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Chemical and Chemotaxonomical Studies of Ferns. LIV.[1] Pterosin Derivatives of the Genus *Microlepia* (Pteridaceae)

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From the fronds of five pteridaceous ferns [*Microlepia speluncae* (L.) MOORE, *M. trapeziformis* (ROXB.) KUHN, *M. obtusiloba* HAYATA, *M. substrigosa* TAGAWA and *M. strigosa* (THUNB.) PRESL], several 1-indanone-type sesquiterpenes (pterosins and pterosides) were isolated. Four of them, isolated from *M. speluncae* were new and were assigned the structures 13-hydroxy-3(*R*)-pterosin D (V), 13-hydroxy-3(*R*)-pterosin D 3-*O*-α-L-arabinopyranoside (VI), 3(*R*)-pterosin D 3-*O*-α-L-arabinopyranoside (VIII) and 2(*R*),3(*R*)-pterosin L 3-*O*-α-L-arabinopyranoside (XI). The structures were elucidated mainly by spectroscopic methods. Compound V was named spelosin. The investigation of two other species, *M. calvescens* (WALL.) PRESL and *M. okamotoi* TAGAWA, gave no identifiable compounds, of this type.

Keywords——*Microlepia speluncae*; Microlepia trapeziformis; *Microlepia obtusiloba*; *Microlepia substrigosa*; *Microlepia strigosa*; *Microlepia calvescens*; *Microlepia okamotoi*; Pteridaceae; chemotaxonomy; pterosin-type sesquiterpene

As a continuation of our chemical and chemotaxonomical investigations on ferns, we have studied seven further species of the genus *Microlepia* [Pteridaceae, *Microlepia speluncae* (L.) MOORE, *M. trapeziformis* (ROXB.) KUHN, *M. obtusiloba* HAYATA, *M. substrigosa* TAGAWA, *M. strigosa* (THUNB.) PRESL, *M. calvescens* (WALL.) PRESL and *M. okamotoi* TAGAWA]. Previous studies on *M. marginata* (PANZER) C. CHR.[2] and *M. hookeriana* (WALL.) PRESL (*Schypholepia hookeriana* J. SMITH)[3] have yielded *ent*-kaurane and *ent*-pimarane diterpenes, but no pterosin-type sesquiterpenes, which are quite common in the family Pteridaceae.[4]

We report here the isolation of several pterosin-type sesquiterpenes from five *Microlepia* species (*Microlepia speluncae, M. trapeziformis, M. obtusiloba, M. substrigosa* and *M. strigosa*), and the structure determination of four new compounds from *M. speluncae*. However, two other species *M. calvescens* (Japanese name: Inu-fumotoshida)[5] and *M. okamotoi* (Japanese name: Niseishikaguma)[5] gave no identifiable compounds of this type (*cf.* Table I).

(1) *Microlepia speluncae* (L.) MOORE (Japanese Name: Ooishikaguma)

From this fern, four new pterosin-type sesquiterpenes, V, VI, VIII and XI, were isolated along with pterosins Z (I), I (II) and H (III), 3(*R*)-pterosin D (VII) and 2(*R*),3(*R*)-pterosin L (X).

TABLE I. The Distribution of Pterosin-Type Sesquiterpenes in the Genus *Microlepia*

	Aglycones		Glycosides[a]	
	C_{15}-Pterosin	C_{14}-Pterosin (=norpterosin)	C_{15}-Pterosin-glycoside	C_{14}-Pterosin-glycoside
M. speluncae	Z, I, H, D, L, Spelosin	—	3-Ara (D, L, spelosin)	—
M. trapeziformis	Z, H	—	3-Ara (L, spelosin)	—
M. obtusiloba	I, H	—	—	—
M. substrigosa	Z, H, A	F	3-Ara (spelosin) 3-Glc (D)	3-Glc (C)
M. strigosa	D, L	B, O, F, P, C	—	3-Glc (C)
M. okamotoi	—	—	—	—
M. calvescens	—	—	—	—
M. marginata	—	—	—	—
M. hookeriana (*Scypholepia hookeriana*)	—	—	—	—

a) Aglycones are shown in parentheses.

TABLE II. ^{13}C-NMR Chemical Shifts (in C_5D_5N) of V, VI, VII, VIII, X and XI

	VI	V	Glycosidation shift	VIII	VII	Glycosidation shift	XI	X	Glycosidation shift
C-1	208.7	209.6		208.7	209.6		207.7	208.4	
C-2	51.7	51.9	−0.2	51.5	51.8	−0.3	56.4	56.4	0.0
C-3	85.1	76.4	+8.7	85.2	76.7	+8.5	83.1	77.2	+5.9
C-4	127.1	126.6		126.0	125.6		126.1	125.3	
C-5	144.9	145.0		144.5	144.7		144.8	144.8	
C-6	141.4	140.6		137.0	137.1		137.0	136.7	
C-7	137.8	137.9		138.3	138.0		138.6	138.0	
C-8	130.6	130.9		130.2	130.6		131.9	132.3	
C-9	151.5	154.2	−2.7	150.8	153.5	−2.7	151.1	154.4	−3.3
C-10	22.7	23.4		22.7	23.5		65.8	67.1	
C-11	22.0	21.1		22.0	21.1		19.4	19.4	
C-12	22.2	22.3		21.1	21.3		21.3	21.3	
C-13	72.2	72.2		33.0	33.1		33.1	33.1	
C-14	65.5	65.1		60.9	61.1		60.9	61.0	
C-15	15.1	15.1		14.0	14.0		14.0	14.0	
C-1′	106.8			106.8			104.5		
C-2′	72.6			72.6			72.2		
C-3′	74.6			74.6			73.9		
C-4′	69.5			69.5			68.2		
C-5′	67.2			67.2			66.2		

Compound V, $C_{15}H_{20}O_4$ (M^+ 264.137), colorless syrup, $[\alpha]_D^{22}$ +83.3° (c=0.7, MeOH), showed ultraviolet (UV) and infrared (IR) absorptions suggesting it to be a pterosin-type compound.[4] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of V is partially similar to that of pterosin D (VII)[4] and showed signals assignable to geminal dimethyl groups at C-2 [$\delta_{C_5D_5N}$ 1.39 (6H, s)], two aromatic methyl groups [δ 2.69 (3H, s) 3.12 (3H, s)], one

carbinyl proton at C-3 [δ 5.10 (1H, s)] and an aromatic proton [δ 7.59 (1H, s)]. The ABX signals in the ^{1}H-NMR spectrum [δ 4.12 (1H, dd, J=11 and 5 Hz), 4.46 (1H, dd, J=11 and 8 Hz) and 5.84 (1H, dd, J=8 and 5 Hz)] demonstrated that V contained a 1,2-glycol side chain at C-6 instead of a hydroxyethylene group.

From the above spectroscopic data, this new sesquiterpene can be formulated as a pterosin D derivative bearing an additional hydroxyl group at the α-position in the side chain at C-6. The absolute configuration at C-3 can be deduced from the similarity of the circular dichroism (CD) curve to that of 3(*R*)-pterosin D.[4] Hence, compound V was assigned the structure 13-hydroxy-3(*R*)-pterosin D, and named spelosin. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum supported this structure (*cf.* Table II).

Compound VI, $C_{20}H_{28}O_8$ (M^+ 396.179), colorless amorphous powder, $[\alpha]_D^{21}$ +56.9° (*c*=1, MeOH), showed a UV spectrum characteristic of pterosin-type sesquiterpenes. Acid hydrolysis afforded L-arabinose and spelosin (V). The ^{13}C-NMR spectrum showed signals at $\delta_{C_5D_5N}$ 106.8, 72.6, 74.6, 69.5 and 67.2[6] characteristic of the α-arabinopyranosyl moiety and the ^{1}H-NMR signal (1H, d, J=8 Hz) at δ 4.99 (in C_5D_5N) indicated that the L-arabinose moiety in VII had an α-glycosidic linkage. A comparison of the ^{13}C-NMR spectrum of VII with that of spelosin (V) showed a downfield shift of the C-3 signal, indicating that the arabinosyl moiety is attached at the C-3 hydroxyl. Thus the structure of VI was determined to be spelosin (13-hydroxy-3(*R*)-pterosin D) 3-*O*-α-L-arabinopyranoside.

Compound VIII, $C_{20}H_{28}O_7$ (M^+ 380.184), colorless needles, mp 164—166 °C, $[\alpha]_D^{21}$ +43.9° (*c*=1, MeOH), showed UV and IR spectra characteristic of pterosin-type compounds. The ^{1}H-NMR spectrum of VIII showed signals assignable to geminal dimethyl groups at C-2 [$\delta_{C_5D_5N}$ 1.35 (3H, s) and 1.50 (3H, s)], two aromatic methyl groups [δ 2.29 (3H, s) and 2.82 (3H, s)], one hydroxyethylene group at C-6 [δ 3.10 (2H, t, J=7.5 Hz) and 3.92 (2H, t, J=7.5 Hz)], one carbinyl proton [δ 5.04 (1H, s)] and one aromatic proton [δ 7.76 (1H, s)]. Furthermore, the presence of a sugar moiety was evident from the signals assignable to one anomeric proton [δ 5.00 (1H, d, J=8 Hz)] and five overlapping protons [δ 3.8—4.6 (5H)]. This was corroborated by the IR absorptions at 3390 (br) and 1070 cm^{-1}. Acid hydrolysis of VIII gave 3(*R*)-pterosin D $\{[\theta]_{328}^{20}$ +3500 (MeOH)$\}$[5] (VII) and L-arabinose. The ^{13}C-NMR spectrum of VIII showed signals at $\delta_{C_5D_5N}$ 106.8, 72.6, 74.6, 69.5 and 67.2 characteristic of the α-L-arabinopyranosyl moiety.[6] The large coupling constant (J=8 Hz) of the anomeric proton signal indicated an α-glycosidic linkage of the arabinosyl moiety. The glycosidation shifts of the C-3 signal showed the attachment of the arabinopyranosyl moiety at the C-3 hydroxyl group. Consequently, compound VIII was assigned the structure 3(*R*)-pterosin D 3-*O*-α-L-arabinopyranoside.

Compound XI, $C_{20}H_{28}O_8$ (M^+ 396.178), colorless needles, mp 209—211 °C, $[\alpha]_D^{20}$ −21.7° (*c*=0.46, MeOH), showed UV and IR spectra indicating it to be a pterosin-type sesquiterpene glycoside. In the ^{1}H-NMR spectrum of XI (in C_5D_5N), an anomeric proton signal (δ 5.27, 1H, d, J=7 Hz) and five overlapping proton signals (δ 3.8—4.6) due to a pentosyl moiety were observed along with the following signals: δ 1.53 (3H, s), 2.30 (3H, s), 2.79 (3H, s), 3.08 (2H, t, J=7.5 Hz), 3.91 (2H, t, J=7.5 Hz), 4.18 (1H, d, J=11 Hz), 4.27 (1H, d, J=11 Hz), 5.25 (1H, s), 7.71 (1H, s).

The enzymatic hydrolysis of XI gave 2(*R*),3(*R*)-pterosin L[4] (X) $\{[\theta]_{330}^{20}$ +19900 (MeOH)$\}$, while acid hydrolysis gave L-arabinose. A comparison of the ^{13}C-NMR spectrum of XI with that of 2(*R*),3(*R*)-pterosin L showed a glycosidation shift (5.9 ppm) of the C-3 signal, indicating that the arabinopyranosyl moiety was attached at the C-3 hydroxyl. The ^{13}C-NMR spectrum showed signals at $\delta_{C_5D_5N}$ 104.5, 72.2, 73.9, 68.2 and 66.2, characteristic of the α-arabinopyranosyl moiety.[7] The coupling constant (J=7 Hz) of the anomeric proton signal confirmed the presence of an α-glycosidic linkage. Hence, compound XI was assigned the structure 2(*R*),3(*R*)-pterosin L 3-*O*-α-L-arabinopyranoside.

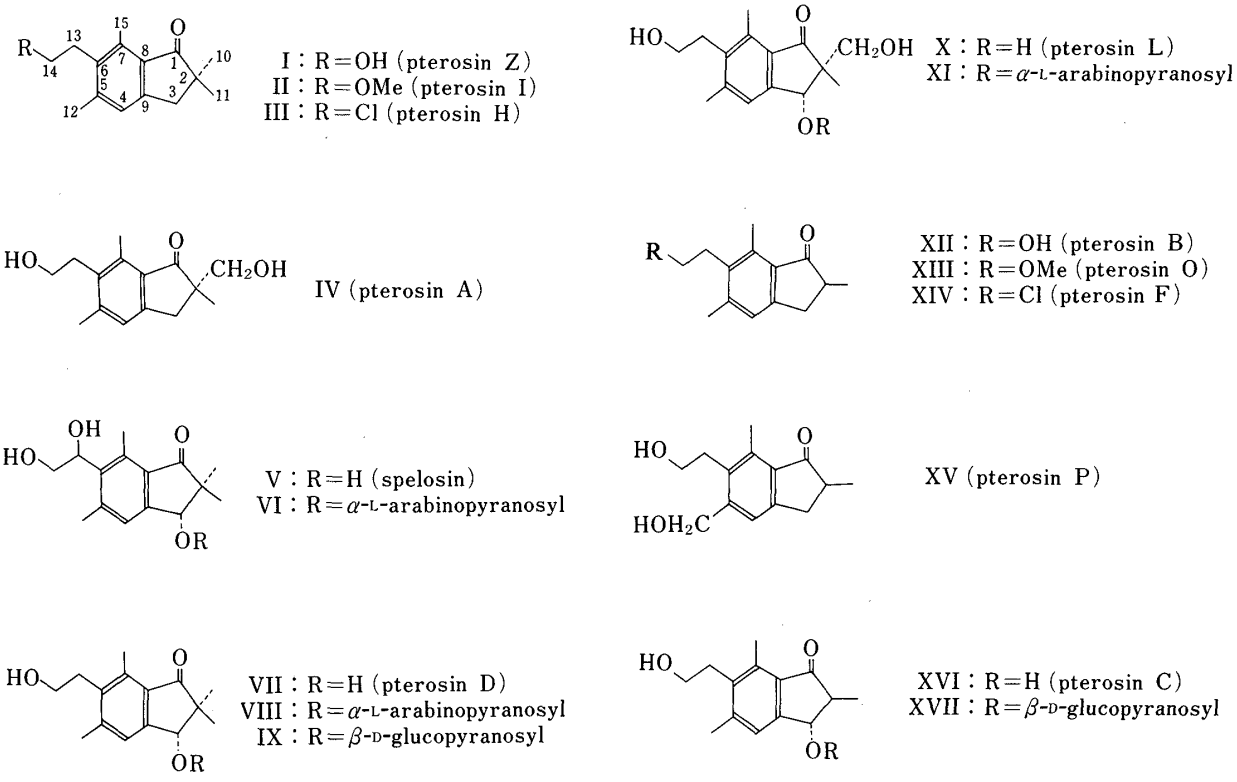

Fig. 1

(2) *Microlepia trapeziformis* (ROXB.) KUHN (Japanese Name: Hikage-ishikaguma)

Pterosin Z (I), H (III), spelosin (13-hydroxy-3(*R*)-pterosin D) 3-*O*-α-L-arabinopyranoside (VI), 2(*R*),3(*R*)-pterosin L 3-*O*-α-L-arabinopyranoside (XI) were isolated and identified.

(3) *Microlepia obtusiloba* HAYATA (Japanese Name: Koosyunshida)

In addition to a new styrene-glycoside,[8] pterosin I (II) and H (III) were isolated and identified.

(4) *Microlepia substrigosa* TAGAWA (Japanese Name: Usuba-ishikaguma)

Pterosins Z (I), H (III), A(IV) and F (XIV), spelosin (13-hydroxy-3(*R*)-pterosin D) 3-*O*-α-L-arabinopyranoside (VI), 3(*R*)-pterosin D 3-*O*-β-D-glucopyranoside (IX)[9] and 2(*S*),3(*S*)-pterosin C 3-*O*-β-D-glucopyranoside (XVII) were isolated and identified.

(5) *Microlepia strigosa* (THUNB.) PRESL (Japanese Name: Ishikaguma)

3(*R*)-pterosin D (VII), 2(*R*),3(*R*)-pterosin L (X), 2(*R*)-pterosin B (XII), 2(*R*)-pterosin O (XIII), 2(*R*)-pterosin F (XIV), 2(*S*)-pterosin P (XV), 2(*S*),3(*S*)-pterosin C (XVI) and 2(*S*),3(*S*)-pterosin C 3-*O*-β-D-glucopyranoside (XVII) were isolated and identified.

In the Pteridaceae species, pterosin 14-*O*-glycosides[4,10] are distributed widely, but 3-*O*-glycosides[9] appear only sporadically. It is remarkable that all glycosides so far isolated from the *Microlepia* species are of the latter type (*cf.* Table I). Further chemotaxonomical considerations regarding the genus *Microlepia* will be presented elsewhere.

Experimental

The instruments, materials and experimental conditions were the same as described in our preceding paper.[1]

Isolation Procedure——(1) *Microlepia speluncae* (L.) MOORE: The air-dried fronds (400 g) of *M. speluncae*, collected in December in Qixingshan, Taiwan, China, were extracted three times with MeOH (2.5 l) under reflux for 8 h each. The combined extracts (7.5 l) and then MeOH (7.5 l) were passed through activated charcoal (50 g) packed in a column of 5.5 cm diameter. The resulting solution (15 l) was concentrated to a syrup under reduced pressure. The

syrup was chromatographed on silica gel (100 g) with $CHCl_3$ (600 ml, frac. 1), 5% MeOH in $CHCl_3$ (600 ml, frac. 2), 10% MeOH in $CHCl_3$ (600 ml, frac. 3) and [20% MeOH in $CHCl_3$ (600 ml) + 30% MeOH in $CHCl_3$ (400 ml), frac. 4] as eluents. Frac. 1 was rechromatographed on alumina with benzene as the eluent to yield pterosin I (II, 40 mg) and pterosin H (III, 45 mg). Frac. 2 was rechromatographed on alumina with $CHCl_3$ (100 ml, frac. 2-1) and 10% MeOH in $CHCl_3$ (150 ml, frac. 2-2) as eluents. Frac. 2-1 was further chromatographed on silica gel (5% MeOH in $CHCl_3$) to give pterosin Z (I, 410 mg). Frac. 2-2 was subjected to preparative layer chromatography (PLC) (solvent system, Et_2O: MeOH = 50 : 1) to yield pterosin D (VII, 8 mg) and pterosin L (X, 6 mg). Frac. 3 was partitioned between the upper and lower layers of a mixture of $CHCl_3$ (80 ml), MeOH (80 ml) and H_2O (60 ml). The upper layer was evaporated under reduced pressure to a syrup, which was chromatographed on silica gel with $CHCl_3$ (300 ml), 7% MeOH in $CHCl_3$ (150 ml, frac. 3-1), 10% MeOH in $CHCl_3$ (150 ml) and 15% MeOH in $CHCl_3$ (150 ml, frac. 3-2). Frac. 3-1 was rechromatographed on alumina with 20% MeOH in $CHCl_3$ as the eluent. Further purification by PLC (solvent, AcOEt) yielded compound V (=spelosin, 28 mg). Frac. 3-2 was rechromatographed on alumina with 50% MeOH in $CHCl_3$ as the eluent. Further purification by PLC (solvent, AcOEt) yielded compound VIII (19 mg). Frac. 4 was distributed between the upper and lower layers of a mixture of $CHCl_3$ (120 ml), MeOH (120 ml) and H_2O (90 ml). The upper layer was concentrated under reduced pressure to a syrup which was subjected to droplet counter current chromatography (DCCC), (solvent system, $CHCl_3$: MeOH : H_2O = 4 : 4 : 3) to yield compounds VI (315 mg), VIII (26 mg) and XI (37 mg).

(2) *Microlepia trapeziformis* (Roxb.) Kuhn: The air-dried fronds (200 g) of *M. trapeziformis*, collected in December in Shanping (Gaoxiong), Taiwan, China, were extracted three times with 1 l of boiling MeOH for 6 h each. The combined extracts (3 l) and then MeOH (6.5 l) were passed through activated charcoal (40 g) packed in a column of 5.5 cm diameter. The resulting solution (9.5 l) was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel (80 g) with $CHCl_3$ (300 ml, frac. 1), 10% MeOH in $CHCl_3$ (300 ml, frac. 2) and 20% MeOH in $CHCl_3$ (400 ml, frac. 3) as eluents. Frac. 1 was rechromatographed on alumina with $CHCl_3$: Et_2O (1 : 1) as the eluent. Further purification by PLC (solvent, $CHCl_3$) yielded pterosin H (III, 4 mg). Frac. 2 was rechromatographed on alumina with 10% MeOH in $CHCl_3$ as the eluent. Further purification by PLC (solvent system, $CHCl_3$: MeOH = 10 : 1) yielded pterosin Z (I, 10 mg). Frac. 3 was subjected to repeated chromatography (silica gel, AcOEt) followed by PLC (solvent system, $CHCl_3$: MeOH = 5 : 1) to give compounds VI (72 mg) and XI (46 mg).

(3) *Microlepia obtusiloba* Hayata: The air-dried fronds (220 g) of *M. obtusiloba*, collected in July in Yakushima, Kagoshima Prefecture, were extracted four times with MeOH (1.5 l) under reflux for 8 h each. The combined extracts (6 l) and then MeOH (6 l) were passed through activated charcoal (25 g) packed in a column of 5.5 cm diameter. The resulting solution (12 l) was chromatographed on silica gel (70 g) with $CHCl_3$ (300 ml, frac. 1), 10% MeOH in $CHCl_3$ (300 ml), 20% MeOH in $CHCl_3$ (300 ml) and 30% MeOH in $CHCl_3$ (300 ml) as eluents. Frac. 1 was rechromatographed on alumina with benzene as the eluent to yield pterosin I (II, 6 mg) and pterosin H (III, 8 mg).

(4) *Microlepia substrigosa* Tagawa: The air-dried fronds (500 g) of *M. substrigosa*, collected in December in Yakushima, Kagoshima Prefecture, were extracted three times with MeOH (1.5 l) under reflux for 7 h each. The combined extracts (4.5 l) and then MeOH (10 l) were passed through activated charcoal (80 g) packed in a column of 7 cm diameter. The resulting solution was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel (100 g) with $CHCl_3$ (700 ml, frac. 1) [10% MeOH in $CHCl_3$ (800 ml) + 30% MeOH in $CHCl_3$ (200 ml), frac. 2], 30% MeOH in $CHCl_3$ (500 ml) and 40% MeOH in $CHCl_3$ (600 ml, frac. 3) as eluents. Frac. 1 was rechromatographed on alumina with *n*-hexane and 10—20% $CHCl_3$ in *n*-hexane as eluents to yield pterosin H (III, 7 mg) and pterosin F (XIV, 9 mg). Frac. 2 was rechromatographed on alumina with $CHCl_3$ and 5% MeOH in $CHCl_3$ as eluents to yield pterosin Z (I, 12 mg) and pterosin A (IV, 9 mg). Frac. 3 was partitioned into the upper and lower layers of a mixture of $CHCl_3$ (80 ml), MeOH (80 ml) and H_2O (60 ml). The upper layer was concentrated under reduced pressure to a syrup, which was chromatographed on silica gel with 10% MeOH in $CHCl_3$ as the eluent. Further purification by PLC (solvent system, $CHCl_3$: MeOH = 3 : 1) yielded pterosin C 3-*O*-glucoside (XVII, 15 mg), pterosin D 3-*O*-glucoside (IX, 20 mg) and compound VI (10 mg).

(5) *Microlepia strigosa* (Thunb.) Presl: The air-dried fronds (2 kg) of *M. strigosa*, collected in Yakushima, Kagoshima Prefecture, were extracted three times with MeOH (6 l) under reflux for 8 h each. The combined extracts (18 l) and then MeOH (10 l) were passed through activated charcoal (100 g) packed in a column of 7 cm diameter. The resulting solution was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel (200 g) with $CHCl_3$ (1 l, frac. 1) 5% MeOH in $CHCl_3$ (1 l, frac. 2), 10% MeOH in $CHCl_3$ (1 l, frac. 3) and 20% MeOH in $CHCl_3$ (1 l, frac. 4). Frac. 1 was further chromatographed on alumina (25 g) with MeOH as the eluent. The resulting solution was concentrated under reduced pressure to a syrup, which was chromatographed on silica gel (30 g) with 80% $CHCl_3$ in benzene as the eluent. Further purification by PLC (solvent system, benzene : $CHCl_3$ = 4 : 1) yielded pterosin O (XIII, 25 mg) and pterosin F (XIV, 30 mg). Frac. 2 was rechromatographed on alumina (25 g) with MeOH as the eluent. The eluate was concentrated under reduced pressure to a syrup, which was chromatographed on silica gel (40 g) with 3% MeOH in $CHCl_3$ (200 ml, frac. 2-1), 5% MeOH in $CHCl_3$ (200 ml, frac. 2-2) and 7% MeOH in $CHCl_3$ (200 ml, frac. 2-3) as eluents. Frac. 2-1 and frac. 2-2 were subjected to PLC (solvent system, $CHCl_3$: MeOH = 9 : 1) to yield pterosin B (XII, 40 mg) and pterosin P (XV, 7 mg), respectively. Frac. 2-3 was subjected to PLC (solvent system, $CHCl_3$: MeOH = 6 : 1) to yield pterosin D (VII, 5 mg) and pterosin C (XVI, 50 mg). Frac. 3 was

rechromatographed on alumina (40 g) with MeOH as the eluent. The resulting solution was concentrated under reduced pressure to a syrup, which was chromatographed on silica gel (70 g) with 10% MeOH in $CHCl_3$ as the eluent. Further purification by PLC (solvent system, MeOH : $CHCl_3$ = 1 : 6) yielded pterosin L (X, 10 mg), Frac. 4 was partitioned into the upper and lower layers of a mixture of $CHCl_3$ (80 ml), MeOH (80 ml) and H_2O (60 ml). The upper layer was concentrated under reduced pressure to a syrup, which was chromatographed on silica gel (70 g) with 20% MeOH in $CHCl_3$ as the eluent to yield pterosin C 3-*O*-glucoside (XVII, 150 mg).

Pterosin Z (I)——Colorless needles from *n*-hexane, mp 89—90 °C. UV λ_{max}^{MeOH} nm (log ε): 217 (4.73), 260 (4.31), 304 (3.34). IR ν_{max}^{KBr} cm^{-1}: 3440, 2965, 2920, 2870, 1675, 1600. ^{1}H-NMR (100 MHz, in C_5D_5N) δ: 1.17 (6H, s), 2.40 (3H, s), 2.75 (2H, s), 2.82 (3H, s), 3.10 (2H, t, *J* = 7.5 Hz), 3.93 (2H, t, *J* = 7.5 Hz), 6.95 (1H, s). MS *m/z*: 232, 217, 201. This product was identical with an authentic sample on direct comparison (gas-liquid chromatography (GLC) and IR).

Pterosin I (II)——Colorless plates from *n*-hexane, mp 59—60 °C. UV λ_{max}^{MeOH} nm (log ε): 218 (4.50), 260 (4.15), 305 (3.39). IR ν_{max}^{KBr} cm^{-1}: 2960, 2925, 2860, 1695, 1600. ^{1}H-NMR (100 MHz, C_5D_5N) δ: 1.17 (6H, s), 2.36 (3H, s), 2.75 (2H, s), 2.79 (3H, s), 2.97 (2H, m), 3.25 (3H, s), 3.44 (2H, m), 6.96 (1H, s). MS *m/z*: 246, 231, 201. This product was identical with an authentic sample on direct comparison (GLC and IR).

Pterosin H (III)——Colorless needles from a mixture of $CHCl_3$ and *n*-hexane, mp 86—87 °C. UV λ_{max}^{MeOH} nm (log ε): 218 (4.43), 260 (4.12), 303 (3.25). IR ν_{max}^{KBr} cm^{-1}: 2950, 2920, 2855, 1690, 1595. ^{1}H-NMR (100 MHz, C_5D_5N) δ: 1.17 (6H, s), 2.32 (3H, s), 2.74 (3H, s), 2.74 (2H, s), 3.13 (2H, m), 3.65 (2H, m), 6.95 (1H, s). MS *m/z*: 252, 250, 237, 235, 215, 201. This product was identical with an authentic sample on direct comparison (GLC, IR and mixed fusion).

Pterosin A (IV)——Colorless amorphous powder, UV λ_{max}^{MeOH} nm (log ε): 217 (4.48), 260 (4.16), 304 (3.30). IR ν_{max}^{KBr} cm^{-1}: 3240, 1700, 1603. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.17 (3H, s), 2.40 (3H, s), 2.62 (3H, s), 2.66 (1H, d, *J* = 18 Hz), 2.95 (2H, t, *J* = 8 Hz), 3.10 (1H, d, *J* = 18 Hz), 3.52 (1H, d, *J* = 11 Hz), 3.68 (2H, t, *J* = 8 Hz), 3.76 (1H, d, *J* = 11 Hz), 7.08 (1H, s). MS *m/z*: 248, 233, 217. This product was identical with an authentic sample on direct comparison (thin layer chromatography (TLC), GLC and ^{1}H-NMR).

Spelosin (= 13-Hydroxy-3(*R*)-pterosin D, V)——Colorless syrup, $[\alpha]_D^{22}$ +83.3° (*c* = 0.7, MeOH). UV λ_{max}^{MeOH} nm (log ε): 220 (4.51), 261 (4.16), 302 (3.44). IR ν_{max}^{KBr} cm^{-1}: 3380, 2970, 2930, 2875, 1695, 1600, 1385, 1210, 1080, 1040, 995, 960, 910. ^{1}H-NMR (100 MHz, C_5D_5N) δ: 1.39 (6H, s), 2.69 (3H, s), 3.12 (3H, s), 4.12 (1H, dd, *J* = 11 and 5 Hz), 4.46 (1H, dd, *J* = 11 and 8 Hz), 5.10 (1H, s), 5.84 (1H, dd, *J* = 8 and 5 Hz), 7.59 (1H, s). MS *m/z*: 264, 233, 215. Calcd for $C_{15}H_{20}O_4$: 264.136 (M), Found 264.137 (M^+). $[\theta]_{328}^{20}$ +15440 (MeOH).

Spelosin 3-*O*-α-L-Arabinopyranoside (VI)——Colorless amorphous powder, $[\alpha]_D^{21}$ +56.9° (*c* = 1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 221 (4.46), 261 (4.11), 303 (3.29). IR ν_{max}^{KBr} cm^{-1}: 3380, 2960, 2930, 2870, 1695, 1600, 1380, 1210, 1140, 1070, 960, 910. ^{1}H-NMR (100 MHz, C_5D_5N) δ: 1.33 (3H, s), 1.49 (3H, s), 2.56 (3H, s), 3.10 (3H, s), 4.08 (1H, dd, *J* = 11 and 5 Hz), 4.42 (1H, dd, *J* = 11 and 8 Hz), 3.8—4.6 (5H), 5.00 (1H, d, *J* = 8 Hz), 5.79 (1H, dd, *J* = 8 and 5 Hz), 7.78 (1H, s). MS *m/z*: 396, 264, 247, 233, 215. Calcd for $C_{20}H_{28}O_8$: 396.178 (M), Found 396.179 (M^+). $[\theta]_{328}^{20}$ +17330 (MeOH).

Enzymatic Hydrolysis of VI——A solution of VI (40 mg) and crude hesperidinase (100 mg, Tanabe Pharm. Co.) in 0.05 M citrate buffer (pH 4.0, 30 ml) was stirred at 38 °C for 4 h. The reaction mixture was extracted with AcOEt. The AcOEt layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was subjected to PLC (solvent system, $CHCl_3$: MeOH = 6 : 1) to yielded spelosin (V, 16 mg), which was shown to be identical with an authentic sample on direct comparison (GLC, IR, ^{1}H-NMR and ^{13}C-NMR).

Acid Hydrolysis of VI——Compound VI (40 mg) was hydrolyzed with 5% HCl (6 ml) at 90 °C for 2.5 h. After cooling, the reaction mixture was neutralized with 5% Na_2CO_3 solution and washed with AcOEt. The water layer was concentrated and the residue was chromatographed on silica gel with 40% MeOH in $CHCl_3$ as the eluent to yield 7 mg of L-arabinose, $[\alpha]_D^{21}$ +86° (*c* = 0.3, H_2O). Its trimethylsilyl ether was identical with an authentic sample (GLC: t_R 8.0, 8.4 and 8.8 min; column temp., 160 °C).

3(*R*)-Pterosin D (VII)——Colorless needles from a mixture of $CHCl_3$ and *n*-hexane, mp 188—190 °C. $[\alpha]_D^{22}$ +5° (*c* = 0.35, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.37), 261 (4.09), 304 (3.11). IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 2920, 2850, 1690, 1600. ^{1}H-NMR (60 MHz, in C_5D_5N): 1.08 (3H, s), 1.25 (3H, s), 2.40 (3H, s), 2.60 (3H, s), 2.95 (2H, t, *J* = 8 Hz), 3.65 (2H, t, *J* = 8 Hz), 4.74 (1H, s), 7.22 (1H, s). MS *m/z*: 248, 233, 217. This product was identical with an authentic sample on direct comparison (GLC, IR, ^{1}H-NMR and mixed fusion).

3(*R*)-Pterosin D 3-*O*-α-L-Arabinopyranoside (VIII)——Colorless needles from a mixture of MeOH and $CHCl_3$, mp 163—165 °C, $[\alpha]_D^{21}$ +43.9° (*c* = 1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 218 (4.55), 260 (4.17), 302 (3.38). IR ν_{max}^{KBr} cm^{-1}: 3390, 2960, 2925, 2860, 1700, 1600, 1345, 1150, 1070, 940, 905. ^{1}H-NMR (100 MHz, in C_5D_5N) δ: 1.35 (3H, s), 1.50 (3H, s), 2.29 (3H, s), 2.82 (3H, s), 3.10 (2H, t, *J* = 7.5 Hz), 3.92 (2H, t, *J* = 7.5 Hz), 3.8—4.6 (5H), 5.00 (1H, d, *J* = 8 Hz), 5.04 (1H, s), 7.76 (1H, s). MS *m/z*: 380, 248, 231, 217. Calcd for $C_{20}H_{28}O_7$: 380.184 (M), Found: 380.184 (M^+). $[\theta]_{328}^{20}$ +3500 (MeOH).

Acid Hydrolysis of VIII——Compound VIII (25 mg) was hydrolyzed with 5% HCl (6 ml) at 90 °C for 3 h. After cooling, the reaction mixture was neutralized with 5% Na_2CO_3 solution and extracted with AcOEt. The AcOEt layer was concentrated and the residue was subjected to PLC (solvent system, $CHCl_3$: MeOH = 6 : 1) to yield pterosin D

(VII, 9 mg), which was shown to be identical with an authentic sample on direct comparison (GLC, IR and mixed fusion). The water layer was concentrated and the residue was chromatographed on silica gel with 40% MeOH in $CHCl_3$ as the eluent to yield 4 mg of L-arabinose, $[\alpha]_D^{22}$ +84° (c=0.15, H_2O). Its trimethylsilyl ether was identical with an authentic sample (GLC: t_R 8.0, 8.4, 8.8 min; column temp., 160 °C).

3(*R*)-Pterosin D 3-*O*-β-D-Glucopyranoside (IX)——Colorless amorphous powder, $[\alpha]_D^{22}$ −20° (c=0.35, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.61), 260 (4.22), 305 (3.39). IR ν_{max}^{KBr} cm^{-1}: 3400, 2910, 2850, 1690, 1600, 1505, 1465, 1380, 1320, 1070, 1030, 985, 900. ^{1}H-NMR (90 MHz, in C_5D_5N) δ: 1.29 (3H, s), 1.44 (3H, s), 2.29 (3H, s), 2.78 (3H, s), 3.07 (2H, t, J=8 Hz), 3.89 (2H, t, J=8 Hz), 3.7—4.7 (6H), 4.99 (1H, s), 5.34 (1H, d, J=8 Hz), 7.76 (1H, s). This product was identical with an authentic sample on direct comparison (TLC, IR and ^{1}H-NMR).

Acid Hydrolysis of IX——Compound IX (15 mg) was hydrolyzed in the same way as described for compound VIII to yield pterosin D (VII, 6 mg) and D-glucose.

2(*R*),3(*R*)-Pterosin L (X)——Colorless needles from a mixture of $CHCl_3$ and n-hexane, mp 132—134 °C, $[\alpha]_D^{21}$ +20° (c=0.25, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.45), 260 (4.11), 302 (3.24). IR ν_{max}^{KBr} cm^{-1}: 3350, 1690, 1600. ^{1}H-NMR (100 MHz, in C_5D_5N) δ: 1.37 (3H, s), 2.42 (3H, s), 2.80 (3H, s), 3.09 (2H, t, J=7.5 Hz), 3.92 (2H, t, J=7.5 Hz), 4.18 (1H, d, J=11 Hz), 4.28 (1H, d, J=11 Hz), 5.18 (1H, s), 7.52 (1H, s). This product was identical with an authentic sample on direct comparison (TLC, GLC and IR).

2(*R*),3(*R*)-Pterosin L 3-*O*-α-L-Arabinopyranoside (XI)——Colorless needles from a mixture of $CHCl_3$ and MeOH, mp 209—211 °C $[\alpha]_D^{20}$ −21.7° (c=0.46, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.51), 261 (4.11), 303 (3.23). IR ν_{max}^{KBr} cm^{-1}: 3520, 3300, 2975, 2880, 1693, 1600, 1385, 1220, 1175, 1010, 980, 945, 910. ^{1}H-NMR (100 MHz, C_5D_5N) δ: 1.53 (3H, s), 2.30 (3H, s), 2.79 (3H, s), 3.08 (2H, t, J=7.5 Hz), 3.91 (2H, t, J=7.5 Hz), 4.18 (1H, d, J=11 Hz), 4.27 (1H, d, J=11 Hz), 3.8—4.6 (5H), 5.25 (1H, s), 5.27 (1H, d, J=7 Hz), 7.71 (1H, s). MS m/z: 396, 275, 248, 217, 185. Calcd for $C_{20}H_{28}O_8$: 396.178 (M), Found: 396.178 (M^+). $[\theta]_{328}^{21}$ +25750 (MeOH).

Enzymatic Hydrolysis of XI——Compound XI (20 mg) was hydrolyzed in the same way as described for compound VI to yield pterosin L (X, 5 mg).

Acid Hydrolysis of XI——Compound XI (15 mg) was hydrolyzed in the same way as described for compound VI to yield L-arabinose.

2(*R*)-Pterosin B (XII)——Colorless prisms from a mixture of $CHCl_3$ and n-hexane, mp 104—106 °C, $[\alpha]_D^{22}$ −46° (c=0.52, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 217 (4.57), 260 (4.21), 303 (3.40). IR ν_{max}^{KBr} cm^{-1}: 3300, 1705, 1670. ^{1}H-NMR (60 MHz, in $CDCl_3$) δ: 1.20 (3H, d, J=7 Hz), 2.42 (3H, s), 2.67 (3H, s), 2.99 (2H, t, J=7 Hz), 3.73 (2H, t, J=7 Hz), 7.01 (1H, s). MS m/z: 218, 103, 187. This product was identical with an authentic sample on direct comparison (GLC, IR and mixed fusion).

2(*R*)-Pterosin O (XIII)——Colorless needles from n-hexane, mp 43—45 °C, $[\alpha]_D^{22}$ −3.2° (c=0.22, benzene). UV λ_{max}^{MeOH} nm (log ε): 217 (4.52), 259 (4.20), 305 (3.42). IR ν_{max}^{KBr} cm^{-1}: 1700, 1603. ^{1}H-NMR (60 MHz, in $CHCl_3$) δ: 1.25 (3H, d, J=7 Hz), 2.40 (3H, s), 2.68 (3H, s), 3.05 (2H, t, J=8 Hz), 3.50 (2H, t, J=8 Hz), 3.33 (3H, s), 2.3—3.5 (3H, m), 7.09 (1H, s). MS m/z: 232, 217, 201. This product was identical with an authentic sample on direct comparison (TLC, GLC and IR).

2(*R*)-Pterosin F (XIV)——Colorless needles from n-hexane, mp 68—70 °C, $[\alpha]_D^{22}$ −8.5° (c=0.53, benzene). UV λ_{max}^{MeOH} nm (log ε): 219 (4.50), 261 (4.20), 304 (3.50). IR ν_{max}^{KBr} cm^{-1}: 2920, 2850, 1695, 1600, 1460, 1110. ^{1}H-NMR (60 MHz, in $CDCl_3$) δ: 1.15 (3H, d, J=7 Hz), 2.40 (3H, s), 2.66 (3H, s), 2.3—3.5 (3H, m), 3.00 (2H, m), 3.50 (2H, m), 7.00 (1H, s). MS m/z: 238, 236, 223, 221, 203, 201, 187. This product was identical with an authentic sample on direct comparison (GLC, IR and MS).

2(*S*)-Pterosin P (XV)——Colorless needles from benzene, mp 121—124 °C, $[\alpha]_D^{20}$ +6.8° (c=0.21, MeOH). UV λ_{max}^{MeOH} nm (log ε): 217 (4.62), 258 (4.30), 304 (3.70). IR ν_{max}^{KBr} cm^{-1}: 3350, 1675, 1600. ^{1}H-NMR (60 MHz, in $CDCl_3$ + CD_3OD) δ: 1.25 (3H, d, J=8 Hz), 2.60 (3H, s), 2.95 (2H, t, J=8 Hz), 3.65 (2H, t, J=8 Hz), 2.3—3.5 (3H, m), 4.65 (2H, s), 7.30 (1H, s). MS m/z: 234, 219, 203. This product was identical with an authentic sample on direct comparison (TLC, IR and mixed fusion).

2(*S*),3(*S*)-Pterosin C (XVI)——Colorless needles from a mixture of $CHCl_3$ and CCl_4, mp 135—137 °C, $[\alpha]_D^{18}$ +102.3° (c=0.75, MeOH). UV λ_{max}^{MeOH} nm (log ε): 218 (4.53), 259 (4.12), 301 (3.23). IR ν_{max}^{KBr} cm^{-1}: 3380, 1700, 1600. ^{1}H-NMR (60 MHz, in $CDCl_3$ + CD_3OD) δ: 1.24 (3H, d, J=8 Hz), 2.3—2.8 (1H, m), 2.35 (3H, s), 2.53 (3H, s), 2.90 (2H, t, J=8 Hz), 3.53 (2H, t, J=8 Hz), 4.57 (1H, d, J=4 Hz), 7.23 (1H, s). MS m/z: 234, 219, 203. This product was identical with an authentic sample on direct comparison (TLC, IR and ^{1}H-NMR).

2(*S*),3(*S*)-Pterosin C 3-*O*-β-D-Glucopyranoside (XVII)——Colorless needles from a mixture of MeOH and H_2O, mp 220—223 °C, $[\alpha]_D^{22}$ +40° (c=0.43, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.75), 260 (4.34), 301 (3.39). IR ν_{max}^{KBr} cm^{-1}: 3450, 1715, 1600, 1080, 1015. ^{1}H-NMR (90 MHz, in C_5D_5N) δ: 1.58 (3H, d, J=7 Hz), 2.27 (3H, s), 2.77 (3H, s), 2.9—3.2 (1H, m), 3.07 (2H, t, J=8 Hz), 3.92 (2H, t, J=8 Hz), 3.8—4.5 (6H), 5.02 (1H, d, J=4 Hz), 5.19 (1H, d, J=8 Hz), 7.69 (1H, s). This product was identical with an authentic sample on direct comparison (TLC, IR, ^{1}H-NMR and mixed fusion).

Acid Hydrolysis of XVII——Compound XVII (15 mg) was hydrolyzed in the same way as described for compound VI to yield D-glucose.

References and Notes

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