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INSECT ANTIFEEDANT SECOAROMADENDRANE-TYPE SESQUITERPENES FROM *PLAGIOCHILA* SPECIES*

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Key Word Index — *Plagiochila fruticosa*; *P. hattoriana*; *P. ovalifolia*; *P. yokogurensis*; Hepaticae; plagiochilines A, B and C; plagiochilide; hanegokedial (plagiochilal A); furanoplagiochilal; *ent*-secoaromadendrane-type sesquiterpenes; insect antifeedant activity; pungency.

Abstract—The characteristic pungency of the liverworts *Plagiochila* species *P. fruticosa*, *P. hattoriana*, *P. ovalifolia* and *P. yokogurensis* is due to a new *ent*-secoaromadendrane-type sesquiterpene hemiacetal, plagiochiline A, which exhibits very strong antifeedant activity against the African army worm, *Spodoptera exempta* at 1–10 ng/cm^2. Two new secoaromadendranes, plagiochilide and furanoplagiochilal A, together with the previously known plagiochiline C were isolated from *P. yokogurensis*. Plagiochilal A, which may be a precursor of plagiochilide and its related hemiacetals, and a bitter principle, plagiochiline B were also isolated from *P. hattoriana*. *P. ovalifolia* contained plagiochilines A, B and C. From *P. fruticosa*, plagiochilide and plagiochilines A, B and C were isolated. The structures of the new secoaromadendrane-type sesquiterpenes were elucidated by extensive ^{1}H NMR and ^{13}C NMR studies.

INTRODUCTION

In spite of Muller's suggestion that the components of the oil bodies found in liverworts consisted of sesquiterpenes [2], the chemical constituents have only been investigated during the last 10 years, because the collection and separation of a large quantity of a pure species and its identification are difficult and time-consuming. Some bryophytes contain biologically active substances, e.g. compounds with allergenic contact dermatitis [3,4], anticancer [5, 6] and antibiotic activities [7]. As part of our investigations on such biologically active substances of Hepaticae, and as an extension of a chemosystematic investigation on their terpenoids and lipophilic aromatic compounds, we have recently reported the isolation and the structures of various sesqui- and diterpenoids which cause allergy and have an intense pungent taste [8–19]. The leafy liverworts, Plagiochila species (Plagiochilaceae), also produce characteristic pungent substances and their crude extracts exhibit plant growth inhibitory activity. In preliminary papers, we have reported the isolation of the unique secoaromadendrane-type sesquiterpenes, plagiochilines A (**1**), B (**2**), C (**3**), D, E and F, and plagiochilide (**4**), together with various mono- and sesquiterpene hydrocarbons from *P. asplenioides*, *P. hattoriana*, *P. semidecurrens* and *P. yokogurensis* [20–22]. These secoaromadendrane-type sesquiterpenes are widely distributed in Plagiochilaceae [23, 24].

In the present paper, we report the structures of five new *ent*-secoaromadendrane-type sesquiterpenes, plagiochiline A (**1**), which is the strong insect antifeedant, plagiochiline B (**2**), plagiochilide (**4**), hanegokedial (plagiochilal A) (**5**) and furanoplagiochilal (**6**) isolated from four *Plagiochila* species.

RESULTS AND DISCUSSION

Column chromatography and PLC on Si gel of the crude extract of *P. yokogurensis* resulted in the isolation of plagiochiline A (**1**), plagiochiline C (**3**) [22], plagiochilide (**4**) and furanoplagiochilal (**6**). By the same method, plagiochilines A (**1**), B (**2**) and C (**3**) were also isolated from P. ovalifolia, four *ent*-secoaromadendrane-type hemiacetals (**1**–**4**) were obtained from *P. fruticosa*, and the hemiacetals **1** and **2** and hanegokedial (plagiochilal A) (**5**) were isolated from *P. hattoriana*.

Plagiochiline A (**1**), $C_{19}H_{26}O_6$ (M^+ at *m/e* 350.1750), showed the presence of two acetoxyl groups [1740, 1237 cm^{-1}; δ 2.03, 2.19 (each 3 H, *s*)] and a non-conjugated double bond [205 nm (ε, 1312)]. In the IR spectrum, absorption bands for carbonyl or hydroxyl groups (except for the acetoxyl group) could not be observed, indicating the additional two oxygens to be ethers. Hydrogenation of **1** in the presence of PtO_2 gave a dihydrodiacetate (**7**) [$C_{19}H_{28}O_6$ (M^+ at *m/e* 352), 1748 cm^{-1}; δ 0.98, 1.11 (each 3 H, *s*)] and dihydromonoacetate (**8**) [$C_{17}H_{26}O_4$ (M^+ at *m/e* 294), 1750 cm^{-1};

* Part of the present work was reported at the 21st Symposium of the Chemistry of Terpenes, Essential Oils and Aromatics of Japan, Tokushima, Symposium Paper 225 (November 1977) and International Congress of Bryology, Bordeaux (November 1977) [1].

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Table 1. ^{1}H NMR chemical shifts of the new secoaromadendrane-type sesquiterpenes (ppm from internal TMS)*

	1		2	4	5	6
H-1	1.70 (*dd*, 10, 4)	−3.39†	1.77 (*dd*, 11, 3)	3.53 (*d*, 5)		
H-2	6.79 (*d*, 10)	−4.16†	6.93 (*d*, 11)		9.67 (*d*, 1.5)	7.06 (*br. s*)‡
H-3	6.28 (*d*, 0.8)	−2.44†	6.47 (*br s*)	6.24 (*d*, 2)	9.57 (*s*)	9.61 (*s*)
H-5	2.10 (*dd*, 10, 5)	−3.20†		1.90 (*dd*, 10, 5)		3.75 (*d*, 10)
H-6	0.55 (*dd*, 10, 10)	−3.90†	0.8 (*dd*, 10, 10)	0.43 (*dd*, 10)		0.90 (*dd*, 10)
H-7	0.90 (*m*)			0.84 (*m*)		
H-8a	1.1–2.1 (*m*)			0.86 (*m*)		
H-8b				2.05 (*m*)		
H-9	1.0–2.0 (*m*)			2.50 (*m*)	2.20 (*m*)	2.80 (*m*)
H-11a	2.42 (*d*, 4.5)	−1.75†	2.55 (*br s*)	4.76 (*d*, 2)	4.50 (*s*)	
H-11b	2.48 (*dd*, 4.5, 1.5)	−2.16†		4.92 (*br s*)	4.85 (*d*, 2)	6.53 (*br s*)‡
H-12a	4.40 (*d*, 12)	−7.28†	4.13 (*d*, 12)		6.20 (*s*)	6.21 (*s*)
H-12b	4.40 (*dd*, 12, 0.8)	−7.24†	4.49 (*d*, 12)	1.74 (*d*, 2)	6.52 (*s*)	6.55 (*s*)
H-14	1.07 (*s*)	−1.76†	4.60 (*br s*)	1.06 (*s*)	1.00 (*s*)	1.06 (*s*)
H-15	1.02 (*s*)	−0.91†	1.20 (*s*)	1.06 (*s*)	1.16 (*s*)	1.06 (*s*)
OAc-2	2.03 (*s*)	−1.59†	2.12 (*s*)§			
OAc-12	2.19 (*s*)	−6.19†	2.15 (*s*)§			
OAc-14			2.20 (*s*)§			

* All assignments were confirmed by double resonance experiments.
† $\Delta Eu = \delta_{CDCl_3} - \delta_{Eu(fod)_3}$, plagiochiline A (10 mg) containing 3 mg of $Eu(fod)_3$.
‡ The top of the signals were complicated.
§ These signals may be interchanged.

Table 2. ^{13}C NMR chemical shifts of the new secoaromadendrane-type sesquiterpenes (ppm from internal TMS)*

	1	*J*†	2	4	6
C-1	49.8‡ *d*	130.6	49.9 *d*	53.7 *d*	128.3 *s*
C-2	91.6 *d*	170.9	91.6 *d*	170.0 *s*	138.0 *d*
C-3	140.2 *d*	190.5	140.9 *d*	135.1 *d*	193.5 *d*
C-4	116.1 *s*		115.9 *s*	124.5 *s*	152. 1 *s*
C-5	31.6‡ *d*	130	31.6 *s*	38.9 *d*	31.9 *d*
C-6	29.7 *d*	174	30.4 *d*	29.3 *d*	29.9 *d*
C-7	27.6 *d*	162	27.7 *d*	27.6 *d*	27.1 *d*
C-8	21.6 *t*	126.4	21.7 *t*	25.4 *t*	21.3 *t*
C-9	33.7 *t*	129.3	33.8 *t*	35.2 *t*	23.8 *t*
C-10	59.8 *s*		59.9 *s*	147.4 *s*	122.4 *s*
C-11	51.1 *t*	184.6§	51.6 *t*	116.9 *t*	140.7 *s*
C-12	63.0 *t*	148.3§	63.4 *t*	16.1 *q*	135.8 *t*
C-13	18.8 *s*		22.7 *s*	19.5 *s*	19.3 *s*
C-14	15.5 *q*	125.7	65.3 *t*	15.5 *q*	14.8 *q*
C-15	28.5 *q*		24.2 *q*	28.7 *q*	28.9 *q*
OAc	20.8 *q*	130.1	20.8 *q*		
	169.1 *s*		171.0 *s*		
	20.6 *q*	129.4	21.2 *q*		
	170.6 *s*		170.0 *s*		
			21.0 *q*		
			171.6 *s*		

* The spectra were obtained at 25.03 MHz in Fourier transform mode in $CDCl_3$ solutions (*ca* 6 mg in 30 μl).
† Coupling constant ($J = {}^{13}C-H$).
‡ Irradiation at 2.10 ppm in ^{1}H NMR collapsed the signal at 31.6 ppm (C-5) into a sharp singlet, while the lower field methine signal at 49.8 (C-1) showed weak residual coupling, thus the lower-field signal is assigned to C-1 and the higher one to C-5.
§ C-11 was differentiated from C-12 by the magnitude of residual coupling. C-11 was decoupled more than C-12.

δ0.80 (3H, *d*, $J = 7$ Hz), 0.97, 1.10 (each 3H, *s*)], suggesting the presence of an allylic acetoxymethyl group in **1**. The treatment of **1** with *m*-chloroperbenzoic acid gave an epoxide (**9**) [$C_{19}H_{26}O_7$ (M^+ at *m/e* 366)] indicating that **1** contained one double bond. Reduction of **1** with $NaBH_4$ gave a labile alcohol (**10**) (3400, 1030 cm^{-1}) showing that one of the two acetoxyl groups had the hemiacetal nature. The ^1H NMR (Table 1) and ^1H NMDR spectra of **1** contained the signals of two acetoxyl groups, two tertiary methyls, one methylene located between an acetoxyl group and a double bond, an olefinic proton on a carbon bearing an ether oxygen, two protons on an oxyrane ring, two protons on a cyclopropane ring and one proton on a carbon bearing an acetoxyl group. The above chemical and spectral evidence, along with the molecular formula, indicated that **1** was a tetracylic sesquiterpene hemiacetal. The structure of plagiochiline A was confirmed as **1** by various ^1H NMR (Table 1) and ^{13}C NMR (Table 2) techniques. The nature of all 19 carbons was determined by ^{13}C NMR of PND, selective decoupling, partial relaxed Fourier transform (PRFT), and gated decoupling (proton-coupled spectra). The complex ^1H NMR spectrum of **1** was analysed with the aid of $Eu(fod)_3$, and the proton systems (Fig. 1) were disclosed. The confirmation of an epoxide ring was based on the following evidence. The ^1H NMR signals of H-11a and H-11b showed typical chemical shifts (δ 2.42, 2.48) and geminal coupling ($J = 4.5$ Hz). The ^{13}C NMR signal of C-11 showed a typical chemical shift (51.1) and coupling J_{C-H} ($J = 184.6$ Hz) of an epoxide. Since one proton at H-11 (2.48) showed *W*-type coupling with H-9, the oxygen atom on C-10 must have the equatorial (β) orientation. The presence of a cyclopropane ring was confirmed by the higher chemical shift of H-6 (0.55) and H-7 (*ca* 0.9) and the higher chemical shift of C-13 (18.8). The ^{13}C NMR signals at 29.7 and 27.6 which become singlets upon irradiation of the proton region at 0.6 were assigned to C-6 and C-7 because of their relation with H-6 and H-7. Moreover, the magnitude of their J_{C-H} coupling also supported this assignment. The signal at 27.6 which showed as an unclear doublet in the CWD, due to the second-order coupling, was assigned to C-7 and the clearer doublet at 29.7 was assigned to C-6. The confirmation of the relationship between C-3 to C-4 and C-12, and between C-2 to C-1 and C-5 was based on double resonance studies. One of the H-12 protons (4.44) showed coupling (0.8 Hz) with H-3 (6.28). Irradiation of H-12 led to 10% NOE for H-3. The proton-coupled ^{13}C NMR signal of C-3 (140.2) showed a much larger J_{C-H} coupling than a normal sp^2 carbon, thus C-3 must be connected with oxygen to C-2. Irradiation of H-2 [10.7 in $CDCl_3/Eu(fod)_3$] collapsed the double-doublet signal at 5.06 (H-1) into a doublet. Irradiation of H-1 led to a doublet for the H-5 which previously was a doublet of doublets ($J = 10$ and 4 Hz). Thus, the gross structure of plagiochiline A was represented as **1**. The fragment ion for $M^+ - C_3H_7$ (obs. *m/e* 307. 1188) and the well-separated IR bands at 1370 and 1390 cm^{-1} (gem-dimethyl), together with biogenetic considerations, further supported structure **1** for plagiochiline A. The absolute configuration of **1** was established by the positive Cotton effect [25] and the coexistence of (+)-cyclocolorenone (**17**) [10].

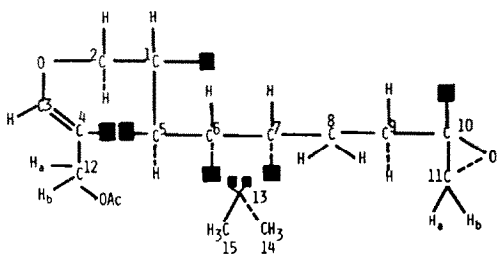


Fig. 1.

The structure of the bitter hemiacetal, plagiochiline B (**2**), has been reported but without the stereochemistry of the C-10 epoxide and C-15 acetoxymethyl group [20]. The ^1H NMR (Table 1) and IR spectra were quite similar to those of plagiochiline A, except for the presence of an additional acetoxyl group instead of one quaternary methyl group indicating that plagiochiline B possessed the same skeleton as that of **1** and one of the gem-dimethyls was substituted by an acetoxymethyl group. The stereochemistry of the C-15 acetoxymethyl group was confirmed by the ^{13}C NMR spectrum. In plagiochiline A (**1**), the C-14 methyl group appeared at 15.5 and the C-15 methyl group at 28.5. On the other hand, in plagiochiline B, a higher signal at 15.5 disappeared and the quartet signal of a tertiary methyl group at 24.2 and a triplet signal at 65.3 assignable to a methylene group bearing an oxygen atom could be observed. The above ^{13}C NMR spectral data clearly showed that the acetoxymethyl group at C-13 in **2** was α-oriented.

Plagiochilide (**4**), mp 110–111°, $C_{15}H_{20}O_2$ (M^+ at *m/e* 232.1454) showed evidence for the presence of a lactone group (1760 cm^{-1}) and an exocyclic double bond [1640 cm^{-1}; δ 4.76, 4.92 (2H)]. Hydrogenation of **4** in the presence of PtO_2 gave two inseparable isomeric tetrahydro derivatives (**11**), $C_{15}H_{24}O_2$, whose IR spectrum showed a band at 1735 cm^{-1}, characteristic of a saturated δ-lactone. Treatment of **4** with *m*-chloroperbenzoic acid afforded a monoepoxide (**12**), ($C_{15}H_{20}O_3$, M^+ at *m/e* 248: 1760 cm^{-1}) and a diepoxide (**13**) ($C_{15}H_{20}O_4$, M^+ at *m/e* 264; 1760 cm^{-1}), indicating the presence of two double bonds. The reduction of **4** with $LiAlH_4$ gave a diol (**14**) (3400 cm^{-1}), which was oxidized by CrO_3–pyridine to afford a δ-lactone (**15**), ($C_{15}H_{22}O_2$, M^+ at *m/e* 234; 1742 cm^{-1}). The above chemical and spectral evidence, coupled with the molecular formula, showed that **4** was a tricyclic sesquiterpene containing a δ-lactone. The structure of plagiochilide was confirmed by a ^1H NMR spin-decoupling experiment and by the ^{13}C NMR spectrum. The ^1H NMR spectrum (Table 1) included signals due to two tertiary methyl groups, a non conjugated exocyclic methylene group, one olefinic proton on a carbon-bearing oxygen, one methine located between a carbonyl group and a double bond, an allylic methylene group and a vinylic methyl group. The presence of the two protons on the cyclopropane ring was also confirmed by the higher chemical shifts at 0.43 and 0.84 in the ^1H NMR spectrum. Irradiation at the centre of a broad triplet at 0.43 ($J = 10$ Hz) (H-6) collapsed a double doublet at 1.90 ($J = 10, 5$ Hz) (H-5) to a doublet and an overlapped multiplet at 0.84 (H-7) to a broad singlet. Saturation of 1.90 (H-5 and H-8a) collapsed a doublet at 3.53 (H-1) to a singlet and a triplet at 0.43 to a broad doublet. Irradiation at a doublet at 3.53 collapsed a double doublet at 1.90 to a doublet.

When the multiplet at 0.84 (H-7 and H-8a) was irradiated it collapsed a triplet at 0.43 (H-6) to a doublet and a complex multiplet at 2.50 (H-9) to a broad AB-type

doublet signal and also affected an overlapped signal at 2.05. Irradiation of the multiplet at 2.50 (H-9) collapsed the broad singlet at 4.92 (H-11$_a$) to a doublet (J = 2 Hz). The arrangement of a lactone moiety was also confirmed by the spin-decoupling experiment between H-3 and the vinylic methyl group (H-12). On the basis of the above ^{1}H NMR spectral data, together with the well-separated absorption bands at 1380 and 1390 cm^{-1} assignable to the gem-dimethyl group and the loss of a C_3H_7 group ($M^+ - 43$, $C_{12}H_{13}O_2$) from the molecular ion, and the coexistence of the hemiacetals (**1** and **2**) and *ent*-cyclocolorenone (**17**), the structure of the new sesquiterpene δ-lactone was established to be **4**. The ^{13}C NMR spectrum (Table 1) further supported this structure. The absolute configuration of the ring junction of **4** was established by the negative Cotton effect and by the coupling constants between H-1 and H-5 (J = 5 Hz) and between H-5 and H-6 (J = 10 Hz). Recently, plagiochilide has been found in *P. asplenioides* [26].

The labile compound, named plagiochilal A (**5**), $C_{15}H_{24}O_2$, indicated the presence of an aldehyde group [1690 cm^{-1}; δ 9.57 (*s*)] conjugated with an exocyclic methylene group [6.20 and 6.52 (*br s*)], and a saturated aldehyde group [1720 cm^{-1}; δ 9.67 (*d*, J = 1.5 Hz)]. The ^{1}H NMR spectrum (Table 1) contained the signals due to two tertiary methyl groups, an additional non-conjugated exocyclic methylene group and one methine group located between a double bond and the aldehyde group. The molecular formula and the above spectral data, together with biogenetic considerations (Fig. 3), led us to structure **5** for plagiochilal A. After our reports on the isolation and the structures of various unique *ent*-secoaromadendrane-type sesquiterpenes from *Plagiochila* species, Atsumi *et al.* [27] recently reported the isolation of hanegokedial and plagiochiline C (**3**) from *P. semidecurrens*. The spectral data of plagiochilal A were identical to those of hanegokedial.

Furanoplagiochilal (**6**), $C_{15}H_{18}O_2$ (M^+ at *m/e* 230), showed a positive Ehrlich test. The IR spectrum showed the presence of an α,β-unsaturated aldehyde group [2720 and 1695 am^{-1}; δ 9.61 (*s*)] and a furane ring (1535 cm^{-1}), The ^{1}H NMR spectrum (Table 1) contained the signals for an exomethylene group conjugated with the aldehyde group, two protons on a β,β'-disubstituted furane ring, one methine located between sp^2 carbons, two tertiary methyl groups and an allylic methylene group. The presence of a cyclopropane ring was supported by the higher chemical shift at δ 0.90 (1H). The above spectral evidence coupled with the molecular formula showed that compound **6** was a tricyclic sesquiterpene aldehyde containing a β,β'-disubstituted furane ring. The structure of **6** was directly established by spin-decoupling experiments of the ^{1}H NMR spectrum and by the ^{13}C NMR spectrum.

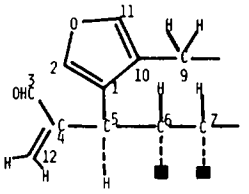


Fig. 2.

Irradiation at an overlapped triplet signal at 0.90 (H-6) collapsed a doublet at 3.75 (J = 10 Hz) to a broad singlet. Long range couplings of two protons on a furane ring could be observed. When the broad singlet-like signal at 6.55 (H-11) was irradiated, the broad doublet at 3.75 collapsed to a sharp doublet. The reverse irradiation caused the signal at 6.55 to collapse to a doublet. Irradiation at the centre of the multiplet at 2.80 (H-9) collapsed the broad singlet-like signal at 7.06 (H-2) to a doublet (J = 1 Hz). Thus, the partial structure of **6** was as shown in Fig. 2. The ^{13}C NMR spectrum (Table 2) showed the presence of fifteen well-separated carbon atoms: two methylenes, three methines, one sp^3 quaternary carbon, two methyls, four carbons of a β,β'-disubstituted furane ring, an exomethylene and a formyl carbon. The chemical shifts of the seven sp^3 carbons were quite similar to those of **1** and **2**. From the above spectral

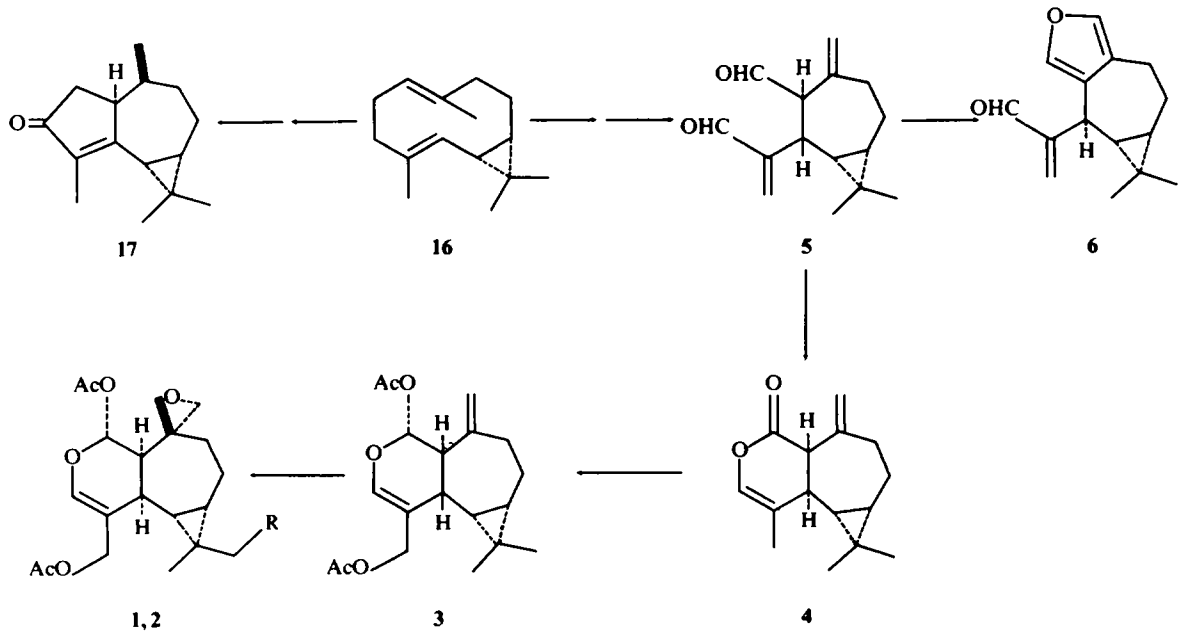
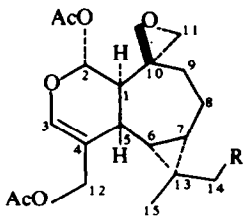
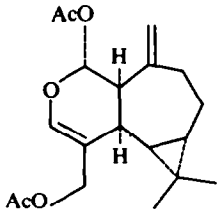


Fig. 3. Possible biogenetic pathways of *ent*-secoaromadendrane-type sesquiterpenes.



1 R = H
2 R = OAc



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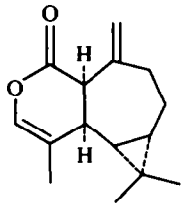

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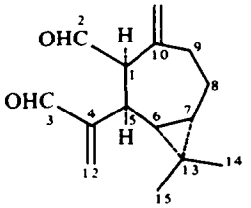

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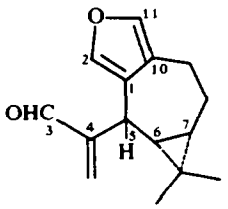

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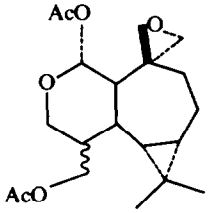

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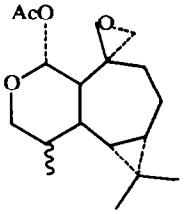

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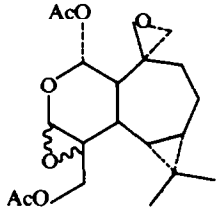

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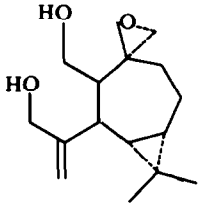

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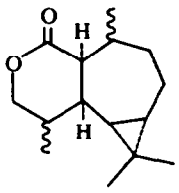
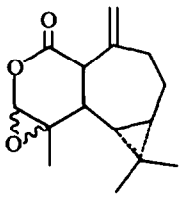
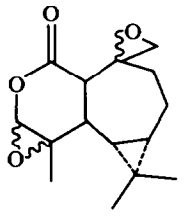


11



12



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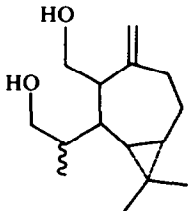

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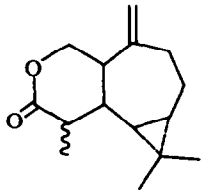

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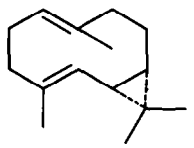

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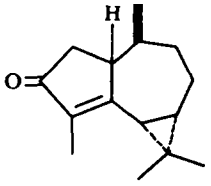

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evidence and biogenetic considerations (Fig. 3), the structure of furanoplagiochilal was confirmed to be **6**.

The present *Plagiochila* species contain (−)-bicyclogermacrene (**16**) from which the *ent*-secoaromadendrane-type sesquiterpene hemiacetals (**1**–**6**) might be formed (Fig. 3). The pungency of *Plagiochila* species is due to plagiochiline A. *P. fruticosa*, *P. hattoriana* and *P. yokogurensis* show an intense pungent taste; on the other hand, the pungency of *P. ovalifolia* is remarkably weak. This is due to the different content of pungent plagiochiline A (**1**) in *Plagiochila* species. Plagiochiline A inhibits the feeding of African army worm, *Spodoptera exempta* at a concentration of 1–10 ng/cm^2 (2 hr). From hydrocarbon fractions of each species, various mono- and sesquiterpene hydrocarbons have been detected by GC–MS [23, 24]. It is considered that the fragrant odor of the present *Plagiochila* species may be due to the large quantity of mono- and sesquiterpene hydrocarbons.

EXPERIMENTAL

The solvents used for spectral determinations were TMS–$CDCl_3$ (^{1}H NMR 60, 80, 90 and 100 MHz; ^{13}C NMR 25.20 MHz); $CHCl_3$ (IR, $[\alpha]_D$); MeCN (CD), EtOH (UV), unless otherwise stated. TLC and PLC: precoated Si gel plates (0.25 mm) F_{254}, *n*-hexane–EtOAc (4:1), C_6H_6–EtOAc (4:1 and 1:1). Spots were detected by UV light (254 nm) and by spraying with 30% H_2SO_4, 2,4-DNP, Ehrlich reagent or I_2 vapor. GC MS: 70 eV, column 1% SE-30, 2 m × 2 mm, temp. programme, 50–270° at 5°/min. CI–MS were obtained at 500 eV, direct inlet method, reaction gas: *iso*-butane. The leaf disk feeding test against African army worm, *Spodoptera exempta*, was carried out by the same method as described in the preceeding paper [28].

Extraction and isolation. *Plagiochila yokogurensis*, *P. hattoriana*, *P. fruticosa* and *P. ovalifolia* collected in Tokushima in 1976–1978 were air-dried for 5 days. The ground materials (2.10 kg of *P. yokogurensis*, 87 g of *P. h.*, 89 g of *P. f.* and 200 g of *P. o.*) were extracted with Et_2O for 2 weeks. The green, intensely pungent extract (100 g) of *P. yokogurensis* was directly chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The crude extracts (12.60 g of *P. h.*, 9.52 g of *P. f.* and 17.63 g of *P. o.*) were treated in the same manner as described above. The fraction of *P. yokogurensis* eluted with *n*-hexane (100%) contained a mixture (10.30 g) of mono- and sesquiterpene hydrocarbons in which α- and β-pinenes, camphene, β-elemene, calamenene, cuparene, β-santalene, β-calacorene and bicyclogermacrene were detected by GC–MS. The fraction eluted with *n*-hexane–EtOAc (19:1) gave unidentified sesquiterpenes, triglycerides and carotenoids (9.20 g). The *n*-hexane–EtOAc (4:1) fraction gave oxygenated sesquiterpene mixtures which were rechromatographed on Si gel using *n*-hexane–EtOAc to afford plagiochilide (**4**) (5.30 g) and (+)-cyclocolorenone (**17**) (30 mg). *Plagiochilide* (**4**): mp 110–111°; $[\alpha]_D$ − 5° (*c*, 1.0); $C_{15}H_{20}O_2$; UV λ_{max} nm: 205 (ε, 1312); $\Delta\varepsilon^{dioxane}_{255nm}$ − 2.74; IR ν_{max} cm^{-1}: 1760, 1110 (δ-lactone), 1675 (C=C–O–), 1630, 910 (C=CH_2), 1390, 1380 (gem-dimethyl), 1180, 1080, 1045, 1020, 995, 935, 837, 820, 655, 632, 550; MS *m/e* (rel. int.): 232.1454 (M^-, 50, calc. 232.1463), 189.0925 ($M^+ - C_3H_7$, calc. 189.0915), 161 (52), 147 (44), 133 (50), 122 (63), 121 (52), 109 (61), 107 (100), 105 (57), 91 (77), 79 (53), 41 (59), 39 (40).

The *n*-hexane-EtOAc (3:1) fraction contained the intensely pungent substances which were rechromatographed on Si gel using the same solvent system described above, to afford plagiochiline A (**1**) (6.00 g), plagiochiline C (**3**) (110 mg) [22, 23] and furanoplagiochilal (**6**) (90 mg). *Plagiochiline A* (**1**): $[\alpha]_D \pm 0°$ (*c*, 5.2); $C_{19}H_{26}O_6$; UV λ_{max} nm: 205 (ε, 1312); $\Delta\varepsilon_{232nm}$ + 2.33; IR ν_{max} cm^{-1}: 1740, 1237 (OAC), 1670 (C=C–O–), 1620 (C=C), 1390, 1370 (gem-dimethyl), 1190, 1165, 1145, 1124, 1107, 1088, 1067, 1010, 975, 962, 911, 886, 870, 843, 592, 550; MS *m/e* (rel. int): 350.1750 (M^+, 1, calc. 350.1729), 307.1188 ($M^+ - 60$,4), 247 ($M^+ - 60 - 43$, 28), 230 ($M^+ - 60 - 60$, 20), 187 (25), 159 (30), 131 (25), 105 (30), 91 (35), 79 (25), 43 (100).

Furanoplagiochilal (**6**): $[\alpha]_D$ − 80° (*c*, 0.83); $C_{15}H_{18}O_2$; IR ν_{max} cm^{-1}: 2720, 1695 (C=C–CHO), 1660 (C=C), 1535 (furane), 1245, 1220, 1135, 1055, 950, 910, 885, 830, 790, 765, 600; MS *m/e* (rel. int.): 230 (M^+, 74), 215 ($M^+ - 15$, 41), 201 ($M^+ - CHO$, 28), 187 ($M^+ - C_3H_7$, 52), 161 (50), 159 (31), 131 (48), 120 (44), 119 (38), 105 (98), 91 (100), 69 (75), 41 (41).

The fraction of *P. hattoriana* eluted with *n*-hexane (100%) gave fragrant mono- and sesquiterpene hydrocarbons (2.80 g) in which α- and β-pinenes, camphene, myrcene, calamenene and bicyclogermacrene were detected by GC–MS [23, 24]. The *n*-hexane–EtOAc (19:1) fraction contained triglycerides and carotenoids (1.5 g). Rechromatography of the crude oil obtained from the *n*-hexane–EtOAc (4:1) fraction on Si gel using the same solvent system described above resulted in the isolation of plagiochilines A (**1**) (2.20 g) and B (**2**) (630 mg). The fourth *n*-hexane EtOAc, (3:2) fraction, which contained sesquiterpene aldehydes, was rechromatographed on Si gel using C_6H_6–EtOAc to afford hanegokedial (plagiochilal A, **5**) (22 mg) and plagiochilal B (32 mg). *Hanegokedial* (plagiochilal A): mp 66–68° (lit. 66–67° [27]); $C_{15}H_{20}O_2$ [CI–MS: M^+ at *m/e* 233]; IR ν_{max} cm^{-1}: 2720 (CHO), 1720 (CHO), 1690 (C=C–CHO), 1230, 1200, 1140, 950.

The first fraction (*n*-hexane 100%) from *P. fruticosa* gave mono- and sesquiterpene hydrocarbons (1.82 g) in which α- and β-pinenes, camphene, γ-cadinene, calamenene, cuparene, bicylcogermacrene and β-barbatene were detected by GC–MS [23, 24]. The *n*-hexane–EtOAc (4:1) fraction contained oxygenated sesquiterpene and sterol mixtures and was rechromatographed on Si gel to give plagiochilines A (**1**) (1.98 g), B (**2**) (45 mg), C (**3**) (50 mg) and plagiochilide (**4**) (2.80 g), and phytosterols (30 mg).

The first fraction of *P. ovalifolia* eluted with *n*-hexane also contained mono- and sesquiterpene hydrocarbons (1.35 g) in which α- and β-pinenes, camphene, *p*-cymene, calamenene, α-elemene, *trans*-β-farnesene, δ-elemene, bicyclogermacrene, β-barbatene, bazzanene (trichodiene), β-bisabolene, α-gurjunene, and α-himachalene were detected [23, 24]. The second *n*-hexane EtOAc (19:1) fraction gave a yellow fragrant oil (2.60 g) which contained unidentified sesquiterpene hydrocarbons. The third *n*-hexane–EtOAc (4:1) fraction gave sesquiterpene hemiacetals which were purified by PLC to afford plagiochiline A (**1**) (70 mg), plagiochiline B (**2**) (45 mg), plagiochiline C (**3**) (50 mg) and phytosterols (80 mg). The fourth *n*-hexane–EtOAc (3:2) fraction contained a mixture of sesquiterpene hemiacetals, labile sesquiterpene aldehydes and monoterpene alcohol. This was rechromatographed on Si gel to afford pure linalool (20 mg) and a mixture of unidentified sesquiterpene dialdehydes and two secoaromadendrane-type sesquiterpenes (270 mg).

Hydrogenation of plagiochiline A (**1**). An EtOAc soln of **1** (50 mg) was hydrogenated in the presence of PtO_2 to give a viscous oil which was further purified by PLC to afford a dihydrodiacetate (**7**) (14 mg) and a dihydromonoacetate (**8**) (22 mg). *Compound* **7**: $[\alpha]_D$ − 2° (*c*, 1.6); $C_{19}H_{28}O_6$; IR ν_{max} cm^{-1}; 1748, 1240 (OAc), 1370, 1175, 1150, 1105, 1090, 1065, 975, 950, 885, 865, 845, 730; ^{1}H NMR: δ 0.98, 1.11 (each 3 H, *s*), 1.52 (1 H, *dd*, *J* = 10, 2 Hz), 2.08, 2.18 (each 3 H, *s*), 2.48 (2 H, *s*), 3.91–4.05 (4 H, *m*), 6.45 (1 *H*, *d*, *J* = 10 Hz); MS *m/e* (rel. int.): 352 (M^+, 0.7), 91 (25), 82 (22), 69 (24), 43 (100). The compound **8**: $[\alpha]_D$ − 4° (*c*, 0.7); $C_{17}H_{26}O_4$; IR ν_{max} cm^{-1}: 1750, 1250 (OAc), 1150, 885, 620, 595, 575; ^{1}H NMR: δ 0.57 (1 H, *dd*, *J* = 10 Hz),

0.80 (3 H, *d*, J = 7 Hz), 0.97, 1.10 (each 3 H, *s*), 1.67 (1 H, *dd*, J = 10, 2 Hz), 2.17 (3 H, *s*), 2.40 (2 H, *s*), 3.85 (2 H, *d*, J = 7 Hz), 6.83 (1 H, *d*, J = 9 Hz); MS *m/e* (rel. int.): 294 (M^+, 0.7), 105 (21), 95 (20), 93 (20), 91 (36), 81 (20), 79 (26), 77 (23), 69 (40), 67 (24), 55 (33), 43 (100).

Epoxidation of **1**. A $CHCl_3$ soln of **1** (60 mg) was treated with *m*-chloroperbenzoic acid (48 mg) at 0° for 1 hr. Work-up as usual gave the crude epoxides, purified by PLC to afford an epoxidized hemiacetal (**9**) (10 mg), mp 181–182°; $C_{19}H_{26}O_7$; IR ν_{max} cm^{-1}: 1740, 1240 (OAc), 1378, 1365, 860, 845, 813; ^1H NMR: δ 1.12, 1.23 (each 3 H, *s*), 2.02, 2.22 (each 3 H, *s*), 4.12, 4.58 (each 1 H, *d*, J = 12 Hz), 6.37 (1 H, *br s*), 6.78 (1 H, *d*, J = 10 Hz); MS *m/e* (rel. int.): 366 (M^+, 0.5), 246 (M^+–60 – 60, 4), 175 (64), 156 (86), 139 (100), 119 (39), 111 (55), 105 (32), 91 (34), 77 (33), 75 (47), 43 (94).

Reduction of **1**. An EtOH soln of $NaBH_4$ (30 mg) was added to the hemiacetal (**1**) (40 mg) with stirring at 0° for 2 hr. Excess $NaBH_4$ was decomposed with 1% HOAc, the reaction mixture was filtered through a short column packed with Si gel and the solvent was evapd to give a diol (10 mg). IR ν_{max} cm^{-1}: 3400, 1030 (OH), 1115, 970, 903, 863, 835, 783; ^1H NMR: δ 0.97, 1.11 (each 3 H, *s*), 2.60 (2 H, *s*), 3.93–4.30 (2 H, *m*), 4.17 (2 H, *s*), 5.10 (1 H, *br s*), 5.23 (1 H, *br s*); MS *m/e* (rel. int.): 253 (M^+ + 1, 4), 221 (M^+–31, 38), 145 (57), 125 (55), 121 (60), 119 (57), 109 (67), 107 (98), 105 (65), 95 (68), 93 (72), 91 (52), 82 (100), 69 (77), 45 (35).

Hydrogenation of plagiochilide (**4**). An EtOH soln of **4** (21 mg) was hydrogenated in the presence of Pd–C (15 mg) for 2 hr. Work-up as usual gave two isomeric tetrahydro derivatives (**11**) (10 mg): GC ratio (4:1), mp 119–121°; $C_{15}H_{24}O_2$; IR ν_{max} 1735 (δ-lactone), 1380, 1130, 1030. 970; ^1H NMR: δ 0.30–0.90 (2 H), 0.93–1.05 (6 H), 1.07 (6 H, *s*), 4.00, 4.57 (each *d*, J = 11 Hz), 4.08, 4.43 (each *d*, J = 11 Hz); Major tetrahydro derivative: GC–MS *m/e* (rel. int.): 236 (M^+, 51), 221 (100), 193 (39), 139 (42), 122 (32), 121 (46), 113 (55), 109 (43), 107 (93), 95 (63), 93 (65), 81 (50), 79 (43), 77 (32), 69 (43), 67 (45), 55 (44), 41 (55); Minor tetrahydro derivative: GC–MS *m/e* (rel. int.): 236 (M^+, 38), 221 (52), 193 (37), 139 (22), 122 (34), 121 (45), 113 (53), 109 (43), 107 (100), 105 (34), 95 (64), 93 (65), 91 (43), 81 (51), 79 (51), 77 (34), 69 (43), 67 (44), 55 (41), 41 (57).

Epoxidation of **4**. Compound **4** (63 mg) in $CHCl_3$ (5 ml) was treated with *m*-chloroperbenzoic acid (70 mg) at 0° for 2 hr. Work-up as usual gave epoxides, purified by PLC to afford a monoepoxide (**12**) (10 mg) and a diepoxide (**13**) (7 mg). *Monoepoxide* (**12**): mp 102–104°; $C_{15}H_{20}O_3$; IR ν_{max} cm^{-1}: 1760 (δ-lactone), 1640 (C=C), 1380, 1358, 1278, 1202, 1115, 1090, 1063, 1028, 935, 920, 894, 855, 838, 790, 768, 708, 665, 632; ^1H NMR: δ 1.08, 1.12 (each 3 H, *s*), 1.45 (3 H, *s*), 3.80 (1 H, *d*, J = 5 Hz), 4.85, 4.95 (each 1 H, *br s*), 5.08 (1 H, *br s*); MS *m/e* (rel. int.): 248 (M^+, 16), 205 (M^+ – 43, 39), 147 (38), 137 (54), 123 (42), 121 (76), 119 (40), 111 (56), 109 (86), 107 (62), 105 (59), 95 (93), 91 (100), 82 (92), 79 (69), 77 (66), 69 (60), 67 (91), 55 (69), 53 (71), 43 (74), 41 (89), 39 (42). *Diepoxide* (**13**): mp 147–151°; $[\alpha]_D$ + 4° (*c*, 0.8); $C_{15}H_{20}O_4$: IR ν_{max} cm^{-1}: 1760 (δ-lactone), 1380, 1360, 1260, 1120, 1105, 1085, 1060, 995, 905, 850, 595, 550. 500; ^1H NMR: δ 1.10, 1.18, 1.45 (each 3 H, *s*), 2.68, 2.82 (2 H, *d*, J = 4 Hz), 5.06 (1 H, *br s*); MS *m/e* (rel. int.): 264 (M^+, 1), 109 (22), 107 (31), 105 (22), 93 (20), 91 (38), 79 (28), 77 (25), 69 (25), 67 (25), 55 (29), 43 (100), 41 (46), 39 (30).

Reduction of **4** *with* $LiAlH_4$. To $LiAlH_4$ (30 mg) in dry Et_2O was added compound **4** (15 mg) at 0° and the mixture stirred for 1.5 hr to give a diol (**14**) (20 mg). IR ν_{max} cm^{-1}: 3400, 1025 (OH), 1640, 895 C=CH_2); ^1H NMR: δ 0.95–1.05 (6 H), 3.35–4.0 (4 H, *m*), 4.75, 4.80 (each 1 H, *br s*).

Oxidation of diol **14**. To the CrO_3–Py complex (50 mg) in CH_2Cl_2 was added the diol **14** (20 mg) and the mixture stirred for 5 hr at room temp. The reaction mixture was filtered through a short column packed with Si gel and the solvent was evapd to afford a yellow oil, purified by PLC to give a δ-lactone (**15**) (18 mg). $C_{15}H_{22}O_2$; IR ν_{max} cm^{-1}: 1742 (δ-lactone), 1640, 885 (=CH_2), 1377, 1354, 1251, 1175, 1111, 1065, 1046, 1634, 848, 800, 730, 720, 620, 590, 575, 460; ^1H NMR: δ 0.20–0.88 (2 H), 1.03, 1.10 (each 3 H, *s*), 1.25 (3 H, *d*, J = 7 Hz), 2.50 (2 H, *m*), 4.37 (1 H, *d*, J = 2 Hz), 4.50 (1 H, *d*, J = 9 Hz), 4.70, 4.97 (each 1 H, *br s*); MS *m/e* (rel. int.): 234 (M^+, 16), 191 (22), 179 (55), 122 (26), 121 (32), 120 (24), 119 (45), 109 (21), 107 (59), 106 (24), 105 (90), 96 (20), 95 (26), 93 (68), 91 (82), 81 (46), 79 (69), 43 (100).

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