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Herbertane-type sesquiterpenoids from the liverwort *Herbertus sakuraii*

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Abstract

Seven herbertane-type sesquiterpenoids, 1,13-dihydroxyherbertene, 1,14-dihydroxyherbertene, 1,15-dihydroxyherbertene, 12-methoxyherebertene-1,2-diol, herberteneacetal, herbertenone A and herbertenone B were isolated from the Japanese liverwort *Herbertus sakuraii*, together with four known herbertane- and three dimeric herbertane-type sesquiterpenoids and *ent*-pimara-8(14),15-dien-19-oic acid. Their structures were elucidated by spectroscopic methods. *H. sakuraii* is chemically similar not only to *H. aduncus* but also to the *Mastigophora* species.

Keywords: *Herbertus sakuraii*; Hepaticae; Liverwort; Herbertane-type sesquiterpenoids; Biogenesis; Chemosystematics

1. Introduction

The Hepaticae contain lipophilic aromatic compounds and terpenoids which can be used as important chemical markers at different taxonomic levels (Asakawa, 1982, 1994, 1995). Furthermore, several compounds isolated from liverwort show interesting biological activities, such as cytotoxic, insect antifeedant, antimicrobial, antifungal, neutotrophic sprouting, piscicidal and muscle relaxing activity, and 5-lipoxygenase, calmodulin, cyclooxygenase, cathepsin B and L inhibitory activity (Asakawa, 1982, 1988, 1990a,b, 1993, 1995, 1997, 1998, 1999; Schwartner et al., 1995).

Liverworts from the genus *Herbertus* contain herbertane-type sesquiterpenoids that can be considered as chemical markers of the genus (Asakawa, 1995; Asakawa et al., 1982; Buchanan et al., 1996; Matsuo et al., 1981, 1982, 1983, 1986).

In this paper we wish to report the structure elucidation of seven new herbertane-type sesquiterpenoids isolated from *H. sakuraii*.

2. Results and discussion

The ether and ethyl acetate extracts of *H. sakuraii* were combined and the resulting mixture was analyzed by TLC and GC-MS to detect the presence of naphthalene, *cis*-calamenene, longifolene and herbertene (**1**), and the previously known α-herbertenol (**2**) and herbertene-1,2-diol (**3**). Further fractionation of the crude extract resulted in the isolation of twelve compounds (**4–14**), including seven new herbertane-type sesquiterpenoids, 1,13-dihydr-oxyherbertene (**4**), 1,14-dihydroxyherbertene (**5**), 1,15-dihydroxyherbertene (**6**), 12-methoxyherbertene-1,2-diol (**7**), herbertenelactol (**8**), herbertenone A (**10**) and herbertenone B (**11**), all of which have been preliminarily described elsewhere (Hashimoto et al., 2000a). Also isolated were the known herbertenolide (**9**) (Wu, 1992), mastigophorene A (**12**), mastigophorene B (**13**), mastigo-phorene C (**14**), *ent*-pimara-8(14)-15-dien-19-oic acid (**15**) (Hashimoto et al., 2000a) and three new macrocyclic bis(bibenzyls), 2,12-dichloroisoplagiochin D, 12,7′-dichloro-isoplagiochin D and 12,10′-dichloro-sioplagiochin D, along with the known isoplagiochin C and isoplagiochin D (Hashimoto et al., 2000a,b). α-Herbertenol (**2**) was the major component comprising a 15% of the total crude extract. The structures of all new herbertane-type sesquiterpenoids were deduced by analysis of their NMR spectra and comparison of their ^{1}H NMR spectral data with those of α-herbertenol (**2**) and herbertene-1,2-diol (**3**) (Matsuo et al., 1986).

2.1. 1,13-Dihydroxyherbertene (4)

Compound **4** was obtained as a colorless oil. The molecular formula, $C_{15}H_{22}O_2$, was established by high resolution mass spectrometry (*m*/*z* $[M]^+$ 234.1636). The

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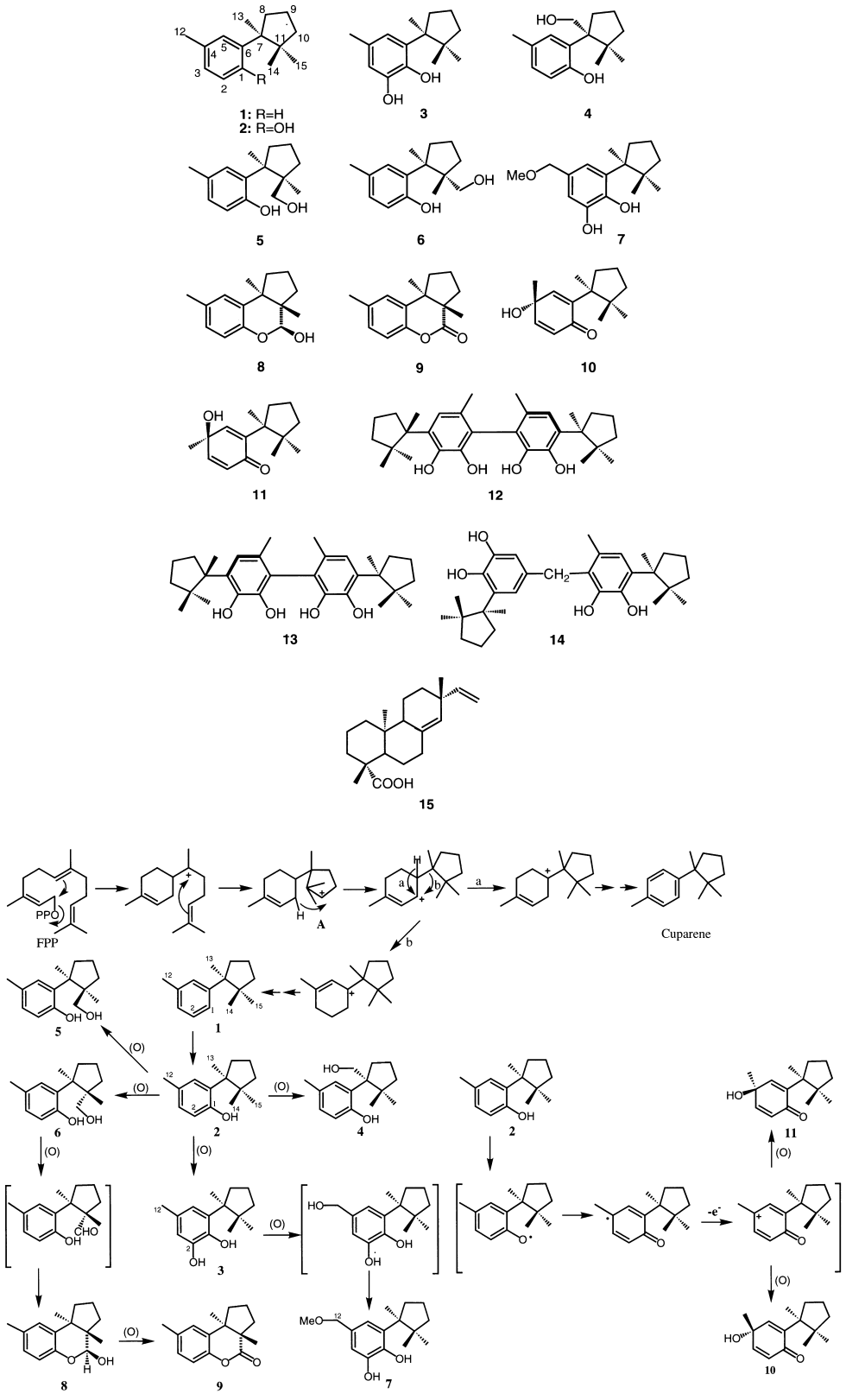

Fig. 1. Possible biogenetic pathways of herbertane-type sesquiterpenoids (**4**–**11**).

presence of a hydroxyl group (3283 cm^{-1}) and a benzene ring (1609 cm^{-1}; 284 nm) was suggested by the IR and UV spectra. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **4** contained signals derived from two tertiary methyl groups, an arylic methyl group and three protons on a 1,2,5-trisubstituted aromatic ring [δ_H 6.69 (*d*, $J=8.2$ Hz), 6.92 (*qdd*, $J=0.6$, 2.1, 8.2 Hz) and 7.02 (*d*, $J=2.1$ Hz)]. The above spectral data were almost identical to α-herbertenol (**2**), except for the presence of a primary alcohol [δ_H 3.78, 4.40, (each *d*, $J=10.7$ Hz, 1H); δ_C 69.0 (*t*)] in place of one tertiary methyl group, indicating that **4** was 13, 14 or 15-hydroxy-α-herbertenol. The location of the hydroxyl group at C-13 was established by the HMBC (Table 3) in which the methylene (H-13) correlated with C-6, C-7, C-8 and C-11 and H-12 with C-3, C-4 and C-5. The NOE spectrum (Table 4) indicated the presence of NOE's between H-13 methylene proton and H-8, and H-15. Thus, the structure **4** was established to be 1,13-dihydroxyherbertene (13-hydroxy-α-herbertenol).

2.2. 1,14-Dihydroxyherbertene (5)

The high resolution mass spectrometry indicated that compound **5** obtained as a colorless oil possessed $C_{15}H_{22}O_2$ (*m/z* $[M]^+$ 234.1660). The IR and UV spectra indicated the presence of a hydroxyl (3214 cm^{-1}) group and a benzene ring (1609 cm^{-1}; 283 nm). The ^1H NMR spectrum (Table 1) was very similar to that of α-herbertenol (**2**) and 1,13-dihydroxyherbertene (**4**), except for the absence of a 14-methyl signal in place of which were two doublets [δ 3.28, 3.37 (each *d*, $J=11.3$ Hz, 1H)] assignable to two protons on a carbon bearing a hydroxyl group, meaning that **5** was 14-hydroxy-α-herberteneol. The location of the hydroxyl group at C-14 was established by the NOE experiments in which the correlation between (i) H-14 and H-15 and (ii) H-13 and H-15 were observed.

2.3. 1,15-Dihydroxyherbertene (6)

The molecular formula, $C_{15}H_{22}O_2$, (*m/z* $[M]^+$ 234.1599) of compound **6** which was obtained as a colorless oil, was established by high resolution mass spectrometry. The ^1H NMR spectrum (Table 1) of **6** resembled that of **2** and was almost identical to that of compound **5**, except for the absence of the C-15 methyl signal in place of a primary alcohol [δ_H 3.50, 4.02 (each *d*, $J=11.0$ Hz, 1H)], indicating that **6** was 15-dihydoxy-α-herbertenol. This was further confirmed by the NOESY spectrum (Table 4) of **6**, in which the NOEs' were observed between (i) H-13 and H-15, (ii) H-14 and H-15. Previously compound **6** was prepared from herbertenolide (**9**) (Matsuo et al., 1986). The spectral data of the natural product (**6**) were identical to **6**.

2.4. 12-Methoxyherbertene-1,2-diol (7)

Compound **7** was obtained as a colorless oil, whose molecular formula, $C_{16}H_{24}O_3$, was established by high resolution mass spectrometry (*m/z* 264.1725 $[M]^+$ required for 264.1726). The IR and UV spectra showed the presence of a hydroxyl group (3430 cm^{-1}) and a benzene ring (1599 cm^{-1}; 285 nm). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) contained signals derived from three tertiary methyl groups, two *meta*-coupled protons [δ_H 6.72 (*d*, $J=1.9$ Hz) and 6.81 (*d*, $J=1.9$ Hz)] on benzene ring, two protons (δ_H 4.34, 2H, *s*; δ_c 75.3, *t*)

Table 1
^1H NMR spectral data for compounds **4**–**8**, **10** and **11** (600 MHz, $CDCl_3$-TMS)

H	4	5	6	7	8	10	11
H-2	6.69 (*d*, 8.2)	6.74 (*d*, 8.0)	6.76 (8.0)		6.72 (*d*, 8.2)	6.03 (*d*, 9.9)	6.03 (*d*, 9.9)
H-3	6.92 (*qdd*, 0.6, 2.1, 8.2)	6.92 (*qdd*, 0.8, 2.1, 8.0)	6.92 (*qdd*, 0.5, 2.2, 8.0)	6.72 (*d*, 1.9)	6.89 (*qdd*, 0.7, 2.2, 8.2)	6.76 (*dd*, 3.0, 9.9)	6.75 (*dd*, 3.0, 9.9)
H-5	7.02 (*d*, 2.1)	6.96 (*d*, 2.1)	1.47–1.75 (*m*)	6.81 (*d*, 1.9)	6.79 (*d*, 2.2)	6.71 (*qd*, 0.5, 3.0)	6.69 (*qd*, 0.3, 3.0)
H-8	2.05 (*m*)	1.84 (*m*)	1.47–1.75 (*m*)	1.73 (*m*)	1.61 (*m*)	1.48–1.74 (*m*)	1.50–1.75 (*m*)
	2.37 (*m*)	2.45 (*m*)	2.68 (*m*)	2.61 (*m*)	2.00 (*m*)	2.21 (*m*)	2.26 (*m*)
H-9	1.82 (*m*)	1.93 (*m*)	1.47–1.75 (*m*)	1.76 (*m*)	1.93 (*m*)	1.48–1.74 (*m*)	1.50–1.75 (*m*)
H-10	1.52 (*m*)	1.27 (*m*)	1.47–1.75 (*m*)	1.53 (*m*)	1.80 (*m*)	1.48–1.74 (*m*)	1.50–1.75 (*m*)
	1.61 (*m*)	1.45 (*m*)		1.65 (*m*)			
H-12	2.27 (*d*, 0.6)	2.27 (*d*, 0.8)	2.27 (*d*, 0.5)	4.34 (*s*)	2.26 (*d*, 0.7)	1.46 (*s*)	1.46 (*s*)
H-13	3.78 (*d*,10.7)	1.56 (*s*)	1.50 (*s*)	1.41 (*s*)	1.12 (*q*, 0.8)	1.25 (*d*, 0.5)	1.28 (*d*, 0.8)
	4.40 (*d*, 10.7)						
H-14	0.86 (*s*)	3.28 (*d*, 11.3)	0.82 (*s*)	0.73 (*s*)	0.72 (*q*, 0.8)	0.72 (*s*)	0.72 (*s*)
		3.37 (*d*, 11.3)					
H-15	1.24 (*s*)	1.23 (*s*)	3.50 (*d*,11.0)	1.17 (*s*)	5.67 (*d*, 7.7)	1.13 (*s*)	1.11 (*s*)
			4.02 (*d*, 11.0)				
OMe				3.37 (*s*)			
OH					3.02 (*d*, 7.7)		

on a carbon bearing ether oxygen atom and a methoxyl group (δ_H 3.37, 3H, *s*). These spectral data were almost identical with those of herbertane-1,2-diol (**3**) except for the presence of a methoxyl signal in place of one arylic methyl, indicating that compound **7** was 12-methoxyherbertene-1,2-diol. This assumption was further confirmed by ^1H–^1H COSY, HMBC (Table 3), HMQC and NOESY spectra (Table 4). The HMBC spectrum showed connectivity between (i) OMe and C-12 and (ii) C-12 and H-3, H-5. In the NOESY spectrum, NOEs' were observed between (i) OMe and H-3, H-5, H-12, (ii) H-13 and H-15 and (iii) H-13 and H-5. Thus the structure of **7** was established to be 12-methoxyherbertene-1,2-diol.

Table 2
^{13}C NMR spectral data for compounds **4**, **7** and **10** (150 MHz, $CDCl_3$-TMS)

C	4	7	10
1	153.1 *s*	143.8 *s*	185.7 *s*
2	117.4 *d*	143.5 *s*	129.3 *d*
3	128.2 *d*	112.7 *d*	148.7 *d*
4	128.7 *s*	127.4 *s*	68.1 *s*
5	130.9 *d*	121.8 *d*	148.0 *d*
6	129.3 *s*	133.1 *s*	142.6 *s*
7	56.7 *s*	51.2 *s*	50.4 *s*
8	36.0 *t*	39.1 *t*	38.7 *t*
9	21.1 *t*	20.3 *t*	20.1 *t*
10	41.7 *t*	41.0 *t*	41.6 *t*
11	45.4 *s*	44.9 *s*	43.7 *s*
12	20.9 *q*	75.3 *t*	27.5 *q*
13	69.0 *t*	22.7 *q*	22.3 *q*
14	26.6 *q*	26.9 *q*	27.5 *q*
15	24.7 *q*	25.5 *q*	25.5 *q*
OMe		57.5 *q*	

Table 3
HMBC correlations for compounds **4**, **7** and **10**

	4	7	10
H-2	C-1,4,6		C-4,6
H-3	C-1,5,12	C-1,5,12	C-1,5
H-5	C-1,3,7,12	C-1,3,7,12	C-1,3,7,12
H-12	C-3,4,5	C-3,4,5,OMe	C-3,4,6
H-13	C-6,7,8,11	C-6,7,8,11	C-6,7,8,11
H-14	C-7,10,11,15	C-7,10,11,15	C-7,10,11,15
H-15	C-7,10,11,14	C-7,10,11,14	C-7,10,11,14
OMe		C-12	

2.5. Herberteneacetal (*8*)

Compound **8** was obtained as a colorless oil. The molecular formula, $C_{15}H_{20}O_2$, was established by high resolution mass spectrometry (*m/z* $[M]^+$ 232.1435). The IR and UV spectra of **8** indicated the presence of a hydroxyl (3451 cm^{-1}) and a benzene ring (281 nm). An additional ether oxygen was suggested to be present since neither the absorption band of ketone nor ester group was observed in the IR spectrum. The ^1H NMR spectra of **8**, which resembled those of herbertenolide (**9**), contained three tertiary methyls, one arylic methyl and three proton signals [δ_H 6.72 (*d, J* = 8.2 Hz), 6.79 (*d, J* = 2.2 Hz), 6.89 (*qdd, J* = 0.7, 2.2, 8.2 Hz)] on a 1,2,5-trisubstituted benzene ring and one doublet proton signal [δ_H 5.67 (*d, J* = 7.7 Hz)] on a carbon bearing a hydroxyl group which collapsed to a broad singlet on addition of D_2O. These spectral data led us to conclude that compound **8** possessed a hemiacetal group derived from herbertenolide (**9**). Conclusive evidence for structure **9** was established by the NOE experiments in which the correlations between (i) H-15 and H-13 and (ii) H-13 and H-5 were observed.

2.6. Herbertenone A (*10*)

Compound **10**, $C_{15}H_{22}O_2$ (*m/z* $[M]^+$ 234.1634 by HR-MS)], which was obtained as a colorless oil, had spectral data similar to those of α-herbertenol (**2**) although it lacked a benzene ring: in place of a tertiary hydroxyl group [3293 cm^{-1}; δ_C 68.1, *s*)], four tertiary methyl group, one (δ_H 1.46, *s*, 3H) of which was bonded on a carbon bearing a hydroxyl group (3293 cm^{-1}) and a cross conjugated enone system [1667 cm^{-1}; 226 nm (log ε) 4.05], together with a disubstituted olefin [δ_H 6.03 (*d, J* = 9.9 Hz), 6.76 (*dd, J* = 3.0, 9.9 Hz)] and a trisubstituted olefin [δ_H 6.71 (*qd, J* = 0.5, 3.0 Hz)]. The ^1H–^1H COSY, HMQC and HMBC spectra (Table 3) gave a herbertane skeleton for **10** and the position of its substituents was confirmed. The relative stereochemistry was confirmed by analysis of the NOESY spectrum

Table 4
NOESY correlations for compounds **4**–**7**, **8**, **10** and **11**

H	4	5	6	7	8	10	11
H-2	H-3	H-3	H-3		H-3	H-3	H-3
H-3	H-2,12	H-2,12	H-2,12	H-12,OMe	H-2,12	H-2,12	H-2,12
H-5	H-8,12,14,15	H-8,12	H-8,12,14	H-8,12,13,14,15,OMe	H-8,12,13	H-8,12	H-8,12
H-12	H-3,5	H-3,5	H-3,5	H-3,5,OMe	H-3,5	H-3,5,14	H-3,5
H-13	H-8,15	H-15	H-14,15	H-5,14,15	H-5,15	H-15	H-15
H-14	H-5,8,15	H-15	H-5,8,13,15	H-5,8,13,15	H-8	H-8,12,15	H-8,15
H-15	H5,8,13,14	H-13,14	H-8,13,14	H-5,8,13,14	H-13	H-13,14	H-13,14

(Table 4). The tertiary methyl at C-4 had correlations with H-3, H-4 and H-5. Furthermore, NOEs' were observed between (i) H-5 and H-8, H-12, (ii) H-13 and H-15, (iii) H-14 and H-15 and (iv) H-12 and H-14. On the basis of the above spectral data, the structure of herbertenone A was determined to be (4*S**)-2-(1,2,2-trimethylcyclopentyl)-4-hydroxy-4-methycylohexa-2,5-diene (**10**).

2.7. Herbertenone B (*11*)

Compound **11** was obtained as a colorless oil and possesed the molecular formula, $C_{15}H_{22}O_2$ (*m*/*z* $[M]^+$ 234.1617 by HR-MS). The UV, IR, ^1H and ^{13}C NMR spectra (Table 1) resembled those of **10**, suggesting that **11** was a C-4 stereoisomer of **10**. This was confirmed by the NOESY spectrum in which correlations between H-12 and H-3, H-5 were observed; however, NOE's between H-12 and H-14 were not seen, indicating that the structure of **11** was the C-4 epimer of herbertenone A (**10**), (4*R**)-2-(1,2,2-trimethylcyclopentyl)-4-hydroxy-4-methycylohexa-2,5-diene. Since compounds **10** and **11** should possess the same S-configuration at C-7 as that of the known co-occurring herbertane sesquiterpenoids (**1**–**3**) (Matsuo et al., 1981, 1982, 1983, 1986; Fukuyama et al., 1988; Fukuyama and Asakawa, 1991), **10** and **11** should have 4*S* and 4*R* configurations, respectively.

The proposed biogenetic pathways for herbertane-type sesquiterpenoids isolated from *H. sakuraii* are shown in Fig. 1: it is envisaged that a hydride shift on the cuparene cation (A), which might be derived from farnesyl phyrophosphate, followed by deprotonation and oxidation, gives cuparene (Scheme 1a). On the other hand, the migration of the cyclopentyl group to the allylic cation, followed by deprotonation and oxydation affords herbertene (**1**) which is further oxidized to furnish α-herbertenol (**2**). The C-13, C-14 and C-15 of (**2**) which has been obtained as the major component might be oxidized to give 13- (**4**), 14- (**5**) and 15-hydroxy-α-herbertenol (**8**), respectively. The hydroxyl group at C-15 of **6** can also be oxidized to afford an aldehyde, followed by acetal formation to afford the hemiacetal (**8**), which might be oxidized to furnish the lactone (**9**). Oxidation of α-herbertenol (**2**) gives herbertene-1,2-diol (**3**) whose arylic methyl group is further oxidized to afford a 12-hydroxy compound, followed by methylation to yield 12-methoxyherbertene-1,2-diol (**3**). For formation of compounds **10** and **11**, we suggest a radical reaction from α-herbertenol (**2**) with subsequent isomerization, where one electron is eliminated, and finally an hydroxyl group is introduced to furnish herbertenone A (**10**) and B (**11**), respectively.

Mastigophorenes A (**12**) and B (**13**) having neurotrophic activity were isolated from the liverwort *Mastigophora diclados* collected in Borneo, together with mastigophorene C (**14**) (Fukuyama et al., 1988; Fukuyama and Asakawa, 1991). This is the first example for the isolation of hebertane-dimers from the Herbertaceae. *M. diclados* produces not only herbertane dimers, but also herbertane monomers and pimarane-type diterpenoids and the same cyclic bis(bibenzyls) as those isolated from *H. sakuraii* (Hashimoto et al., 2000a,b). Thus *H. sakuraii* is closely related chemically to the *Mastigophora* species although two genera are classified into two different families.

3. Experimental

3.1. General

TLC was carried out on silica gel precoated glass plates (Kiesel gel 60 F_{254}, Merck) with *n*-hexane–EtOAc (1:1, 2:1 and 4:1) and CH_2Cl_2–EtOAc (2:1 and 3:1). Detection was with the Godin reagent. For normal phase CC, silica gel 60 (70–230 μm, Merck) and silica gel C-300 (230–400 μm, Wako) were used. A mixture of $CHCl_3$–MeOH (1:1) was used as solvent for CC on Sephadex LH-20. UV and CD spectra were measured in EtOH and $[\alpha]_D$ measurements used MeOH or $CHCl_3$. The ^1H and ^{13}C NMR spectra were obtained with a Varian Unity 200 (200 MHz) or a Varian Unity 600 (600 MHz) spectrometer at room temperature in $CDCl_3$ solution unless otherwise stated. All chemical shifts were reported as δ values (ppm), relative to residual $CDCl_3$ δ_H (7.26 ppm), $CDCl_3$ δ_c (77.0 ppm) and CD_3OD δ_c (49.3 ppm) as internal standards, respectively. Mass spectra were measured at 70 eV. The temp. programming of GC–MS analysis was performed from 80°C, then 80–250°C at 15°C min^{-1} and finally isothermal at 250°C for 13 min. Injection temp. was 260°C. A fused silica column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm) using He as carrier gas (1 ml min^{-1}).

3.2. Plant materials

Herbertus sakuraii (No. H9604002) was collected in Toyo-cho, Kochi, Japan, in April 1996 and identified by Y.A. and confirmed by Dr. M. Mizutani. The voucher specimen was deposited at the Herbarium of Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

3.3. Separation and isolation

The powdered material (259.1 g) was extracted with Et_2O for 1 week, followed by MeOH for 1 month, at room temp. Each extract was filtered and the solvents were evaporated in vacuo to give green oils. The latter extract was partitioned between EtOAc and *n*-BuOH. The EtOAc extract was combined with the Et_2O extract to obtain a viscous oil a small part of which was analyzed

by GC–MS to detect herbertene (**1**), naphthalene, longifolene, cis-calamenene. The remaining oil (9.32 g) was subjected to silica gel chromatography using a *n*-hexane–EtOAc gradient to afford six fractions. Fr. 1 (148.8 mg), Fr. 2 (2.78 g), Fr. 3 (672.7 mg), Fr. 4 (89.9 mg), Fr. 5 (91.8 mg) and Fr. 6 (673.2 mg).

Fr. 2 was applied to a Sephadex LH-20 column and then the silica gel chromatography using *n*-hexane–EtOAc gradient to give α-herbertenol (**2**) (1.40 g) and herbertenolide (**9**) (18.1 mg). Fr. 3 was further subjected to silica gel chromatography (*n*-hexane–EtOAc gradient) and silica gel (CH_2Cl_2–EtOAc gradient), with each fraction of interest purified by HPLC (*n*-hexane–EtOAc 1:1) and Diol HPLC (*n*-hexane–EtOAc 9:1) to afford mastigophorene A (**12**) (2.7 mg), mastigophorene B (**13**) (5.3 mg), *ent*-pimara-8(14),15-dime-19-oic acid (**15**) (119 mg), herbertene-1,2-diol (**3**) (235 mg) and herbertenelactol (**8**) (2.3 mg). Fr. 4 was applied to a Sephadex LH-20 column, with each fraction of interest purified by Diol HPLC (*n*-hexane–EtOAc 7:1 and 9:1) to furnish 1,13-dihydroxyherbertene (**4**) (3.0 mg), 1,15-dihydroxyherbertene (**6**) (3.3 mg), 1,14-dihydroxyherbertene (5.6 mg) (**5**), herbertenone A (**10**) (1.7 mg) and herbertenone B (**11**) (0.6 mg). Fr. 5 was subjected to Sephadex LH-20 and then Diol HPLC (*n*-hexane–EtOAc 7:3) to give 12-methoxyherbertene-1,2-diol (**7**) (92 mg). Fr. 6 was applied to a Sephadex LH-20 column, with each fraction of interest purified by HPLC ($CHCl_3$–EtOAc: 3:2, 7:3, 97:3) and Diol-HPLC (*n*-hexane–EtOAc 1:1, 7:3) to afford five cyclic bis(bibenzy) derivatives, isoplagiochin C (11.3 mg), isoplagiochin D (91.8 mg), 2,12-dichloroisoplagiochin D (16.8 mg), 12,7′-dichloroisoplagiochin D (7.9 mg) and 12,10′-dichloroisoplagiochin C (11.2 mg) whose relative configurations were previously reported (Hashimoto et al., 2000a,b).

3.4. 13-Hydroxy-α-herbertenol (*4*)

Colourless oil; $[\alpha]_D^{20}$ −26.0° ($CHCl_3$, *c* 0.18,); HR–MS: *m/z* 234.1636 (required for $C_{15}H_{22}O_2$, 234.1619); EI–MS *m/z* (rel. int.): 234 $[M]^+$ (62), 216 (13), 203 (52), 187 (6), 173 (100), 159 (33), 147 (48), 135 (29), 121 (83), 105 (8), 95 (21), 77 (9), 69 (30), 55 (7), 41 (9), 32 (4); FT–IR ν_{max} (KBr) cm^{-1}: 3283 (OH), 2959, 1609, 1252, 1020; UV (EtOH) λ_{max} nm (log ε): 202 (4.43), 284 (3.26); 600 MHz ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data: see Tables 1 and 2.

3.5. 14-Hydroxy-α-herbertenol (*5*)

Colourless oil; $[\alpha]_D^{21}$ +33.5° ($CHCl_3$, *c* 0.19); HR–MS: *m/z* 234.1660 (required for $C_{15}H_{22}O_2$, 234.1619); EI–MS: *m/z* (rel. int.): 234 $[M]^+$(38) 216 (10), 201 (21), 187 (5), 173 (12), 161 (37), 135 (100), 121 (21), 105 (10), 91 (10), 77 (6), 41 (6), 32 (14); FT-IR ν_{max} (KBr) cm^{-1}: 3214 (OH), 2963, 1609, 1231, 1024; UV (EtOH) λ_{max} nm (log ε): 202 (4.53), 220 (3.74), 283 (3.31); ^1H NMR (600 MHz) spectral data: see Table 1.

3.6. 15-Hydroxy-α-herbertenol (*6*)

Colourless oil; $[\alpha]_D^{21}$ −66.9° ($CHCl_3$, *c* 0.24); HR–MS: *m/z* 234.1599 (required for $C_{15}H_{22}O_2$, 234.1619); EI–MS *m/z* (rel. int.): 234 $[M]^+$ (42), 216 (15), 201 (35), 187 (5), 173 (15), 161 (38), 135 (100), 121 (23), 105 (10), 99 (5), 83 (12), 77 (6), 55 (5), 32 (10); FT–IR ν_{max} (KBr) cm^{-1}: 3279(OH), 2957, 1609, 1231, 1044; UV (EtOH) λ_{max} nm (log ε): 203 (4.46), 221 (3.71), 281 (3.27); ^1H NMR (600 MHz) spectral data: see Table 1.

3.7. 12-Methoxyherbertenediol (*7*)

Colourless oil; $[\alpha]_D^{21}$ −43.2° ($CHCl_3$, *c* 0.44); HR–MS: *m/z* 264.1725 (required for $C_{16}H_{24}O_3$, 264.1726); EI–MS *m/z* (rel. int.): 264 ($[M]^+$ (99), 232 (15), 194 (41), 181 (100), 163 (67), 150 (56), 137 (39), 123 (10), 91 (10), 69 (11), 55 (11), 45 (37), 32 (17); FT–IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 3117(OH), 2953, 1599, 1292, 1242, 1073; UV (EtOH) λ_{max} nm (log ε): 205 (4.58), 285 (3.40); ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data: see Tables 1 and 2.

3.8. Herberteneacetal (*8*)

Colourless oil; $[\alpha]_D^{20}$ +19.0° ($CHCl_3$, *c* 0.14); HR–MS: *m/z* 232.1435 (required for $C_{15}H_{20}O_2$, 232.1463); EI–MS *m/z* (rel. int.): 232 $[M]^+$ (39), 199 (5), 161 (100), 148 (19), 121 (9), 91 (6), 77 (5), 55 (3), 41 (4), 32 (11); FT–IR ν_{max} (KBr) cm^{-1}: 3451 (OH), 2963, 1493, 1219, 1161; UV (EtOH) λ_{max} nm (log ε): 203 (4.28), 228 (3.68), 281 (3.21); ^1H NMR spectral data: see Table 1.

3.9. Herbertenone A (*10*)

Colourless oil; $[\alpha]_D^{20}$ −11.9° ($CHCl_3$, *c* 0.68); HR–MS: *m/z* 234.1634 (required for $C_{15}H_{22}O_2$, 234.1620); EI–MS *m/z* (rel. int.): 234 $[M]^+$ (36), 219 (58), 191 (100), 165 (82), 151 (66), 135 (93), 123 (76), 109 (60), 95 (42), 77 (23), 69 (63), 55 (33), 43 (43); FT–IR ν_{max} (KBr) cm^{-1}: 3293 (OH), 1667 (C=O), 1628 (C=C), 1140, 1057; UV (EtOH) λ_{max} nm (log ε): 226(4.05); ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data: see Tables 1 and 2.

3.10. Herbertenone B (*11*)

Colourless oil; $[\alpha]_D^{20}$ −3.9° ($CHCl_3$ *c* 0.41); HR–MS: *m/z* 234.1617 (required for $C_{15}H_{22}O_2$, 234.1620); EI–MS *m/z* (rel. int.): 234 $[M]^+$ (24), 219 (47), 191 (100), 165 (66), 151 (52), 135 (91), 123 (71), 109 (55), 95 (44), 77 (22), 69 (64), 55 (33), 43 (47); FT–IR ν_{max} (KBr) cm^{-1}: 3380 (OH), 1667(C=O), 1630 (C=C), 1128, 1057; UV (EtOH) λ_{max} nm (log ε): 230(3.95); ^1H NMR (600 MHz) spectral data: see Table 1.

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