidal iridals are now under investigation.

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9) The ^{1}H-NMR ($CDCl_3$) data of **8**; δ: 10.24 (1H, s, H-25), 5.94 (1H, d, *J*=16 Hz, H-16), 5.65 (1H, dd, *J*=10, 16 Hz, H-17), 5.16 (1H, br s, H-14), 4.72 (1H, br s, H-29), 4.48 (1H, br s, H-29), 4.09 (1H, d, *J*=11.5 Hz, H-27), 3.96 (1H, d, *J*=11.5 Hz, H-27), 3.24 (1H, d, *J*=14 Hz, H-8), 3.04 (1H, t, *J*=7.5 Hz, H-6), 2.70 (1H, br t, *J*=11 Hz, H-8), 2.38 (1H, d, *J*=10 Hz, H-18), 1.82, 1.74, 1.33, 0.85, 0.64 (3H each, s, CH_3), 0.84 (3H, d, *J*=7.5 Hz, $C\underline{H}_3CH$); Data of **10**; δ: 10.25 (1H, s, H-25), 5.97 (1H, d, *J*=15.5 Hz, H-16), 5.43 (1H, br s, H-20), 5.34 (1H, dd, *J*=10.5, 15.5 Hz, H-17), 5.16 (1H, t, *J*=7.5 Hz, H-14), 4.08 (1H, d, *J*=11.5 Hz, H-27), 3.96 (1H, d, *J*=11.5 Hz, H-27), 3.24 (1H, d, *J*=14 Hz, H-8), 3.04 (1H, t, *J*=7 Hz, H-6), 2.70 (1H, br t, *J*=11 Hz, H-8), 1.83, 1.69, 1.50, 1.33, 0.83, 0.63 (3H each, s, CH_3), 0.85 (3H, d, *J*=7 Hz, $C\underline{H}_3CH$).
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