Pergamon

Tetrahedron 56 (2000) 3153–3159

TETRAHEDRON

New Chlorinated Cyclic Bis(bibenzyls) from the Liverworts *Herbertus sakuraii* and *Mastigophora diclados*

Toshihiro Hashimoto, Hiroshi Irita, Shigeru Takaoka, Masami Tanaka and Yoshinori Asakawa*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-1854, Japan

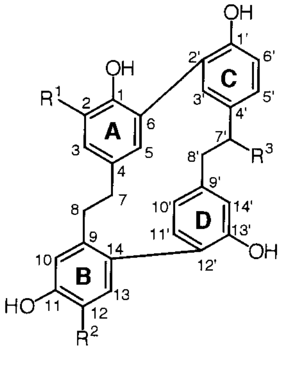
Received 16 February 2000; accepted 21 March 2000

Abstract—Three new cyclic bis(bibenzyls), 2,12-dichloroisoplagiochin D, 12,7′-dichloroisoplagiochin D and 12,10′-dichloroisoplagiochin C have been isolated from the liverwort *Herbertus sakuraii* belonging to the Herbertaceae, together with isoplagiochin C and isoplagiochin D which have been isolated from the liverwort *Plagiochila fruticosa.* Their structures have been elucidated by a combination of 2D-NMR, CD spectra, X-ray crystallographic analysis and comparison of the spectral data with those of the known isoplagiochins. 2,12-Dichloroisoplagiochin D and 12-chloroisoplagiochin D have also been isolated from the liverwort *Mastigophola diclados*, belonging to the Mastigophoroideae. *H. sakuraii* is chemically closely related to *Mastigophora diclados*. © 2000 Elsevier Science Ltd. All rights reserved.

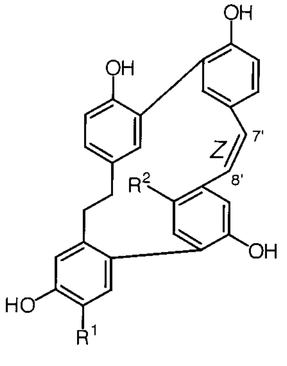
Introduction

We are continuing to study the chemical constituents of bryophytes, particularly liverworts (Hepaticae) from the viewpoint of structural characterization and biological activity of the isolated compounds and their chemosystematics.[1–13] At present 700 terpenoids and 250 aromatic compounds have been found in the bryophytes.[1–10]

The *Herbertus* S. Gray and *Mastigophora* Nees are morphologically very similar and relatively primitive liverworts. The former group is classified to the Herbertaceae Fulf. et Hatch. and the latter to the subfamily Matigophoroideae (Nees) Grolle, the family Lepicoleaceae Schus. Only two *Herbertus* species, *H. aduncus* (Dicks.) S. Gray and *H. sakuraii* (Warnst.) Hatt. and only one species of *M. diclados* (Brid.) Nees are known in Japan. Both *Herbertus* and *Mastigophora* species produce herbertane-type sesquiterpenoids such as herbertene (**9**) and α-herbertenol (**10**) which are the most important chemical markers of these genera.[2,14–19] During the course of the investigation of the chemical constituents of *H. sakuraii* and *M. diclados*, we found that the former species produced three new chlorinated cyclic bis(bibenzyls), 2,12-dichloroisoplagiochin D (**1**), 12,7′-dichloroisoplagiochin D (**2**) and 12,10′-dichloroisoplagiochin C (**5**), together with isoplagiochin D (**4**) and isoplagiochin C (**6**); the latter species elaborated 2,12-dichloroisoplagiochin D (**1**) and 12-chloroisoplagiochin D (**3**), together with the known sesquiterpenoids herbertene (**9**) and α-herbertenol (**10**).[2,14] Here we wish to report the isolation and structure elucidation of the three new cyclic bis(bibenzyls), and the chemosystematics of each liverwort.



1: R^1=R^2=Cl, R^3=H
2: R^1=H, R^2=R^3=Cl
3: R^1=R^3=H, R^2=Cl
4: R^1=R^2=R^3=H



5: R^1=R^2=Cl
6: R^1=R^2=H

Keywords: biaryl; phenolics; terpenes and terpenoids; X-ray crystal structures.

* Corresponding author. Tel.: +81-88-622-9611; fax: +81-88-655-3051; e-mail: asakawa@ph.bunri-u.ac.jp

PII: S0040-4020(00)00236-2

Table 1. ^{1}H NMR spectral data for compounds **1**, **2** and **5** (600 MHz)

H	**1** *J*(Hz)[a]	**2** *J*(Hz)[b]	**5** *J*(Hz)[b]
2		6.74 d (8.2)	6.78 d (8.2)
3	7.08 d (2.2)	6.98 dd (8.2, 2.2)	7.01 dd (8.2, 2.2)
5	6.28 d (2.2)	6.32 d (2.2)	6.52 d (92.2)
7	2.47 m	2.53 m	2.59 m
	2.64 m	2.63 m	
8	2.64 m	2.63 m	2.59 m
	2.69 m	2.78 m	2.72 m
10	6.89 s	7.06 s	7.05 s
13	7.04 s	7.24 s	7.27 s
3′	6.40 d (2.2)	6.45 d (2.2)	7.15 d (2.2)
5′	7.12 dd (8.2, 2.2)	7.11 dd (8.2, 2.2)	7.19 dd (8.2, 2.2)
6′	6.84 d (8.2)	6.87 d (8.2)	6.92 d (8.2)
7′	2.96 m	3.00 t (5.9)	6.69 d (12.1)
	3.02 m		
8′	2.76 m	2.84 m	6.56 d (12.1)
	2,90 m	2.94 m	
10′	6.72 d (7.4)	6.73 d (7.7)	
11′	7.02 d (7.4)	7.08 d (7.7)	7.21 s
14′	6.63 br s	6.81 br s	6.97 s

[a] Measured in CD_3OD.
[b] Measured in $CDCl_3$.

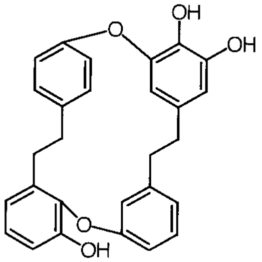
Results and Discussion

The dried *H. sakuraii* was extracted with ether and then methanol. The latter extract was partitioned between ethyl acetate and *n*-butanol and the ethyl acetate extract was combined with the ether extract to give a green oil which was chromatographed on silica gel. Each fraction was purified by HPLC to give an optically inactive new cyclic bis(bibenzyl), 2,12-dichloroisoplagiochin D (**1**) and two new optically active 12,7′-dichloroisoplagiochin D (**2**) and 12,10′-dichloroisoplagiochin C (**5**), together with two known related compounds, isoplagiochin D (**4**) and isoplagiochin C (**6**)[20] which possess optically active forms. *M. diclados* was extracted with *n*-hexane, ether and methanol, successively. *n*-Hexane and ether extract were combined and then chromatographed on silica gel to give herbertene (**9**)[2,14] and α-herbertenol (**10**).[2,14,18,19] The methanol extract was chromatographed on Sephadex LH-20 to afford 2,12-dichloroisoplagiochin D (**1**) and 12-chloroisoplagiochin D (**3**)[21] as optically inactive forms, along with α-herbertenol (**10**).

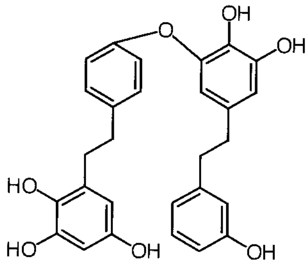
Table 2. ^{13}C NMR spectral data for compounds **1**, **2** and **5** (150 MHz)

C	**1**[a]	**2**[b]	**5**[b]
1	148.7	150.1	150.0
2	122.5	115.9	116.1
3	128.7	127.9	128.2
4	137.2	135.5	135.6
5	133.1	134.1	133.8
6	130.6	126.5	126.3
7	38.3	37.7[c]	37.4[c]
8	39.0	37.8[c]	37.5[c]
9	143.2	143.5	143.2
10	117.7	117.7	117.2
11	153.3	151.5	151.8
12	118.7	117.9	118.0
13	132.9	131.5	131.0
14	132.1	128.6	127.4
1′	152.2	150.4	152.0
2′	125.7	124.7	125.5
3′	134.8	133.8	132.8
4′	134.8	134.1	129.5
5′	130.4	129.2	130.8
6′	116.7	116.7	117.2
7′	37.0	35.9	131.3
8′	39.3	38.1	125.6
9′	143.8	143.4	138.5
10′	122.1	122.4	124.8
11′	132.5	130.6	131.6
12′	126.9	124.2	127.1
13′	155.7	152.9	151.9
14′	118.1	116.5	117.3

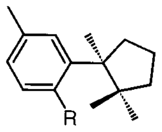
[a] Measured in CD_3OD.
[b] Measured in $CDCl_3$.
[c] Values may be interchangeable.



7



8



9: R=H
10: R=OH

2,12-Dichloroisoplagiochin D (1)

Compound **1** was obtained as colorless prisms, mp 172–175°C (140–143°C). The molecular formula [*m/z* found 492.0904; calcd for $C_{28}H_{22}O_4Cl_2$ 492.0895] was established by high resolution mass spectrometry. The IR and UV spectra showed the presence of a hydroxyl group (3329 cm^{-1}) and a benzene ring (288 nm). The ^{1}H and ^{13}C NMR spectra contained four benzyl methylene signals [δ_H 2.47–2.69, 8H; δ_C 38.3, 39.0, 37.0, 39.3 (each *t*)], four protons [δ_H 6.28 (d, J=2.2 Hz), 6.89 (s), 7.04 (s) and 7.08 (d, J=2.2 Hz)] on two tetrasubstituted benzene rings and six protons [δ_H 6.40 (d, J=2.2 Hz), 6.63 (br s), 6.72 (d, J=7.4 Hz), 6.84 (d, J=8.2 Hz), 7.02 (d, J=7.4 Hz) and 7.12 (dd, J=2.2, 8.2 Hz)] on two trisubstituted benzene rings. The ^{1}H and ^{13}C NMR spectra (see Tables 1 and 2) of **1** were similar to those of isoplagiochin D (**4**) and almost identical to those of 12-chloroisoplagiochin (**3**),[21] except for the presence of

Table 3. HMBC and NOE correlations of compound **1** (CD_3OD)

	HMBC	NOE
H-3	C-1, 2, 5, 7	H-7, 8
H-5	C-1, 2, 3, 7, 2′	H-7, 8, 3′, 10′, 11′, 14′
H-7	C-3, 4, 5, 8, 9	H-3, 5, 10
H-8	C-4, 7, 9, 10, 14	H-3, 5, 10
H-10	C-8, 11, 12, 13, 14	H-7, 8
H-13	C-9, 11, 12, 12′	H-11′
H-3′	C-6, 1′, 5′, 7′	H-5′, 7′, 10′, 14′
H-5′	C-1′, 3′, 7′	H-7′
H-6′	C-1′, 2′, 4′	H-5′
H-7′	C-3′, 5′, 8′, 9′	H-3′, 5′, 14′
H-8′	C-4′, 7′, 9′, 10′	H-10′, 14′
H-10′	C-8′, 12, 14′	H-5, 3′, 8′, 14′
H-11′	C-14, 9′, 13′	H-5, 13
H-14′	C-8′, 10′, 12′, 13′	H-5, 3′, 7′, 8′, 10′

the different chemical shifts of H-3, C-1 and C-2 in the A-ring, indicating that **1** might be 12-chloroisoplagiochin-type cyclic bis(bibenzyl) with one chlorine atom at C-2 in the A-ring. This assumption was confirmed by HMBC spectra (Table 3) in which H-5 was correlated with C-1, C-3, C-7 and C-2′ carbons and H-13 with C-9, C-11 and C-12′. In addition, the NOE experiment (Table 3) showed the NOE's between (i) H-3 and H-7, H-8, (ii) H-5 and H-7, H-3′, H-10′ and (iii) H-13 and H-11′. On the basis of the above spectral data, the structure of compound **1** was suggested to be 2,12-dichloroisoplagiochin D. The conclusive evidence for the structure of **1** was established by X-ray crystallographic analysis. The ORTEP drawing (Fig. 1) shows that **1** is present as a chlathrate compound formed by two molecules of compound **1** and dichloromethane as the solvent for recrystallization to give the racemic crystal. Furthermore, the compound **1** has neither specific rotation nor Cotton effect of CD spectrum, indicating that **1** is present in racemic form in the solution condition. Compound **1** displayed double melting points: colorless crystals melted at 140–143° to give a white solid which melted at 172–175°.

12,7′-Dichloroisoplagiochin D (2)

Compound **2** was obtained as a colorless oil whose molecular formula, $C_{28}H_{22}O_4Cl_2$, was established by high resolution mass spectrometry (*m/z* found: 492.0923; calcd 492.0895). The UV and IR spectra revealed the absorption bands attributable to a hydroxyl group (3262 cm^{-1}) and a benzene ring (288 nm). The ^{1}H NMR spectrum indicated the presence of two protons [δ_H 7.06 (s) and 7.24 (s)] on one tetrasubstituted benzene ring and nine protons [δ_H 6.32 (d, J=2.2 Hz), 6.45 (d, J=2.2 Hz), 6.73 (d, J=7.7 Hz), 6.74 (d, J=8.2 Hz), 6.81 (br s), 6.87 (d, J=8.2 Hz), 6.98 (dd, J=2.2, 8.2 Hz), 7.08 (d, J=7.7 Hz) and 7.11 (dd, J=2.2, 8.2 Hz)] on three trisubstituted benzene rings, three benzylic methylene protons (δ_H 2.53–2.94, 6H), and a benzylic methine proton [δ_H 3.00 (*t*, J=5.9 Hz, 1H] which were also confirmed by ^{13}C NMR spectral data. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) resembled those of 12-chloroisoplagiochin D (**3**), except for the presence of the different chemical shifts of the benzylic methine proton (H-7′, δ_H 3.00 *t*, J=5.9 Hz), suggesting that compound **2** might be 12,7′-dichloroisoplagiochin D. Further evidence for the structure of **2** was obtained from the HMBC and NOE spectra (Table 4). The HMBC showed that H-5 correlated with C-1 and C-2′ and H-13 with C-9, C-11 and C-12′, and H-7′ methine proton with C-3′, C-5′ and C-9′, respectively. The NOE experiment showed the presence of NOE's between (i) H-5 and H-7, H-3′ and H-10′, and (ii) H-13 and H-11′ (iii) H-7′ and H-3′, H-5′, H-10′ and H-14′. These spectral data led us to conclude that compound **2** was 12,7′-dichloroisoplagiochin D. Compound **2** is present in the optically active form since it showed the negative specific optical rotation ($[\alpha]_D=-2.7°$) and the first positive, the second negative and the third positive Cotton effects at $\Delta\epsilon$ 300, 281 and 234 nm in CD spectrum, respectively. On the basis of the above evidence, compound **2** was estimated to be an optically active cyclic bis(bibenzyl) possessing planar chirality.

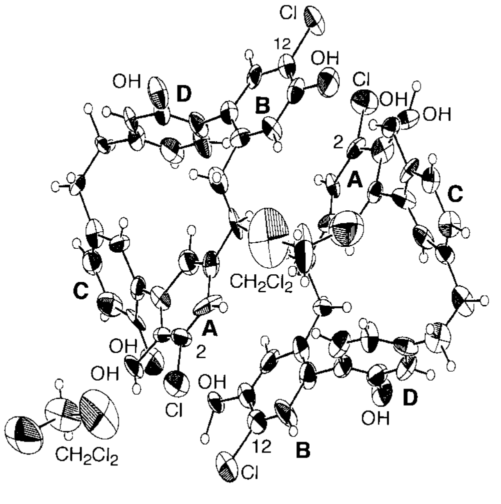


Figure 1. ORTEP drawing of 2,12-dichloroisoplagiochin D (**1**).

12,10′-Dichloroisoplagiochin C (5)

The third new compound **5** obtained as a colorless oil gave the molecular formula $C_{28}H_{20}O_4Cl_2$, which was established by high resolution mass spectrometry (*m/z* found: 490.0714; calcd 490.0738). The presence of a hydroxyl (3277 cm^{-1}) and a *cis*-stilbene group [294 nm; δ_H 6.56 (d, J=12.1 Hz) and 6.69 (d, J=12.1 Hz)] in **5** were confirmed by the IR, UV and ^{1}H NMR spectra. The ^{1}H NMR spectra also contained the signals assignable to two benzylic methylene protons (δ_H 2.59–2.72, m, 4H), four protons [δ_H 6.97 (s), 7.05 (s), 7.21 (s) and 7.27 (s)] on two tetrasubstituted benzene ring

Table 4. HMBC and NOE correlations of compound **2** ($CDCl_3$)

	HMBC	NOE
H-2	C-1, 4, 6	
H-3	C-1, 5, 7	H-7, 8
H-5	C-1, 3, 7, 2′	H-7, 8, 3′, 10′, 11′, 14′
H-7	C-3, 4, 5, 8, 9	H-3, 5, 10
H-8	C-4, 7, 9, 10, 14	H-3, 5, 10
H-10	C-8, 11, 12, 14	H-7, 8
H-13	C-9, 11, 12, 12′	H-11′
H-3′	C-6, 1′, 5′, 7′	H-5′, 7′, 10′, 14′
H-5′	C-1′, 3′, 7′	H-7′
H-6′	C-1′, 2′, 4′	H-5′
H-7′	C-3′, 4′, 5′, 8′, 9′	H-3′, 5′, 10′, 14′
H-8′	C-4′, 7′, 9′, 10′	H-10′, 14′
H-10′	C-8′, 12, 14′	H-5, 3′, 7′, 8′, 14′
H-11′	C-14, 9′, 13′	H-5, 13
H-14′	C-8′, 10′, 12′, 13′	H-5, 3′, 7′, 8′, 10′

Table 5. HMBC and NOE correlations of compound **5** ($CDCl_3$)

	HMBC	NOE
H-2	C-1, 4, 6	
H-3	C-1, 5, 7	H-7, 8
H-5	C-1, 3, 7, 2′	H-7, 8, 3′, 11′, 14′
H-7	C-3, 4, 5, 8, 9	H-3, 5, 10
H-8	C-4, 7, 9, 10, 14	H-3, 5, 10
H-10	C-8, 11, 12, 14	H-7, 8
H-13	C-9, 11, 12, 12′	H-11′
H-3′	C-6, 1′, 5′, 7′	H-5′, 7′, 14′
H-5′	C-1′, 3′, 7′	H-7′
H-6′	C-1′, 2′, 4′	H-5′
H-7′	C-3′, 4′, 5′, 8′, 9′	H-3′, 5′, 14′
H-8′	C-4′, 7′, 10′, 14′	H-14′
H-11′	C-14, 9′, 10′, 13′	H-5, 13
H-14′	C-8′, 10′, 12′, 13′	H-5, 3′, 7′, 8′

and six protons [δ_H 6.52 (d, J=2.2 Hz), 6.78 (d, J=8.0 Hz), 6.92 (d, J=8.2 Hz), 7.01 (dd, J=2.2, 8.0 Hz), 7.15 (d, J=2.2 Hz) and 7.19 (dd, J=2.2, 8.2 Hz)] on two trisubstituted benzene ring. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) also resembled those of isoplagiochin C (**6**), meaning that compound **5** possessed the same skeleton as that of compound **6**. The HMBC spectra (Table 5) of **5** showed the correlation between (i) H-7′ and C-3′, C-4′, C-5′, C-8′ and C-9′, (ii) H-13 and C-9, C-11 and C-12′. In addition, NOE's (Table 5) of **5** were observed between (i) H-3, 5 and H-7, 8, (ii) H-13 and H-11′, (iii) H-7′ and H-3′, H-5′ and (iv) H-8′ and H-14′. Thus the location of two chlorine atoms and a *cis*-double bond was confirmed at C-12 and C-10′, and C-7′ and C-8′, respectively. On the basis of the above spectral data, the structure of **5** was established to be 12,10′-dichloroisoplagiochin C. The specific optical rotation showed a negative value ([α]=−19.1°) indicating that compound **5** was present in the optically active form. This assumption was further confirmed by the presence of the first positive, the second negative, the third positive and the fourth negative Cotton effects at $\Delta\epsilon$ 322, 282, 244 and 214 nm in CD spectrum, respectively. The above physical and spectroscopic evidence indicated that compound **5** possessed planar chirality as described in compound **2**. However, it is difficult to establish the absolute configuration of both **2** and **5**, and optically active non-chlorinated bis (bibenzyls) **4** and **6** using CD spectral data since the presence of four atrop isomers is possible in each compound.[22]

It is considered that the new chlorinated compounds **1**, **2** and **5** might be the artifacts originated from compounds **4** and **6** which co-occurred in *H. sakuraii* during chromatographic procedures. In order to exclude the above hypothesis, isoplagiochin D (**4**) and isoplagiochin C (**6**) were dissolved in a mixture of chloroform and methanol, respectively and Sephadex LH-20 was added and then refluxed for 4 hours. No isoplagiochin series with chlorine atom were detected and the two starting materials were recovered in this experiment. Thus these chlorinated cyclic bis(bibenzyls) are naturally occurring compounds although the origin of the chlorine atom remains to be clarified.

Occurrence of the chlorinated compounds is very rare in the Hepaticae. Only one chlorinated drimane-type sesquiterpene lactone, 7α-chloro-6β-hydroxyconfertifolin has been isolated from the liverwort *Makinoa crispata*.[2] This is the first isolation and characterization of the structures of halogenated and non-halogenated bis(bibenzyls) from the Herbertaceae and the Mastigophoroideae. Recently Anton et al.[21] reported that the liverwort *Plagiochila oresitropha* produced 12-chloroisoplagiochin D (**3**). Furthermore eleven isoplagiochin C and D-type cyclic bis(bibenzyls) with one to six chlorine atoms have been isolated from the liverwort *Bazzania trilobata*.[23]

It is considered that cyclic and acyclic bis(bibenzyls) have been found only in the Hepaticae as secondary metabolites. However, an acyclic bis(bibenzyl), perrottetin E (**8**) has been isolated from a fern, *Hymenophyllum barbatum*.[24] The present characteristic bis(bibenzyls) are very important chemical markers to consider not only in chemosystematics of the Herberrtaceae and the Lepicoleaceae but also in phylogeny of the Hepaticae and the evolutionary processes of the lower terrestrial spore-forming green plants.

Isoplagiochin C (**6**) and isoplagiochin D (**4**) have been isolated from the liverwort *Plagiochila fruticosa* as the optically inactive forms.[20] Neither such compounds nor their related halogenated cyclic bis(bibenzyls) have been found in any other *Plagiochila* species in our laboratory. It is noteworthy that the same cyclic bis(bibenzyls) (**4** and **6**) isolated from *H. sakuraii* exist in the optically active forms.

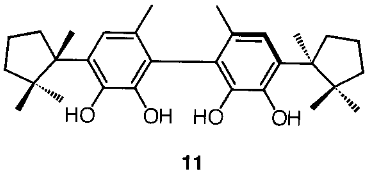
The *Herbertus* species produce many herbertane-type sesquiterpenoids,[2] but these compounds have not been detected in the *Plagiochila* species so far examined (Table 6). Aromadendranes and 2,3-secoaromadendranes are the significant chemical markers of some *Plagiochila* species.[1,2] This chemical difference is reflected in the morphological difference between the Herbertaceae and the Plagiochilaceae.

H. sakuraii is very similar chemically to *H. aduncus*, since

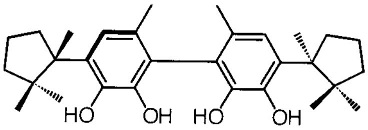
Table 6. Distribution of sesquiterpenoids and cyclic bis(bibenzyls) in *Herbertus, Mastigophora* and *Plagiochila* species (*H.a: Herbertus aduncus*; *H.s: Herbertus sakuraii; M.d*(J): *Mastigophora diclados* collected in Japan; *M.d*(M): *Mastigophora diclados* collected in East Malaysia; *P.f: Plagiochila fruticosa*.

Compounds		*H.a*	*H.s*	*M.d(J)*	*M.d(M)*	*P.f*
Bis(bibenzyls)						
2,12-Dichloroiso-Plagiochin D	(**1**)		+	+		
12,7′-Dichloroiso-Plagiochin D	(**2**)		+			
12-Chloroiso-Plagiochin D	(**3**)			+		
Isoplagiochin D	(**4**)		+			+[20]
12,10′-Dichloroiso-Plagiochin C	(**5**)		+			
Isoplagiochin C	(**6**)		+			+[20]
Sesquiterpenoids						
Herbertene	(**9**)	+[2,14,15]	+[17]	+	+[18,19]	
α-Herbertenol	(**10**)	+[2,14,15]	+[17]	+	+[18,19]	
Herbertane dimers						
Mastigophorene A	(**11**)		+[17]		+[18,19]	
Mastigophorene B	(**12**)		+[17]		+[18,19]	
Mastigophorene C	(**13**		+[17]		+[18,19]	
Aromadendranes						+[1,2]
Secoaromadendranes						+[1,2]
Diterpenoids			+[17]		+[18,19]	
Pimaranes						

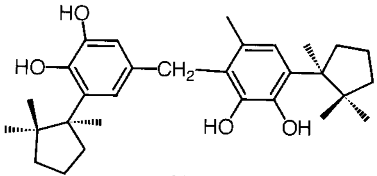
both species produce the same herbertane-type sesquiterpenoids,[2] although neither monomeric nor dimeric bibenzyls have been found in the latter species (Table 6). *H. sakuraii* is rather more similar *to M. diclados* because both species produce the same herbertane-type sesquiterpenoids and cyclic bis(bibenzyls) (**1**) and its related compound (**3**).[21] Herbertane- and herbertane dimers, mastigophorenes A–C (**11**–**13**) have been isolated from the East Malaysian *M. diclados* and neither isoplagiochin C nor isoplagiochin D-type bis(bibenzyls) have been found (Table 6).[18,19] However, the herbertane dimers have not been detected in the Japanese *M. diclados*. Thus it is considered that there are at least two chemo-types of *M. diclados* in Asia.



11



12



13

More recently, two cytochromes P-450 which biosynthesize marchantin A (**7**) series, the cyclic bis(bibenzyls) with two biphenyl ether linkages, from a bibenzyl, lunularic acid, have been isolated from a cell suspension culture of the liverwort *Marchantia polymorpha*.[25,26] We are currently studying the biosynthetic pathway of the chlorinated and non-chlorinated isoplagiochin-type bis(bibenzyls) with two biphenyl linkages.

Experimental

General

Mps were uncorrected. TLC was carried out on silica gel precoated glass plates (Kieselgel 60 F_{254}, Merck) with *n*-hexane–EtOAc (1:1, 2:1and 4:1) and CH_2Cl_2–EtOAc (2:1 and 3:1). Detection was with Godin reagent. For normal phase CC, silica gel 60 (70–230 μm, Merck) and silica gel C-300 (230–400 μm, Wako) were used. The 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. $[\alpha]_D$ was measured in 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 for $CDCl_3$ solution unless otherwise stated. All chemical shifts were reported as δ values (ppm) as 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 temperature 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 temperature 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}).

Plant materials

Herbertus sakuraii and *Mastigophora diclados* were collected in Toyo-cho, Kochi, in April 1996 and in Umajison, Kochi, in December 1996, Japan, respectively, and identified by YA and confirmed by Dr M. Mizutani. The voucher specimens were deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Separation and isolation

The powdered material (259.1 g) was extracted with ether for one week, followed by MeOH for 1 month. 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 ether extract and the combined oil (9.32 g) was chromatographed on silica gel using *n*-hexane–EtOAc gradient to divide into six fractions: Fr. 1 (148.8 mg), Fr. (2.78 g), Fr. 3 (672.7 g), Fr. 4 (89.9 mg), Fr. 5 (91.8 mg) and Fr. 6 (673.2 mg). Fractions 1–5 contained herbertane-type sesquiterpenoids whose structures were elucidated.[17] The last fraction was chromatographed on Sephadex LH-20 and each fraction obtained was purified by HPLC (CHC13–EtOAc: 3:2, 7:3, 97:3) and Diol-HPLC (*n*-hexane–EtOAc 1:1, 7:3) to afford isoplagiochin C (**6**)[20] (11.3 mg), isoplagiochin D (**4**)[20] (91.8 mg), 2,12-dichloroisoplagiochin D (**1**) (16.8 mg), 12,7′-dichloroisoplagiochin D (**2**) (7.9 mg) and 12,10′-dichloroisoplagiochin C (**5**) (11.2 mg).

Isoplagiochin C (6).[20] $[\alpha]_D=+74.8°$ (*c* 0.67, MeOH); CD: λnm ($\Delta\epsilon$): 231 (+24.4), 213 (−63.2) (*c* 1.6×10^{-5}, EtOH)

Isoplagiochin D (4).[20] $[\alpha]_D=+47.5°$ (*c* 0.67, MeOH); CD: λnm ($\Delta\epsilon$): 252 (+2.4), 225 (+22.5) (*c* 1.2×10^{-5}, EtOH)

The dried *M. diclados* (295.1 g) was ground and extracted with *n*-hexane for one week, followed by filtration and evaporation of solvent to give a dark green oil (3.07 g). The residue was further extracted with ether for one week. The extract was treated in the same manner as described above to afford a dark green oil (3.02 g). The residue was further extracted with MeOH for one month and the extract was treated in the same manner to furnish a dark brown oil (12.5 g). The n−hexane and ether extracts were combined and then analyzed by GC–MS to detect herbertene (**9**) and α-herbertenol (**10**).[2] The oil was chromatographed on silica gel using the same solvent system as described above to divide into two fractions. Fr. 1 (29.3 mg) contained herbertene (**9**) as a pure state. Fr. 2 (2.40 g) was rechromatographed on Sephadex LH-20 to give α-herbertenol (**10**)

(485 mg). The methanol extract was rechromatographed on Sephadex LH-20 to give 16 fractions. Frs. 9–16 (2.73 g) was further chromatographed on silica gel using $CHCl_3$–MeOH gradient to divide 4 fractions. Fr. M-1 (1.12 g), Fr.M-2 (87 mg), Fr. M-3 (61 mg) and Fr. M-4 (58 mg). Fr. M-1 contained α-herbertenol (**10**) as a pure state. Fr. M-2 was purified by Diol-HPLC using MeOH–$CHCl_3$ (3:97) to yield 12-chloroisoplagiochin D (**3**) (2.8 mg)[21]; $[\alpha]_D=\pm 0°$ (*c*, 0.2 $CHCl_3$); CD: no Cotton effect and 2,12-dichloroisoplagiochin D (**1**) (2 mg);.$[\alpha]_D=\pm 0°$ (*c*, 0.1 $CHCl_3$); CD: no Cotton effect.

2, 12-Dichloroisoplagiochin D (1). Colorless prisms, mp 172–175° (140–143°); $[\alpha]_D=\pm 0°$ (*c*, 0.8 MeOH); CD: λnm($\Delta\epsilon$): no Cotton effect; HR-MS: *m/z* found: 492.0904, $C_{28}H_{22}O_4Cl_2$ requires 492.0895; EI-MS: *m/z* 496($[M]^+$+4, 14%), 494($[M]^+$+2, 59), 492($[M]^+$, 88), 457(10), 247(54), 245(100), 211(13), 181(8); FT-IR (KBr) ν cm^{-1}: 3329(OH), 1607, 1566, 1415, 1213, 820; UV (EtOH) λ_{max} nm (log ϵ): 207(4.91), 288(3.95); 600 MHz ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR(CD_3OD) (see Tables 1 and 2) Crystal data for 1: Crystal dimensions=0.35×0.2×0.15 mm, Triclinic, space group *P*1 with *a*=11.977 (0) Å, *b*=11.989 (0) Å, *c*=11.820 (0) Å, α=62.154 (0)°, β=73.782 (0)°, γ=66.421 (0)° *V*=1366.4 (0) $Å^3$, *Z*=2, *D*x=1.53 Mg m^{-3}, *D*m=1.50 mg m^{-3}, and μ(Mo K-α)=4.665 mm^{-1} by Mac Science MXC 18 diffractometer. Final *R* value was 0.064 for 2452 reflections.

12, 7′-Dichloroisoplagiochin D (2). Colorless oil; $[\alpha]_D^{20}$= −2.7° (*c* 0.47, MeOH); CD: λnm ($\Delta\epsilon$): 300(+0.74), 281(−0.39), 234(+5.34) (*c* 9.7×10^{-6}, EtOH); HR-MS: *m/z* found: 492.0923, $C_{28}H_{22}O_4Cl_2$ requires 492.0895; EI-MS: *m/z* 496($[M]^+$+4, 1%), 494($[M]^+$+2, 5), 492($[M]^+$, 8), 458(67), 245(11), 211(100), 44(21); FT-IR (KBr) cm^{-1}: 3262(OH), 1607, 1566, 1416, 1231, 822, 756; UV (EtOH) λ_{max}nm (log ϵ): 205(5.08), 288(4.16); ^1H NMR (600 MHz in $CDCl_3$) and ^{13}C NMR (150 MHz $CDCl_3$) (see Tables 1 and 2).

12, 10′-Dichloroisoplagiochin C (5). Colorless oil; $[\alpha]_D^{21}$= −19.1°(*c* 0.38, $CHCl_3$); CD: λnm ($\Delta\epsilon$): 322(+7.80), 282(−7.47), 244 (+48.08), 214 (−157.72) (*c* 6.1×10^{-6}, EtOH); HR-MS: *m/z* founds: 490.0714, $C_{28}H_{20}O_4Cl_2$ requires 490.0738; EI-MS: *m/z* 494($[M]^+$+4, 16%), 492($[M]^+$+2, 74), 490($[M]^+$, 100), 455(24), 437(13), 420(10), 401(9), 227(23), 210(18), 44(16); FT-IR(KBr) cm^{-1}: 3277(OH), 1605, 1568, 1414, 1235, 826, 733; UV (EtOH) λ_{max}nm (log ϵ): 203(4.96), 294(4.30); 600 MHz ^1H NMR (600 MHz in $CDCl_3$) and ^{13}C NMR (150 MHz $CDCl_3$) (see Tables 1 and 2).

Treatment of isoplagiochin D (4) and isoplagiochin C (6) with a mixture of chloroform and methanol in the presence of Sephadex LH-20

Isoplagiochin D (**4**) (1 mg) and isoplagiochin C (**6**) (1 mg) were dissolved in a mixture (1 ml) of chloroform and methanol (1:1), respectively; Sephadex LH-20 (2 g) was added and the mixture stirred for 4 days at rt. Each mixture was filtered, followed by evaporation of the solvent to recover the starting material. Neither mono- nor dichloroisoplagiochins were detected in each residue by their ^1H NMR spectra.

Acknowledgements

We thank Dr M. Mizutani (The Hattori Botanical Laboratory, Nichinan, Miyazaki, Japan) for his confirmation of the liverwort species. This work was supported in part by a Grant-in-Aid for Scientific Research (B) (No.08459026) from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Asakawa, Y. Progress in the Chemistry of Organic Natural Products. In *Chemical Constituents of Hepaticae*, Herz, W.; Grisebach, H.; Kirby, G. W., Eds.; Springer: Vienna, 1982; 42, pp 1–285.
2. Asakawa, Y. Progress in the Chemistry of Organic Natural Products. In *Chemical Constituents of Bryophytes*, Herz, W.; Kirby, G. M.; Moore, R. E.; Steglich, W.; Tamm, Ch., Eds.; Springer: Vienna, 1995; 65, pp 1–618.
3. Nagashima, F.; Asakawa, Y. Chemical constituents of the liverworts Jungermannioideae and Myliioideae (Jungermanniaceae). In *Recent Research Developments in Phytochemistry, Part II*, Pandalai, S. G., Ed.; Research Signpost: India, 1998; 2, pp 327–382.
4. Bardon, A.; Kamiya, N.; Toyota, M.; Takaoka, S.; Asakawa, Y. *Phytochemistry* **1999**, *52*, 1323–1329.
5. Hashimoto, T.; Ikeda, H.; Takaoka, S.; Tanaka, M.; Asakawa, Y. *Phytochemistry* **1999**, *52*, 501–509.
6. Nagashima, F.; Murakami, Y.; Asakawa, Y. *Phytochemistry* **1999**, *51*, 1101–1104.
7. Toyota, M.; Konoshima, M.; Asakawa, Y. *Phytochemistry* **1999**, *52*, 105–112.
8. Toyota, M.; Saito, T.; Asakawa, Y. *Phytochemistry* **1999**, *51*, 913–920.
9. Asakawa, Y. *Heterocycles* **1997**, *46*, 795–848.
10. Asakawa, Y. Phytochemistry of bryophytes biologically active terpenoids and aromatic compounds in liverworts. Recent Advances in Phytochemistry. In *Phytochemicals in Human Health Protection, Nutrition, and Plant Defense*, Romeo, J., Ed.; Kluwer Academic/Plenum Publishers: New York, 1999; Vol. 33, pp 319–342.
11. Asakawa, Y. *J. Hattori Bot. Lab.* **1998**, *84*, 91–104.
12. Schwartner, C.; Bors, W.; Michel, C.; Franck, U.; Jakic, B. M.; Nenninger, A.; Asakawa, Y.; Wagner, H. *Phytomedicine* **1995**, *2*, 113–117.
13. Asakawa, Y. Terpenoids and aromatic compounds with pharmacological activity from bryophytes. In *Bryophytes: Their Chemistry and Chemical Taxonomy*; Zinsmeister, D. H.; Mues, R., Eds.; Clarendon Press: Oxford, 1990, pp 369–410.
14. Matsuo, A.; Yuki, S.; Nakayama, M. *J. Chem. Soc. Perkin Trans. 1* **1986**, 701–710.
15. Asakawa, Y.; Matsuda, R.; Schofield, W. B.; Gradstein, S. R. *Phytochemistry* **1992**, *21*, 2471–2473.
16. Buchanan, M. S.; Connolly, J. D.; Rycroft, S. S. *Phytochemistry* **1996**, *43*, 1245–1248.
17. Irita, H. Phytochemical Studies on the Japanese Liverworts, *Porella acutifolia*, subsp. *tosana* (Steph.) Hatori, *Herbertus*

sakuraii (Warnst.) Hatt. and *Plagiochasma pterospermum* Mass. The Master Thesis, Tokushima Bunri University, 1999.

18. Fukuyama, Y.; Toyota, M.; Asakawa, Y. *J. Chem. Soc. Chem. Commun.* **1988**, 1341–1342.
19. Fukuyama, Y.; Asakawa, Y. *J. Chem. Soc. Perkin Trans. 1* **1991**, 2737–2741.
20. Hashimoto, T.; Kanayama, S.; Kan, Y.; Tori, M.; Asakawa, Y. *Chem. Lett.* **1996**, 741–742.
21. Anton, H.; Kraut, L.; Mues, R.; Morales, Z. M. I. *Phytochemistry* **1997**, *47*, 1069–1075.
22. Harada, N. Tohoku University, Private communication.
23. Martini, U.; Zapp, J.; Becker, H. *Phytochemistry* **1998**, *47*, 89–96.
24. Oiso, Y.; Toyota, M.; Asakawa, Y. *Chem. Pharm. Bull.* **1999**, *47*, 297–298.
25. Friederich, S.; Maier, U.; Deus-Neumann, B.; Asakawa, Y.; Zenk, M. H. *Phytochemistry* **1999**, *50*, 589–598.
26. Friederich, S.; Rueffer, M.; Asakawa, Y.; Zenk, M. H. *Phytochemistry* **1999**, *52*, 1195–1202.