Pergamon

PII: S0031-9422(96)00257-9

Phytochemistry, Vol. 43, No. 1, pp. 83–93, 1996

0031-9422/96 $15.00 + 0.00

ALLOAROMANDENDRANES, BICYCLOGERMACRANE AND 2,3-SECOALLOAROMANDENDRANES IN CULTURED CELLS OF THE LIVERWORT, *HETEROSCYPHUS PLANUS*

KENSUKE NABETA, SHINICHI OHKUBO, REIKO HOZUMI, YUKIHARU FUKUSHI,* HIROSHI NAKAI† and KENJI KATOH†

Department of Bioresource Chemistry, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan; *Department of Applied Bioscience, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan; †Shionogi Aburahi Laboratory, Kohga-cho, Shiga-ken 520-34, Japan

(*Received* 23 *February* 1996)

Key Word Index—*Heteroscyphus planus*; Jungermanniales; suspension cultures; sesquiterpenes; *ent*-alloaromandendranes; *ent*-2,3-secoalloaromandendranes; *ent*-bicyclogermacrane.

Abstract—Novel *ent*-alloaromandendranes, planotriol and its acetates, and four *ent*-2,3-secoalloaromandendranes including two novel compounds in nature and one novel *ent*-bicyclogermacrene were isolated from cultured cells of the liverwort, *Heteroscyphus planus*. The absolute configuration of planotriol and its acetates were determined by a single X-ray analysis and ^{1}H NMR spectroscopy after derivatization with an axially chiral agent, 2-(2′-methoxy-1′-naphthyl)-3,5-dichlorobenzoic acid (MNCB). Planotriol diacetate was chemically converted into the known *ent*-2,3-secoalloaromandendrane, (−)-hanegokedial, suggesting that the 2,3-dihydroxy-alloaromandranes might be immediate precursors for 2,3-secoalloaromandendranes. The absolute stereochemistry of the bicyclogermacrane and the 2,3-secoalloaromandendranes was also determined by ^{1}H NMR analysis of the MNCB esters and chemical correlation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

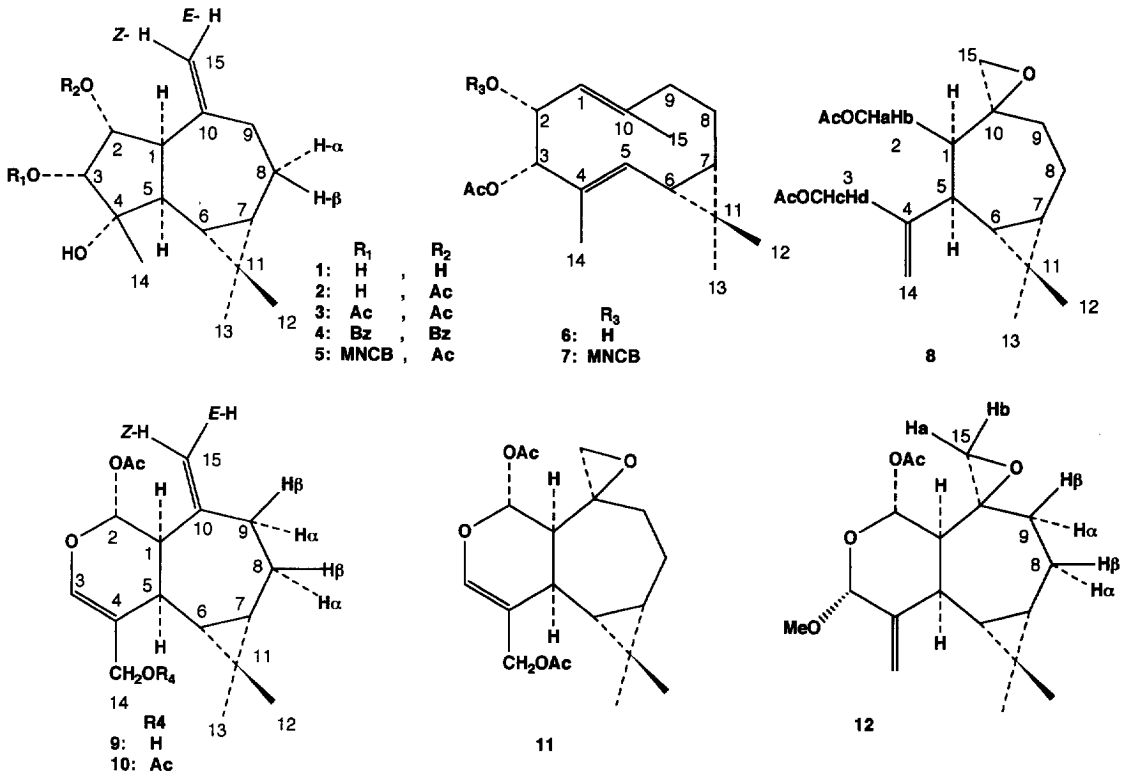
Liverworts frequently contain terpenoids of remarkable interest because of their structure [1], biogenesis [2–9] and biological activity [10, 11]. Since terpenoids enantiomeric to the corresponding compounds from vascular plants often occur in liverworts, attention must be particularly paid to the determination of absolute configuration. We have previously reported the isolation of sesquiterpenes of cadinane-type [12] and diterpenes of clerodane-type [13, 14]. Hashimoto *et al.* [15] have very recently reported diterpenes of the *epi*-neoverrucosane and *ent*-clerodane types, and sesquiterpenes of the *ent*-2,3-secoalloaromandendrane and cadinane types from *Heteroscyphus planus* harvested from the field. As a continuation of our work on terpenoids accumulated in cultured cells of the liverwort *H. planus*, we isolated three new *ent*-alloaromandendranes, plantotriol (**1**) which might be an immediate precursor for *ent*-2,3-secoalloaromandendrane biosynthesis, its acetates (**2** and **3**), one novel *ent*-bicyclogermacrane (**6**) and four *ent*-secoalloaromandendranes (**8**, **9**, **11**, **12**) including two novel compounds (**8**, **9**) in nature. In this communication, we report the elucidation of the absolute configuration of the *ent*-alloaromandendranes and the *ent*-bicyclogermacrane by X-ray analysis and ^{1}H NMR spectroscopy using axially chiral 2-(2′-methoxy-1′-naphthyl)-3,5-dichlorobenzoic acid (MNCB) which one of us employed for discrimination of enantiomeric alcohols by ^{1}H NMR [16, 17]. The chemical conversion of planotriol (**1**) to the known *ent*-2,3-secoalloaromandendrane, (−)-henegokedial [18], (plagiochilal A [19]) is also described.

RESULTS AND DISCUSSION

The methanol extract of the cultured cells and gametophytes of *H. planus*, obtained as described previously [12], was further separated by sequential HPLC and liquid chromatography to give a novel *ent*-trihydroxy-alloaromandendrane (**1**), which we have named planotriol, its acetates (**2** and **3**), four *ent*-secoalloaromandendranes (**8**, **9**, **11** and **12**) including the known plagiochiline A (**11**) [20] and methoxy-plagiochiline A_2 (**12**) [21]. A novel *ent*-bicyclogermacrene (**6**) was also isolated from the freshly harvested calli. However, compound **6** was not detected in the fractions stored at −30°C for over two years, indicating its instability.

ent-*Alloaromandendranes*

Planotriol diacetate (**3**) (17.5 mg from 918 g cultured cells) was obtained as an oil, $[\alpha]_D^{19}$ − 1.55. The molecular formula was determined by FIHR-mass spectrometry as $C_{19}H_{28}O_5$ (*m/z* 336.1927; calc. 336.1937). Planotriol monoacetate (**2**) (3.1 mg) was obtained as an



oil, $[\alpha]_D^{24}$ + 13.3. The molecular formula was established by EIHR-mass spectrometry as $C_{17}H_{26}O_4$ (*m/z* 294.1861; calc. 294.1832). Planotriol (**1**) (0.7 mg), was obtained as needles from Et_2O–*n*-hexane, mp 129–131°C, $[\alpha]_D^{24}$ + 8.60 (*c* 0.11 MeOH). The molecular formula of **1**, $C_{15}H_{24}O_3$, was deduced by EIHR-mass spectrometry ($[M]^+$ 252.1735, calc. 252.1726). Hydrolysis of **2** and **3** with 16.7% aq. Cs_2CO_3 at room temp. for 1.5 h gave planotriol (**1**).

The NMR spectra (^1H, ^{13}C, DEPT, ^1H–^1H and ^1H–^{13}C COSY) of **3** confirmed the proton-line assignments of H-1 to H-4 and H-5 to H-9, and the presence of two acetyls (two methyls at δ_C 20.8 and δ_H at 2.13 and 2.03, and two C=Os at δ_C 170.0 and 170.1), three methyls attached to quaternary Cs and a quaternary C attached to an OH group (see Table 1). The presence of a cyclopropane ring was confirmed by the ^1H peaks resonating at δ ^1H 0.59 (*ddd*, $J_{H\text{-}6,H\text{-}7}$ = 11.2 Hz, H-7) and 0.26 (*dd*, $J_{H\text{-}5,H\text{-}6}$ = 9.4 Hz, H-6). The ^1H–^1H long range connectivity between H-2 and methylene protons at C-15 indicated that a C-10 = C-15 H_2 unit is attached to C-1. Segments as described above were assembled by HMBC connectivities (Fig. 1), that are in agreement with an alloaromandendrane or aromandendrane skeleton. Difference NOE and NOESY experiments performed on **3** established the relative configurations of protons and methyl group in the C-1–C-7 region and of exocyclic methylene at C-10 and H-2 according to Figure 1. The strong NOE between H-1 and H-5 confirmed the *cis*-ring junction, that is, alloaromandendrane skeleton for **1**, while NOE between H-1 and H-5 was not usually observed in the aromandendranes [22, 23]. However, contradictory data on the ring junction was obtained by decoupling experiments which showed the coupling constant between H-1 and H-5 to be 10.2 Hz (the value is in the corresponding *trans*-fused system). The *cis*-orientation of H-1 and H-5 was finally proven by a single crystal X-ray analysis of the dibenzoate (**4**) of **1**, which was obtained by the esterification of **1** with benzoyl chloride. The crystallographic data and perspective view of the molecular structure of **4** (Figure 2) conclusively indicate the *cis*-fused ring system. The larger coupling constants (*ca.* 10 Hz) were so far believed to indicate the *trans*-ring junction of bicyclo [5.3.0] decane skeleton. However, the findings described here demonstrated that the larger coupling constant between the brideghead protons does not indicate the *trans*-ring junction at C-1 and C-5 of this tricyclo[6.3.0.0] undecane skeleton. The larger coupling constant between H-1 and H-5 may be explained by the smaller dihedral angle between the H-1–C-1 and H-5–C-5 bond (−28° obtained by X-ray analysis, $J_{H\text{-}1,H\text{-}5}$ in **4**: 9.6 Hz).

The absolute configuration of planotriol monoacetate (**2**) was determined by esterification of the secondary hydroxyl group at C-3, the presence of which was proved by the doublet proton resonance at δ_H 3.98, with axially chiral MNCB. The monoacetate (**2**) was esterified with (a*R*)- and (a*S*)-MNCB by the method previously reported [16]. Figure 3 shows the configurational correlation models for (a*S*)- (**5a**) and (s*R*)-MNCB (**5b**) esters of **2**. The NOE enhancement of the acetoxy

Table 1. ^{13}C (67.8 MHz) and ^{1}H (270 MHz) NMR spectral data for planotriol (**1**), monoacetate (**2**) and diacetate (**3**) in $CDCl_3$

	1		2		3	
C	δ ^{13}C	δ ^{1}H (*J* Hz)	δ ^{13}C	δ ^{1}H (*J* Hz)	δ ^{13}C	δ ^{1}H (*J* Hz)
1	53.2	2.94 (*dd*, 4.6, 9.6)	49.9	3.20 (*dd*)	49.5	3.18 (*dd*, 6.9, 10.2)
2	74.2	4.36 (*dd*, 4.6, 5.9)	75.2	5.44 (*dd*)	72.9	5.60 (*dd*, 6.9, 5.9)
3	76.1	3.76 (*d*, 5.9)	75.6	3.98 (*d*, 5.9)	77.2	5.48 (*d*, 5.9)
4	81.4		79.7		79.6	
5	45.9	2.19 (*dd*, 9.6, 11.5)	45.5	2.12 (*dd*, 11.6)	45.8	2.19 (*dd*, 10.2, 11.2)
6	26.1	0.14 (*dd*, 11.5, 9.2)	25.1	0.19 (*dd*, 11.6)	25.1	0.26 (*dd*, 11.2, 9.4)
7	25.2	0.56 (*ddd*, 9.2, 12.0, 5.0)	25.0	0.60 (*ddd*, 9.2, 5.6)	25.0	0.59 (*ddd*, 9.4, 9.2, 5.0)
8	21.1	α1.21	21.2	α1.19	21.0	α1.17
		β1.78		β1.82		β1.80
9	37.4	2.38	36.3	2.37	36.9	2.38
10	147.9		147.1		146.2	
11	17.7		17.8		17.8	
12	28.4	1.00 (*s*)	28.5	1.01 (*s*)	28.4	1.01 (*s*)
13	15.9	1.03 (*s*)	15.8	1.03 (*s*)	15.8	1.03 (*s*)
14	22.3	1.26 (*s*)	22.8	1.26 (*s*)	22.8	1.26 (*s*)
15	110.2	*Z*4.81 ($d < 1$ Hz)	110.9	*Z*4.80 ($d < 1$ Hz)	110.8	*Z*4.84 ($d < 1$ Hz)
		*E*4.91 ($d < 1$ Hz)		*E*4.88 ($d < 1$ Hz)		*E*4.91 ($d < 1$ Hz)
OAc-2			21.0	2.11	20.8	2.13
			171.2		170.0	
OAc-3					20.8	2.03
					170.1	

Assignments were based on DEPT, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, NOESY, difference NOE and HMBC experiments.

methyl protons by the irradiation of the methoxy protons in **5a** clearly suggests that the methoxy group is in proximity to the acetoxy group in the case of the (a*S*)-MNCB ester [17], while no NOE enhancement between the acetoxy methyl protons and the methoxy protons was observed in **5b** as expected. These NOE correlations clearly indicate the *R*-configuration at the C-3 position of **2**. The *R*-configuration at the C-3 position was also proven by chemical shift differences defined as $\Delta\delta$(ppm) = δa*S*-δa*R* of MNCB esters (**5**) (see Table 2) [16]. The positive $\Delta\delta$ values of H-1 (+0.28), H-2 (+0.05) and acetyl methyl protons (+0.04) indicate that these protons are located on the left hand side of the naphthalene ring in the case of the (a*S*)-MNCB ester (Fig. 3, **5a**), demonstrating the *R*-configuration at C-3 in **2**. Thus the absolute configuration of **2** is concluded to be as indicated in the structure.

ent-*Bicyclogermacrane*

The molecular formula of the new bicyclogermacrene (*ent*-3β-acetoxy-2β-hydroxybicyclogermacrene, **6**) was $C_{17}H_{26}O_3$ as determined by EIHR-mass spectrometry. IR absorptions at 3400 cm^{-1} and 1740 cm^{-1}, together with the ^{13}C peaks resonated at δ 170.7 (CH_3C^*OO–), 21.2 (C^*H_3COO), 82.1 (*sec* C attached

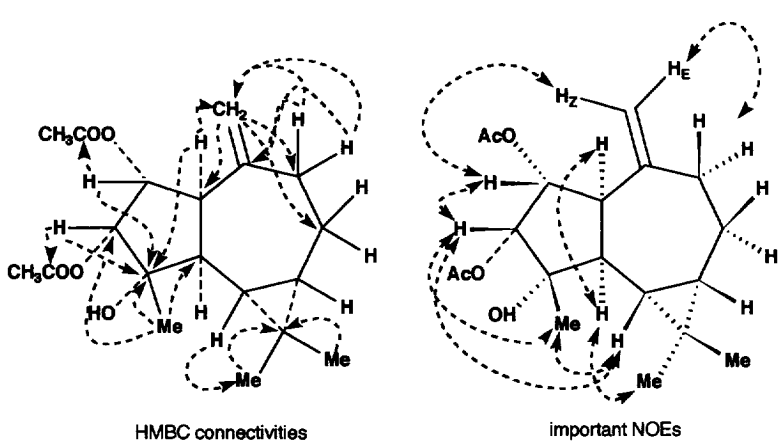


Fig. 1. HMBC connectivities and important NOEs observed in planotriol diacetate (**3**).

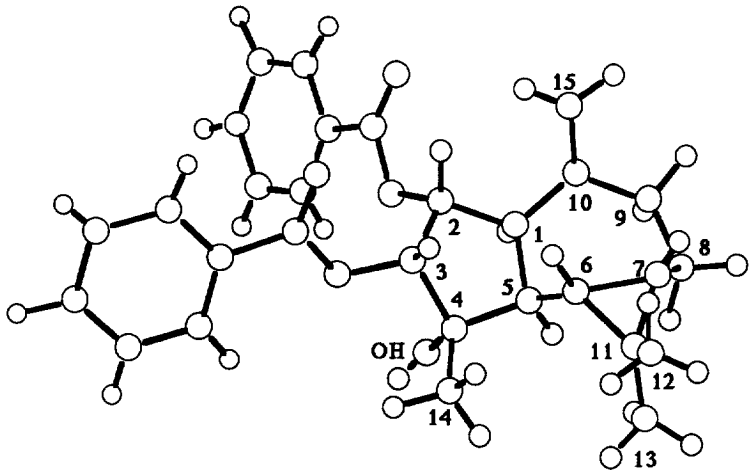


Fig. 2. The perspective view of the molecular structure of planotriol dibenzoate (**4**).

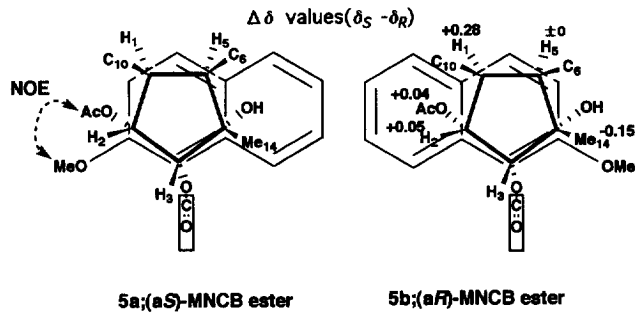


Fig. 3. The configurational correlation models for MNCB esters (**5a** and **5b**) of planotriol monoacetate (**2**) and observed NOE correlation.

to an acetoxy group) and 70.4 (*sec* C attached to an hydroxy group), and the ^{1}H peaks at δ 5.26 (*d*, $J_{H\text{-}2,H\text{-}3}$ = 3.3 Hz, H-3) and 4.49 (*dd*, H-2) indicated the presence of the acetoxy and hydroxy groups vicinal to each other. Analysis of the ^{1}H and ^{13}C NMR spectra (see Table 3), aided DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, NOESY, different NOE and COLOC experiments enabled establishment of the structure of **6**. The ^{1}H NMR spectrum of **6** indicated the presence of two trisubstituted ethylenic double bonds {δ_H 5.07 (*d*,

Table 2. ^{1}H NMR data of MNCB esters **5a** and **5b**

Proton	(a*S*)-MNCB (**5a**)	(a*R*)-MNCB (**5b**)	Δδ (ppm)
H-1	2.43	2.15	+0.28
H-2	5.31	5.26	+0.05
H-3	4.92	4.96	−0.04
H-5	1.25	1.25	0
H-6	0.04	0.07	−0.03
H-7	0.45	0.43	−0.02
H-8α	1.04	0.94	+0.10
H-8β	1.73	1.68	+0.05
H-9	2.30	2.18	+0.12
Me-12	0.83	0.90	−0.07
Me-13	0.88	0.91	−0.03
Me-14	0.65	0.80	−0.15
H-15*Z*	4.61	4.52	+0.09
H-15*E*	4.79	4.72	+0.07
OAc	1.90	1.86	+0.04

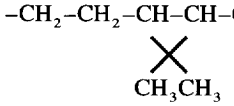
Assignments were based on DEPT, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, NOESY and difference NOE experiments.

Table 3. NMR data of compound **6** and its (a*S*)- and (a*R*)-MNCB esters **7a** and **7b**

		6	**7**		
C	$\Delta^{13}C$	$\Delta^{1}H$ (*J* HZ)	(a*S*)-MNCB (**7a**)	(a*R*)-MNCB (**7b**)	$\Delta\delta$ (ppm)
1	122.9	5.07 (*d*, 3.3)	4.40	4.61	−0.21
2	70.4	4.49 (*dd*, 3.3, 1.3)	5.22	5.21	+0.01
3	82.1	5.26 (*d*, 1.30)	4.95	4.95	0
4	126.6				
5	128.4	4.89 (*d*, 11.9)	4.71	4.77	−0.06
6	27.1	1.30	1.25	1.25	0
7	31.2	0.74 (*ddd*)	0.68	0.68	0
8	27.1	α1.35	1.16	1.20	−0.04
		β1.95	1.91	1.91	0
9	37.6	α2.49	2.34	2.33	+0.01
		β1.80 (*dd*, 12.9, 4.0)	1.71	1.69	+0.02
10	143.3				
11	21.0				
12	29.0	1.07 (*s*)	1.04	1.04	0
13	15.3	1.02 (*s*)	0.96	0.98	−0.02
14	15.9	1.74 (*d*, 1.3)	1.60	1.60	0
15	21.9	1.57 (*d*, 1.3)	1.45	1.42	+0.03
OAc	21.2	2.09	1.81	1.96	−0.15
	170.7				

Assignments were based on DEPT, ^{1}H–$^{1}COSY$, ^{13}C–^{1}H COSY, NOESY, difference NOE and COLOC experiments.

$J_{H\text{-}1,H\text{-}2}$ = 9.9 Hz, H-1) and 4.89 (*d*, $J_{H\text{-}5,H\text{-}6}$ = 11.9 Hz, H-5)} and a cyclopropane ring {1.30 (*m*, H-6) and 0.77 (*ddd*, H-7)}. Two partial structures, Cq–CH(CHOAc)–CH(OH)–CH=C(CH_3)–(Cq:quaternary C) and



were joined to the bicyclogermacrene skeleton as indicated on the basis of the long range connectivities as shown in Fig. 4. *Trans*-substituted C=C bonds at C-1/C-10 and C-4/C-5 were confirmed by the observed NOEs between H-2 and Me-15 and between H-6 and Me-14 (see Figure 5). Additional NOEs of H-2/H3, Me-14/H-3, Me-13/H-5, Me-12/H-6 and H-7, Me-14/Me-15 confirmed the established conformation of the bicyclogermacrene [24], indicating that the acetoxy and the hydroxy groups were *cis*-oriented on the same side as the cyclopropane ring. Based on the above findings, the relative configuration of **6** was elucidated as indicated.

The absolute configuration of the *ent*-bicyclogermacrene **6** was determined by esterification of the secondary hydroxyl group at C-2 with (a*S*)- and (a*R*)-MNCB. Fig. 6 shows the configurational correlation models for (a*S*)- (**7a**) and (a*R*)-MNCB (**7b**) esters of **6**. We tentatively ascribe the preference of conformation 7A of (a*S*)- and (a*R*)-MNCB esters of **6**, in which the 3,5-dichlorobenzoyloxy group staggered between the C-3/C-4 bond and the H-3 to the conformation 7B eclipsing the H-3 and the 3,5-dichlorobenzoyloxy group. This is supported by the observation that protons, H-1 ($\Delta\delta = \delta S - \delta R$: −0.21 ppm), acetoxy Me (−0.15) and H-5 (−0.06) suffered very substantial upfield shifts in the a*S*-isomer, while protons, Me-15 (+0.03) and H-2 (+0.01) suffered a slightly downfield shift in the (a*S*)-isomer. The negative $\Delta\delta$ values of H-1,

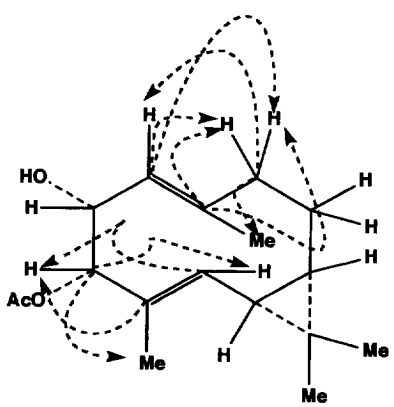


Fig. 4. COLOC connectivities in compound **6**.

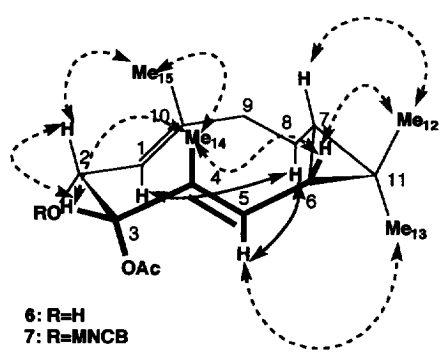


Fig. 5. Important NOEs observed in compound **6** (◀- -▶) and its MNCB ester Z(7) (◀- -▶ and ◀- -▶).

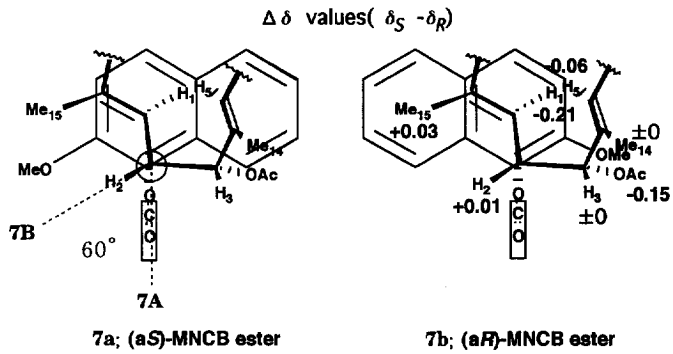


Fig. 6. The configurational correlation models for MNCB esters (**7a** and **7b**) of compound **6**.

H-5, acetoxy Me indicate that these protons are located on the right hand side of the naphthalene ring in the (a*S*)-MNCB ester (Figure 6, **7a**), demonstrating the *R*-configuration at C-2 in **6**. Thus the absolute confiuration of **6** is concluded as indicated.

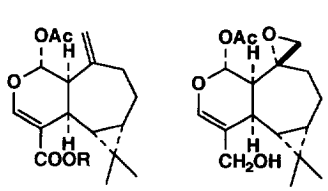
ent-2,3-*Secoalloaromandendranes*

ent - 2,3 - Secoalloaromandendranes, plagiochiline L (**18**) and M (**19**), have been isolated from *H. planus* harvested in the field [15]. Four additional *ent*-2,3-secoalloaromandendranes (**8**, **9**, **11**, **12**) were isolated from cultured cells of *H. planus*. Two of them were identified as the known plagiochiline A (**11**) [20] and methoxyplagiochiline A_2 (**12**) [21] by comparison of the spectroscopic data (^{1}H and ^{13}C NMR. EI-mass spectrometry and $[\alpha]_D$) with those of published data [19–21]. The structure of deacetylplagiochiline C (*ent*-2β-acetoxy - 14 - hydroxy - 2,3 - epoxy - 2,3 - secoalloaromandendrene, **9**) has been elucidated by EI-mass spectrometry, UV, IR and NMR (^{1}H and ^{13}C 1D NMR, DEPT, ^{1}H–^{1}H and ^{1}H–^{13}C COSY, NOESY, difference NOE and HMBC, see Experimental. Deacetylplagiochiline C (**9**) was isolated for the first time from a natural source. Although compound **9** has been reported as a reduced product of plagiochiline L [15], no spectral data was given in the literature. The structure of **9** was conclusively confirmed by acetylation of **9** to give the known plagiochiline C (**10**) [18, 25–27].

The ^{1}H NMR spectrum of the novel diacetate **8** (*ent*-2,3-diacetoxy-10α,15α-epoxy-2,3-secoalloaromandendra - 4(14) - ene) showed signals due to an acetoxy methyl attached to a methine C {δ 2.03 (3H, *s*, acetyl Me), 4.43 (1H, *dd*, J = 11.2 and 6.2 Hz, H-2) and 4.51 (1H, *dd*, J = 11.2 and 8.3 Hz, H-2)}, an isolated acetoxymethyl attached to a C=C bond {δ 2.08 (3H, *s*, acetyl Me), 4.52 (1H, *d*, J = 13.2 Hz, H-3) and 4.66 (1H, *d*, J = 13.2 Hz, H-3)}, a cyclopropane ring {δ 0.61 (1H, *dd*, J = 9.2 and 11.5 Hz, H-6) and 0.84 (1H, *m*, J = 11.5 Hz, H-7)}, geminal dimethyls {δ 0.95 (3H, *s*, Me-13) and 1.10 (3H, *s*, Me-12)} and an epoxy ring {δ 2.52 (2H, *s*, 2 × H-15s), ^{13}C signals at δ_C 52.9 (C-15) and 62.5 (C-10)}. These fragments were connected by ^{1}H–^{1}H and ^{1}H–^{13}C COSY experiments to give two partial structures as indicated in Figure 7, which were further assembled to the structure **8** by HMBC connectivities (see Figure 8). The smaller coupling constant (<1 Hz) between H-5 and H-1, and the larger coupling constant (9.2 Hz) between H-5 and H-6 together with the observed NOEs (Figure 8) indicated that H-5 and H-6 were *trans*-diaxially oriented, and that H-1 was *cis*-oriented to H-5. Thus the relative stereochemistry at C-1, C-5, C-6 and C-7 was determined as indicated. Concerning the stereochemistry of the epoxy ring at C-10, comparison of the δ_C values of C-15 and C-10 with published data [26, 28] indicated that they should be the same as for the regular series of *ent*-10α, 15α-epoxy-2,3-secoalloaromandendrenes from liverworts. Chemically prepared *ent*-10β,15β-epoxy-isomer of plagiochiline A (**20**), for example, gave signals due to C-15 in a lower field (δ_C 57.4 [25]) than did plagiochiline A (δ_C 51.7).

Chemical correlation of planotriol diacetate (**2**) *to* (−)-*hanegokedial* (*plagiochilal A*, **14**)

The *ent*-2,3-secoalloaromandendranes frequently co-occur with the *ent*-bicyclogermacrenes in liverworts. Fukuyama and Asakawa [18] proposed the possible



18: R=H
19: R=Me

20

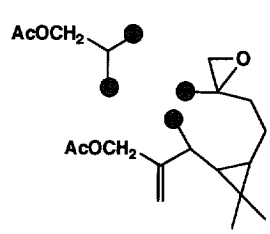


Fig. 7. Partial structures of compound **8**.

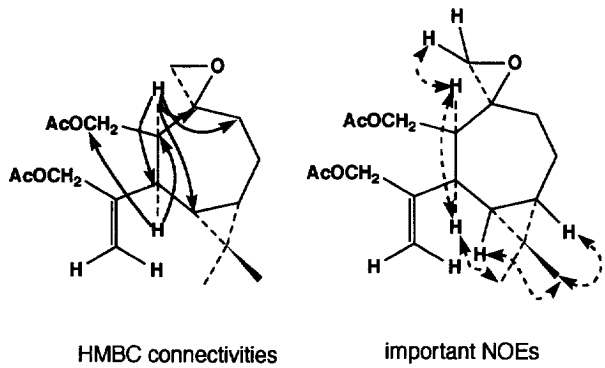
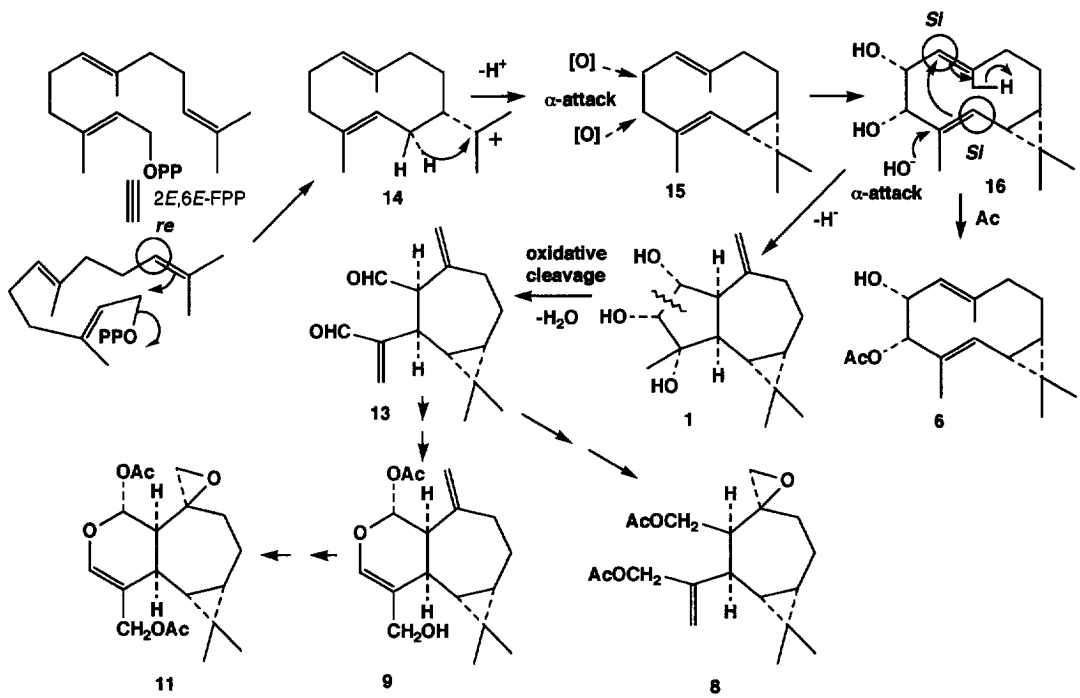


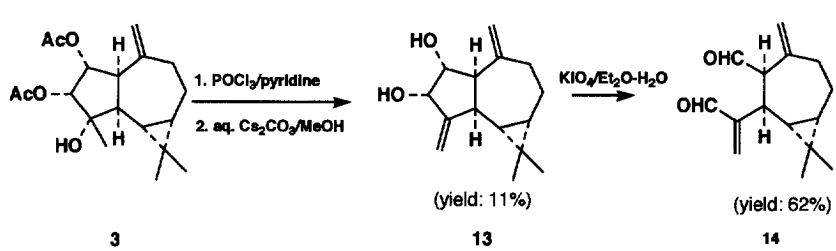
Fig. 8. HMBC connectivities and important NOEs observed in compound **8**.

biosynthetic route of the *ent*-2,3-secoalloaromandendranes via (−)-hanegokedial (plagiochilal A [19]) from (−)-bicyclogermacrene [28]. The *ent*-alloaromandendranes (**1** to **3**) which co-occurred with the above compounds in cultured cells of *H. planus* possess 'absolute stereochemical homogeneity' [29] to the 2,3-secoalloaromandendranes. Thus we propose that the 2,3-dihydroxy-alloaromandendranes such as the diol **13** and triol **1** are the 'missing links' in the *ent*-2,3-secoalloaromandendrane biogenesis from (−)-bicyclogermacrenes as indicated in Scheme 1.

To prove this hypothesis, the 1,2-diol **13** was prepared by dehydration of planotriol diatetate (**3**) with $POCl_3$ in pyridine, followed by hydrolysis with aqueous Cs_2CO_3 in methanol in 11% yield (see Scheme 2). The structure of **13** was confirmed by EIHR-mass



Scheme 1. Possible biosynthetic pathway of the *ent*-2,3-seco-alloaromandendranes in cultured cells of *H. planus*.



Scheme 2. Chemical conversion of planotriol (**1**) to (−)-hanegokedial (**14**).

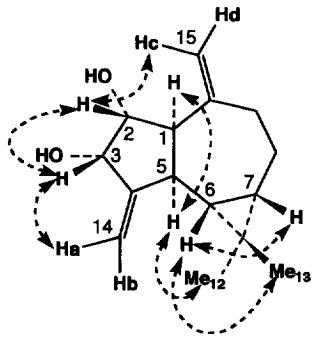


Fig. 9. Important NOEs observed in an intermediate **13**.

spectrometry, ^1H and ^{13}C NMR, and ^1H–^1H COSY and ^1H–^{13}C COSY experiments. Significant NOEs observed by NOESY experiment on **13** (see Figure 9) indicated that the absolute configurations at the C-1, C-2, C-3 and C-5 positions were retained in the formation of **13**. The 1,2-diol **13** was then oxidatively cleaved under mild conditions (potassium periodate in Et_2O–H_2O at 0°C for 32 hr) to afford hanegokedial **14** in 62% yield (Scheme 2). The ^1H and ^{13}C NMR spectra compound **14** chemically prepared from **3** are identical to those reported for (−)-hanegokedial isolated from natural materials. Thus we believe that the *ent*-2,3-dihydroxy-alloaromandendrane is an immediate precursor for 2,3-secoalloaromandendrane biosynthesis.

EXPERIMENTAL

General. ^1H NMR: 270 MHz in $CDCl_3$ or C_6D_6; ^{13}C NMR: 67.8 MHz, solvent peak as the int. standard. Bond type was distinguished by DEPT. ^1H connectivities were determined by means of ^1H–^1H COSY and one bond and long-range heteronuclear ^1H–^{13}C connectivities were determined by ^1H–^{13}C COSY and HMBC, respectively. The usual pulse sequences were used in NOESY. IR: KBr pellet.

Isolation of the ent-*alloaromandendranes* (**1–3**) *and the* ent-2,3-*secoalloaromandendranes* (**8**, **9**, **11**, **12**). Gametophytes grown on MSK-4 and AP-media (201 g and 180 g, fresh wt, respectively) and suspension cells (600 g, fresh wt) were extracted with MeOH (×4) [12]. The combined MeOH soln was partitioned with *n*-pentane (×3). The resulting MeOH soln was evapd to dryness under red. pres., and chromatographed on silica gel (1.5 kg). Elution of the column with *n*-hexane–EtOAc (7:3, 2400 ml) and then EtOAc (500 ml) gave frs containing compound **3** (250 mg, frs 432–489), a mixture of **1** and **2** (348 mg, frs 490–535 and EtOAc fr.). a mixture of **8**, **9** and **11** (175 mg, frs 321–431) and **12** (371 mg frs 79–108). Compound **3** (18.4 mg) was isolated by successive HPLC of frs 432–489 on an ODS column (30 cm × 4.0 cm, i.d.) eluted with MeCN and a LiChroprep Si gel column (24 cm × 10 cm) eluted with $CHCl^3$–Me_2CO (91:9). Frs containing **1** and **2** were further sepd by HPLC on an ODS column (30 cm × 1.5 cm) eluted with MeCN and then MeOH. Compound **2** was eluted with MeCN, while **1** with MeOH. Compound **2** (3.1 mg) was further purified by CC on a silica gel column (8 g) eluted with $CHCl_3$–Me_2CO (9:1) and then HPLC on a silica gel column (24 cm × 0.46 cm) with *n*-hexane–Et_2O (3:2). Compound **1** (0.7 mg) in the MeOH eluates was isolated by HPLC on an ODS column (30 cm × 4 cm) eluted with H_2O–MeOH (1:4) and then repeated CC (×2) on a silica gel column (5 g) with $CHCl_3$–Me_2CO (3:2). The combined frs 321–431 containing **8**, **9** and **11** were further fractionated by HPLC on an ODS column (30 cm × 4 cm) with MeCN to give frs containing **8** (12.3 mg), **9** (14.5 mg) and **11** (11 mg). Each fr. was separately rechromatographed on a silica gel column with $CHCl_3$–Me_2CO to afford purified **8** (3.5 mg), **9** (4.5 mg) and **11** (2.3 mg). The combined frs 79–109 were fractionated by CC on a silica gel column (250 g) with $CHCl_3$–EtOAc (97:3), HPLC on a ODS column (30 cm × 4 cm) with MeOH and then CC on a silica gel column (5 g) with *n*-hexane–EtOAc (7:3) to yield **12** (21.2 mg).

Isolation of the ent-*bicyclogermacrane* (**6**). Freshly harvested calli (fr. wt 170 g) grown on MSK-4 medium were extracted with MeOH (×2, 340 ml) at room temp. The combined MeOH extracts were concd and fractionated on a silica gel column (150 g) with successive elution with *n*-hexane (300 ml, *n*-hexane–EtOAc (9:1, 4:1, 7:3, 3:2 and 1:1, each 300 ml). The *n*-hexane–EtOAc (1:1) eluates were rechromatographed by HPLC on a ODS column (30 cm × 4 cm, i.d.) with MeOH–H_2O (4:1) and then on a silica gel column (20 g) with $CHCl_3$–Me_2CO (10:1) to give pure **6** (27.2 mg) and **3** (27.9 mg).

Planotriol (**1**). Needles (from *n*-hexane–Et_2O), mp 129–131°; $[\alpha]_D^{19}$ + 8.60 (*c* 0.11, MeOH); CD (MeCN *c* 0.023): $\Delta\varepsilon_{207.5}$ − 2.56. EIHR-MS *m/z* (rel. int.); 252.1735 $[M]^+$ (calc. for $C_{15}H_{24}O_3$: 252.1726) (4), 234 $[M - H_2O]^+$ (23), 216 $[M - 2H_2O]^+$ (29), 191 (57), 173 (40), 161 (37), 149 (61), 135 (44), 121 (50), 107 (66), 93 (70), 91 (53), 69 (56), 43 (100). UV λ_{max}^{MeOH} nm (log ε): 205 (3.28). *IR* ν_{max}^{KBr} cm^{-1}: 3500 (OH), 2950 (CH), 1640, 1160, 1140 (tertiary OH), 1110 (secondary OH), 885 (exocyclic = CH_2), ^1H and ^{13}C NMR (see Tables 1 and 2).

Planotiol monoacetate (**2**). Oil $[\alpha]_D^{24}$ + 13.3 (*c* 0.12, MeOH). EIHR-MS *m/z* (rel. int.); 294.1861 $[M]^+$ (calc. for $C_{17}H_{26}O_4$: 294.1832) (6), 234 $[M - AcOH]^+$ (22), $[M - AcOH - H_2O]^+$ (37), 216 $[M - AcOH - 2H_2O]^+$ (37), 191 (54), 173 (55), 161 (100), 147 (27), 131 (26), 119 (28), 105 (43). 91 (30), 69 (32). UV λ_{max}^{MeOH} nm (log ε): 198 (3.27). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2900 (CH), 1730 (CH_3COO–), 1240 (CH_3COO–), 1130 (tertiary OH), 1110 (secondary OH), 885 (exocyclic = CH_2), ^1H and ^{13}C NMR (see Table 1).

Planotriol diacetate (**3**). Oil, $[\alpha]_D^{24}$ − 1.55 (*c* 0.19, MeOH). FIHR-MS *m/z* (rel. int.): 336.1927 $[M]^+$ (*m/z*; calc. for $C_{19}H_{28}O_5$, 336.1937) and FD-MS *m/z*: 336. UV λ_{max}^{MeCN} nm (log ε): 198 (3.37). *IR* ν_{max}^{KBr} cm^{-1}: 3460 (–OH), 2900 (CH), 1740 (CH_3COO–), 1245

(CH_3COO–), 1120 (tertiary OH), 885 (exocyclic = CH_2); ^1H and ^{13}C NMR (see Table 1).

Hydrolysis of compound **2** *and* **3**. Compounds **3** (1.4 mg) and **2** (0.5 mg) were dissolved in 2.5 ml and 1.0 ml of 16.7% aq. Cs_2CO_3–MeOH (25:1) soln, respectively, and stirred at room temp. for 1.5 hr. The reaction mixtures were acidified to pH 4.0 with 1N HCl and then extracted with Et_2O (×3). The Et_2O extracts were dried over dry Na_2SO_4, concd *in vacuo* to afford **1** (1.0 mg from **3** and 0.3 mg from **2**); ^1H and ^{13}C NMR, IR and $[\alpha]_D$: identical to those of **1** isolated from the cultured cells.

Esterification of planotriol (**1**) *with benzoyl chloride*. Compound **1** (2.2 mg, 8.7 μmol) was dissolved in dry pyridine (1.0 ml) containing 80 μl of benzoyl chloride (0.87 mmol) and 4-diaminopyridine (1.1 mg, 8.7 μmol) and stirred at room temp. for 18.5 hr. The usual work-up and CC on a silica gel column (15 g) eluted with *n*-hexane–Et_2O (1:1) afforded the dibenzoate **4**.

Dibenzoate of planotriol (**4**). Needles from H_2O–MeOH; mp 144–145°; CD (MeCN *c* 0.014): $\Delta\varepsilon_{235.8}$ + 1.66, $\Delta\varepsilon_{213.2}$ − 2.61. UV λ_{max}^{MeCN} nm (log ε): 238 (3.85), 273 (3.16), 280 (3.06) and λ_{max}^{MeOH} nm (log ε): 228 (4.33), 272 (3.33), 280 (3.25); ^1H NMR (270 MHz): δ 0.38 (1H, *dd*, *J* = 11.6 and 9.2 Hz, H-6), 0.68 (1H, *ddd*, *J* = 10.6, 9.2 and 5.0 Hz, H-7), 1.06 (3H, *s*, 13-Me), 1.09 (3H, *s*, 12-Me), 1.29 (1H, *m*, H-8), 1.37 (3H, *s*, 14-Me), 1.86 (1H, *m*, H-8), 2.33 (1H, *dd*, *J* = 11.6 and 9.6 Hz, H-5), 2.44 (2H, *m*, H-9s), 3.50 (1H, *dd*, *J* = 9.6 and 5.6 Hz, H-1), 4.97 (1H, *br s*, H-15Z), 5.00 (1H, *br s*, H-15*E*), 5.49 (1H, *d*, *J* = 6.3 Hz), 5.97 ((1H, *dd*, *J* = 6.3 and 5.6 Hz, H-2), 7.33, 7.51 and 7.91 (10H, aromatic Hs). ^{13}C NMR (67.8 Hz): δ 15.8 (C-13), 17.9 (C-11), 21.1 (C-8), 23.0 (C-14), 25.0 (C-7), 25.4 (C-6), 28.5 (C-12), 36.7 (C-9), 45.9 (C-5), 50.4 (C-1), 73.7 (C-2), 77.0 (C-3), 80.4 (C-4), 111.2 (C-15), 128.2, 128.3, 129.6, 129.7, 133.0, 133.2, 134.0 and 134.5 (aromatic Cs), 146.3 (C-14), 165.6 and 165.7 (carbonyl Cs).

Crystallographic data of planotriol dibenzoate (**4**). $C_{29}H_{32}O_5$: M_r = 460.57, triclinic *P*1, *a* = 13.990 (1), *b* = 15.195 (1), *c* = 6.124 (2) Å, α = 95.59 (1)°, β = 91.99 (1)°, γ106.66 (1)°, *V* = 1238.6 (4) Å^3, *Z* = 2, D_{calc} = 1.230 g cm^{-3}, Cu Kα radiation, λ = 1.54178 Å, μ = 6.7 cm^{-1}, *F*(000) = 492. Prisms were obtained from MeOH–H_2O soln. A crystal with the dimensions 0.20 × 0.30 × 0.05 mm^3 was used for X-ray measurement at 295 K on a Rigaku AFC5R diffractometer equipped with a graphite monochromator. The data were collected to a maximum 2θ of 140° by w/2θ scanning. The total number of independent reflections measured was 4516. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods [30]. The non-hydrogen atoms were refined anisotropically. The positional parameters of H-atoms in the OH groups were refined, while the rest were included in fixed positions. The final cycle of full-matrix least-squares refinement was based on 3685 observed reflections [$I \geq \sigma(I)$] and 613 variable parameters. The weighting scheme was w/ σ^2(F). The final *R* and *wR* values were 0.061 and 0.051, respectively. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.22 and −0.21 e Å^{-3}, respectively. Neutral atom scattering factors were taken from Cromer and Waber [31]. All crystallographic calcns were performed using TeXsan [32] crystallographic software package and VAX 3100 workstation.

A list of atomic coordinates, thermal parameters, bond distances and bond angles is deposited at the Cambridge Crystallographic Data Center.

Esterification of compound **2** *with* (a*R*)*-MNCB and* (a*S*)*-isomer*. Compound **2** (1.0 mg, 3.4 μmol) was dissolved in 120 μl of CH_2Cl_2 containing (a*S*)-MNCB (2.2 mg, 6.4 μmol), 1,3-dicyclohexylcarbodiimide (1.75 mg, 8.5 μmol) and 4-pyrrolidinylpyridine (0.5 mg, 3.4 μmol) and stirred at room temp. for 8 hr. The reaction mixt was concd *in vacuo* and chromatographed on a silica gel column (5 g) eluted with *n*-hexane–AcOEt (4:1) to afford 1.4 mg of purified (a*S*)-MNCB ester (**5a**) of **2**. (a*R*)-MNCB ester (**5b**) of **2** was also prepared with (a*R*)-MNCB by the identical procedure. Compound **5a** and **5b**; ^1H NMR: see Table 1; ^{13}C NMR (67.8 Mz, in $CDCl_3$) of **5a**: δ 15.7 (C-13), 17.5 (C-11), 20.6 (acetyl Me), 20.8 (C-8), 23.0 (C-14), 24.6 (C-7), 24.9 (C-6), 28.3 (C-12), 36.5 (C-9), 45.4 (C-5), 48.7 (C-1), 71.9 (C-2), 77.9 (C-3), 78.8 (C-4), 110.6 (C-15), 145.8 (C-14), 170.2 (acetyl C=O), {δ_Cs of carbons in MNCB moiety 56.6, 113.4, 123.4, 124.4, 127.8, 128.6, 128.7, 130.0, 130.5, 132.5, 132.5, 133.2, 134.6, 137.2, 136.9, 165.2}.

ent-3β-*Acetoxy*-2β-*hydroxy*-*bicyclogermacrene*(**6**). $[\alpha]_D^{21}$ + 69.3 ($CHCl_3$, *c* 0.221), UV λ_{max}^{EtOH} nm (log ε): 229 (3.81), EIHR-MS *m/z* (rel. int.): 278.1846 ([M]$^+$: calcd for $C_{19}H_{28}O_5$; 278.1883) (1), 236 (7), 218 (32), 194 (28), 175 (9), 152 (100), 137 (45), 123 (29), 109 (74), 95 (42), 84 (37), 43 (91), IR ν_{max}^{KBr} cm^{-1}: 3400, 2990, 2905, 2885, 1740, 1368, 1235, 938, 895, 838, ^1H and ^{13}C NMR (see Table 2).

Esterification of compound **6** *with* (a*S*)*-MNCB and* (a*R*)*-isomer*. (a*S*)- (**7a**) and (a*S*) and (a*R*)-MNCB by the identical procedure for **5**. Compound **7a** and **7b**; ^1H NMR: see Table 2, **7a**; ^{13}C NMR (67.8 Mz, in $CDCl_3$): 15.3 (C-13), 15.6 (C-14), 20.9 (acetyl Me), 21.2 (C-11), 21.8 (C-15), 27.0 (C-8), 27.1 (C-6), 29.0 (C-12), 31.2 (C-7), 37.6 (C-9), 72.5 (C-2), 78.8 (C-3), 118.3 (C-3), 125.5 (C-4), 128.9 (C-5), 144.8 (C-10), 169.7 (acetyl C=O), {δ_Cs of carbons in MNCB moiety 56.4, 113.3, 119.9, 123.5, 124.1, 126.7, 128.1, 128.7, 129.1, 129.9, 132.4, 132.7, 133.9, 134.2, 135.7, 136.9, 153.6, 165.1}.

ent - 2,3 - *Diacetoxy* - 10α,15α - *epoxy* - 2,3 - *seco-alloaromandendra* - 4(14) - *ene* (**8**). Oil, $[\alpha]_D^{23}$19.8 ($CHCl_3$, *c* 0.353), UV λ_{max}^{EtOH} nm (log ε): 210.5 (3.32). EIHR-MS *m/z* (rel. int.): 336.1905 ([M]$^+$: calc. for $C_{19}H_{28}O_5$; 336.1937) (0.3), 293 (0.5), 277 (4), 263 (2), 216 (12), 201 (11), 185 (13), 173 (22), 143 (29), 131 (20), 107 (69), 91 (37), 79 (25), 69 (20), 43 (100). ^1H NMR δ (270 MHz, in $CDCl_3$): 0.61 (1H, *dd*, *J* = 11.6 and 9.2 Hz, H-6), 0.84 (1H, *m*, H-7), 0.95 (3H, *s*,

13-Me), 1.10 (3H, *s*, 12-Me), 1.14 (2H, *m*, H-8α and H-9α), 1.84 (1H, *dd*, $J = 6.2$ and 8.3 Hz, H-1) 1.98 (1H, *m*, H-8β), 2.03 (3H, *s*, acetyl Me), 2.08 (3H, *s*, acetyl Me), 2.18 (1H, *d*, $J = 9.2$ Hz, H-5), 2.27 (1H, *m*, H-9β), 2.52 (2H, *s*, 2 × H-15), 4.43 (1H, *dd*, $J = 11.2$ and 6.3 Hz, H-2a), 4.51 (1H, *dd*, $J = 11.2$ and 8.3 Hz, H-2b), 4.55 (1H, *d*, $J = 13.2$ Hz, H-3c), 4.62 (1H, *d*, $J = 13.2$ Hz, H-3d), 5.14 and 5.20 (2H, 2 × *b s*, 2 × H-14). ^{13}C NMR δ (67.8 Mz, in $CDCl_3$): 14.4 (C-13), 18.5 (C-11), 21.0 (2 × acetyl Me), 21.3 (C-8), 25.4 (C-6), 26.1 (C-7), 28.6 (C-12), 33.0 (C-9), 38.0 (C-5), 47.1 (C-1), 52.9 (C-15), 62.5 (C-10), 62.6 (C-2), 66.5 (C-3), 114.1 (C-14), 145.1 (C-4), 170.5 and 171.2 (2 × acetyl C=O). ^{1}H and ^{13}C assignments were based on DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, NOESY, difference NOE and HMBC experiments.

4-O-Deacetylplagiochiline C {ent-2β-*acetoxy*-14-*hydroxy*-2,3-*epoxy*-2,3-*secoalloaromandendrene* (**9**)}. Oil, $[\alpha]_D^{25}$ + 13.0 ($CHCl_3$, *c* 0.247), UV λ_{max}^{EtOH} nm (log ε): 214.5 (3.33), *IR* ν_{max}^{KBr} cm^{-1}: 3400, 1755, 1730, 1665, 1630, 1180, 1140, 990, 895, 855, EIHR-MS *m/z* (rel. int.): 292.1681 ([M]$^+$: calc. for $C_{17}H_{24}O_4$, 292.1675) (6), 275 (5), 249 (7), 232 (100), 214 (25), 199 (2), 189 (94), 171 (28), 161 (20), 150 (28), 143 (23), 135 (16), 119 (15), 109 (28), 91 (29), 79 (19), 69 (14), 43 (47). ^{1}H NMR δ (270 MHz, in $CDCl_3$): 0.53 (1H, *dd*, $J = 9.9$ and 8.6 Hz, H-6), 0.88 (1H, *m*, H-7), 0.94 (1H, *m*, H-8α), 1.06 (3H, *s*, 12-Me), 1.07 (3H, *s*, 13-Me), 2.06 (1H, *m*, H-9α), 2.09 (3H, *s*, acetyl Me), 2.13 (1H, *m*, H-8β), 2.21 (1H, *dd*, $J = 3.6$ and 9.9 Hz, H-5), 2.33 (1H, *dd*, H-9β), 2.79 (1H, *dd*, $J = 9.9$ and 3.6 Hz, H-1), 4.00 (2H, *br s*, 2 × H-14s), 4.76 (1H, *d*, $J = 2.3$ Hz, H-15*Z*), 4.79 (1H, $J = 2.3$ Hz, H-15*E*), 6.26 (1H, *s*, H-3), 6.58 (1H, *d*, $J = 9.9$ Hz, H-2). ^{13}C NMR δ (67.8 Mz, in $CDCl_3$): 15.8 (C-13), 19.6 (C-11), 21.0 (acetyl Me), 25.6 (C-8), 28.8 (C-12), 29.3 (C-7), 29.6 (C-6), 33.4 (C-5), 35.2 (C-9), 51.6 (C-1), 61.7 (C-14), 91.4 (C-2), 116.3 (C-15), 120.8 (C-4), 137.8 (C-3), 148.5 (C-10) and 169.8 (acetyl C=O). ^{1}H and ^{13}C assignments were based on DEPT, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, NOESY, difference NOE and HMBC experiments.

Acetylation of compound **9**. 4-*O*-deacetoxyplagiochiline C (**9**) was acetylated with Ac_2O–pyridine to afford plagiochiline C (**10**); $[\alpha]_D^{22}$: +23.8 ($CHCl_3$, *c* 0.264, lit. [26, 27], +24.5 or +28), and ^{1}H [18, 27] and ^{13}C NMR [25] spectra were identical to those reported for plagiochiline C from natural sources.

Plagiochiline A (**11**). $[\alpha]_D^{24}$ {+34 (*c* 0.18, lit [26], +32.3)}, EI-MS [26] and ^{1}H [20] and ^{13}C NMR spectra [19] of compound **11** were identical to those reported for plagiochiline A.

Methoxyplagiochiline A_2(**12**). Oil, $[\alpha]_D^{26}$ + 9.0 (*c* 0.20, $CHCl_3$). EI-MS *m/z* (rel. int.): 262 (9), 159 (55), 131 (74), 121 (53), 109 (44), 105 (76), 91 (100), 79 (48), 77 (47), 69 (67), 43 (82) and ^{1}H NMR (in $CDCl_3$) spectra of compound **12** were identical to those reported for methoxyplagiochiline A_2 [21]. UV λ_{max}^{EtOH} nm (log ε): 208 nm (4.5). ^{1}H NMR δ [270 MHz, in $CDCl_3$ (in C_6H_6)]: 0.82 (0.39, 1H, *m*, H-7), 0.84 (0.86, 1H, *m*, H-6), 1.03 (0.92, 3H, *s*, Me-12), 1.08 (0.76, 1H, *m*, H-8α), 1.09 (0.92, 3H, *s*, Me-13), 1.10 (0.90, 1H, *m*, H-9α), 2.0 (1.59, *m*, H-8β), 2.09 (2.31, 1H, *m*, $J = 9.6$ Hz, H-1), 2.16 (1.91, 3H, *s*, acetyl Me), 2.31 (2.44, 1H, *m*, $J = 2.0$ Hz, H-9β), 2.40 (2.26, 1H, *m*, H-5), 2.42 (1.90, 1H, *d*, $J = 4.6$ Hz, H-15 *E*), 2.48 (1.94, 1H, *dd*, $J = 4.6$ and 2.0 Hz, H-15 *Z*), 3.45 (3.36, 3H, *s*, methoxy Me), 4.97 and 5.01 (4.87 and 4.92, each 1H, *s*, 2 × H-14s), 5.18 (5.16, 1H, *s*, H-3), 6.70 (7.23, 1H, *d*, $J = 9.6$ Hz, H-2). ^{13}C NMR δ (67.8 MHz, in $CDCl_3$): 15.7 (C-13), 18.3 (C-11), 21.4 (acetoxy Me), 21.6 (C-8), 27.8 (C-7), 28.9 (C-12), 29.4 (C-6), 33.8 (C-9), 35.7 (C-5), 47.2 (C-1), 52.2 (C-15), 55.5 (methoxy C), 60.2 (C-10), 91.4 (C-2), 101.0 (C-3), 112.7 (C-14). 147.9 (C-4), 170.6 (acetoxy C=O). ^{1}H and ^{13}C assignments were based on DEPT, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, NOESY, difference NOE and HMBC experiments.

Conversion of planotiol diacetate (**3**) *to compound* **13**. The diacetate **3** (4.0 mg, 12.0 μmol) was dissolved in 0.5 ml dry pyridine containing 54 μl (0.6 mmol) of $POCl_3$, stirred at room temp. for 72 hr and extracted with Et_2O after addition of 1 ml H_2O. The Et_2O extracts were washed with 1N HCl, dried over dry Na_2SO_4, and passed through a silica gel column (3 g) in $CHCl_3$–Me_2CO to remove the unreacted **3**. A mixture containing the dehydrated compounds in MeOH (2.0 ml) was treated with 25 μl of 16.7% aq. Cs_2CO_3 for 3 hr. Usual work-up and chromatography of the residue on a silica gel column (8 g) with $CHCl_3$–acetone (4:1) afforded *ent*-2β,3β-dihydroxyalloaromandendra-4 (14), 10 (15)-diene (**13**) (0.3 mg), $[\alpha]_D^{23}$ + 28 (*c* 0.03, $CHCl_3$). EI-MS *m/z* (rel. int.): 234.1608 ([M]$^+$: calc. for $C_{15}H_{22}O_2$, 234.1621) (11), 275 (5), 216 (62), 201 (26), 191 (50), 173 (94), 159 (30), 145 (66), 131 (54), 109 (62), 105 (62), 91 (79), 79 (58), 69 (95), 59 (95), 55 (75), 43 (63) and 41 (100). ^{1}H NMR δ (270 MHz, in $CDCl_3$): 0.37 (1H, *dd*, H-6), 0.54 (1H, *ddd*, H-7), 1.02 (3H, *s*, Me-12), 1.06 (3H, *s*, Me-13), 1.21 (1H, *m*, H-8α), 1.78 (1H, *m*, H-8β), 2.43 (2H, *m*, 2 × H-9s), 2.87 (1H, *m*, H-5), 2.92 (1H, *m*, H-1), 4.27 (1H, *m*, H-2), 4.35 (1H, *br s*, H-3), 4.90 (1H, *s*, H-15 c), 4.99 (2H, *m*, H-15d and H-14b), 5.32 (1H, *d*, $J = 1.6$ Hz, H-14a). ^{13}C NMR δ (67.8 MHz, in $CDCl_3$): 15.7 (C-13), 17.4 (C-11), 21.4 (C-8), 25.1 (C-7), 28.6 (C-12), 29.7 (C-6), 37.6 (C-9), 39.0 (C-5), 50.7 (C-1), 74.8 (C-2), 75.9 (C-3), 109.9 (C-15), 112.7 (C-14). 147.2 (C-10), 154.7 (C-4). ^{1}H and ^{13}C assignments were based on DEPT, ^{1}H–^{1}H COSY, NOESY and difference NOE.

Oxidative cleavage of **13** *to* (−)-*hangeokedial* (**14**). The 1,2-diol **13** (1.3 mg, 5.6 μmol) was treated with 20 mg KIO_4 (87 μmol) in a two layer soln of Et_2O–H_2O (1.5 ml, 2:1, v/v) [33] and stirred at 0° for 32 hr. Usual work-up and prep. TLC {an ODS plate, C_6H_6–Et_2O (9:1)} afforded hanegokedial (0.8 mg) containing a trace amount of concomitant which could not be removed by chromatography; $[\alpha]_D^{22}$ ±0 (lit. [18] − 10.0), ^{1}H NMR spectrum δ (270 MHz, in $CDCl_3$): 0.88 (4H, unresolved *s* and *m*, Me-13 and H-7), 1.00 (1H,

dd, $J = 11.9$ and 9.2 Hz, H-6), 1.08 (3H, *s*, Me-12), 1.14 (1H, *m*, H-8α), 2.08 (1*H*, *m*, H-8β), 2.32 (1H, *m*, H-9α), 2.51 (1H, *m*, H-9β), 2.62 (1H, *d*, $J = 11.9$ Hz, H-5), 3.36 (1H, *br s*, H-1), 4.89 and 4.98 (each 1H, each *s*, 2 × H-15s), 6.22 and 6.57 (each 1H, each *d*, $J = 1.0$H, 2 × H-14s), 9.62 (1H, *s*, H-3), 9.74 (1H, *d*, $J = 1.0$ Hz, H-2).

Acknowledgement—This research has been financially supported by the Suhara Memorial Foundation.

REFERENCES

1. Asakawa, Y. (1982) *Progress in the Chemistry of Organic Natural Products* (Herz, W., Griesebach, H. and Kirby, G., eds), Vol. 42, p. 2. Springer-Verlag, Wien.
2. Takeda, R. and Katoh, K. (1983) *J. Am. Chem. Soc.* **105**, 4056.
3. Nabeta, K., Mototani, Y., Tazaki, H. and Okuyama, H. (1994) *Phytochemistry* **35**, 915.
4. Nabeta, K., Ishikawa, T., Kawae, T. and Okuyama, H. (1994) *J. Chem. Soc. Perkin I*, 3277.
5. Tazaki, H., Nabeta, K., Okuyama, H. and Becker, H. (1995) *Biosci. Biotech. Biochem.* **59**, 158.
6. Nabeta, K., Ishikawa, T., Kawae, T. and Okuyama, H. (1995) *J. Chem. Soc., Chem. Commun.*, 681.
7. Nabeta, K., Kigure, K., Fujita, M., Nagoya, T., Ishikawa, T. and Okuyama, H. (1995) *J. Chem. Soc. Perkin I*, 1935.
8. Nabeta, K., Ishikawa, T. and Okuyama, H. (1995) *J. Chem. Soc. Perkin I*, 3111.
9. Nabeta, K., Kawae, T., Kikuchi, T., Saitoh, T. and Okuyama, H. (1995) *J. Chem. Soc., Chem. Commun.* 2529.
10. Asakawa, Y. (1990) in *Bryophytes: Their Chemistry and Chemical Taxonomy* (Zinsmeister, H. D. and Mues, R., eds). Oxford University Press, Oxford.
11. Zinsmesiter, H. D., Becker, H. and Eicher, T. (1991) *Angew. Chem. Int. Ed. Engl.* **30**, 130.
12. Nabeta, K., Katayama, M., Nakagawara, S. and Katoh, K. (1993) *Phytochemistry* **32**, 117.
13. Nabeta, K., Oohata, N., Izumi, N. and Katoh, K. (1994) *Phytochemistry* **37**, 1263.
14. Nabeta, K., Oohata, N., Ohkubo, S., Sato, T. and Katoh, K. (1996) *Phytochemistry* **41**, 581.
15. Hashimoto, T., Nakamura, I., Tori, M., Takaoka, S., Asakawa, Y. (1995) *Phytochemistry* **38**, 119.
16. Fukushi, Y., Yajima, C. and Mizutani, J. (1994) *Tetrahedron Letters* **35**, 599.
17. Fukushi, Y., Yajima, C. and Mizutani, J. (1994) *Tetrahedron Letters* **35**, 9417.
18. Matsuo, A., Atsumi, K., Nakayama, M. and Hayashi, S. (1979) *J. Chem. Soc., Chem. Commun.* 1010.
19. Asakawa, Y., Toyota, M., Takemoto, T., Kubo, I. and Nakanishi, K. (1980) *Phytochemistry* **19**, 2147.
20. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Tetrahedron Letters* 1553.
21. Asakawa, Y., Toyota, M. and Takemoto, T. (1980) *Phytochemistry* **19**, 2141.
22. Ramanoella, A. R. P., Rakotonirainy, O., Bianchini, O. and Gaydou, E. M. (1991) *Mag. Reson. Chem.* **29**, 967.
23. Weenen, H., Nkunya, M. H. H., Mgani, Q. A., Posthumus, M. A., Waibel, R. and Achenbach, H. (1991) *J. Org. Chem.* **56**, 5865.
24. Nishimura, K., Horibe, I. and Tori, K. (1973) *Tetrahedron* **29**, 271.
25. Matsuo, A., Atsumi, K., Nadaya, K., Nakayama, M. and Hayashi, S. (1981) *Phytochemistry* **20**, 1065.
26. Matsuo, A., Atsumi, K., Nakayama, M. (1981) *J. Chem. Soc. Perkin I*, 2816.
27. Asakawa, Y., Toyota, M., Takemoto, T. and Suire, C. (1979) *Phytochemistry* **18**, 1355.
28. Fukuyama, Y. and Asakawa, Y. (1991) *Phytochemistry* **30**, 4061.
29. Andersen, N. H., Ohta, Y. and Syrdal, D. D. (1978) in *Bioorganic Chemistry*, Vol. II (van Tamelen, E. E. ed.), P.I. Academic Press, New York.
30. MULTAN 88: Dabaerdemaeker, T., Germain, G., Main, P., Rafaat, L. S., Tate, C. and Woolfson, M. M. (1988) Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data. University of York, York, U.K.
31. Cromer, D. T. and Waber, J. T. (1974) in *International Table for X-ray Crystallography*, vol. IV, Table 2.2A. The Kynoch Press, Birmingham, England.
32. teXan: Crystal Structure Analysis Package, Molecular Structure Corporation.
33. Brewster, D., Meyers, M., Ormerod, J., Otter, P., Smith, A. C. B., Spinner, M. E. and Turner, S. (1973) *J. Chem. Soc. Perkin I*, 2796.