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PLAGIOCHILINES C, D, E AND F, FOUR NOVEL SECOAROMADENDRANE-TYPE SESQUITERPENE HEMIACETALS FROM *PLAGIOCHILA ASPLENIOIDES* AND *PLAGIOCHILA SEMIDECURRENS*

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Key Word Index—*Plagiochila asplenioides*; *Plagiochila semidecurrens*; Jungermanniales; Hepaticae; plagiochilines C, D, E and F; secoaromadendrane-type sesquiterpene hemiacetals; (−)-bicyclogermacrene.

Abstract—Four novel secoaromadendrane-type sesquiterpene hemiacetals, plagiochilines C, D, E and F, together with the previously known (−)-bicyclogermacrene, have been isolated from the liverwort, *Plagiochila asplenioides* and their structures have been spectroscopically elucidated. From *Plagiochila semidecurrens* plagiochilines A and C have been obtained along with (−)-bicyclogermacrene and its related sesquiterpene hydrocarbons.

INTRODUCTION

Some *Plagiochila* species, belonging to Jungermanniales (Hepaticae) elaborate characteristic pungent substances. Recently, we have reported the isolation and structures of the unique secoaromadendrane-type sesquiterpenes, plagiochiline A (**1**), plagiochiline B (**2**) and plagiochilide (**7**) from *Plagiochila yokogurensis* and *P. hattoriana*, and the pungency of these species was due to the hemiacetal (**1**) [1, 2]. In this paper we wish to report the isolation and structures of four new secoaromadendrane-type sesquiterpene hemiacetals and the previously known sesquiterpene hydrocarbons from French *Plagiochila asplenioides* and Japanese *P. semidecurrens*.

RESULTS AND DISCUSSION

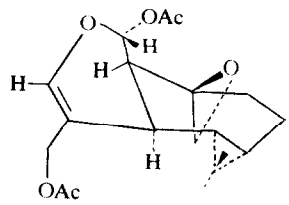
Air-dried ground material of each species was extracted with ether. The viscous oil of *P. semidecurrens* was directly chromatographed on Si gel to give a new sesquiterpene hemiacetal, named plagiochiline C, together with the previously known plagiochiline A (**1**) and an unknown secoaromadendrane-type sesquiterpene hemiacetal having one aldehyde group. The same treatment of the extract of *P. asplenioides* afforded four new sesquiterpene hemiacetals, named plagiochilines C, D, E and F.

Plagiochiline C (**3**)

The colorless oil, plagiochiline C, $C_{19}H_{26}O_5$ (CI–MS: $M^+ + 1$, *m/e* 335), showed the presence of two acetoxyl groups [1757, 1740, 1230 cm^{-1}; δ 2.07, 2.10 (6H, each *s*)] and a double bond (1638 cm^{-1}). The ^1H NMR and double resonance spectra also contained signals attributable to two tertiary methyl groups (δ 1.01 and 1.07), a methylene group (4.45) located between a double bond and an acetoxyl group, a vinylic proton (6.35) located between a double bond and an ether oxygen, one proton (6.61) on a carbon bearing an acetoxyl group and equivalent protons (4.83) of an exomethylene group. The ^1H NMR spectrum was quite similar to that of plagiochiline A (**1**), except for the presence of the signal of the exomethylene group instead of that of an epoxide methylene group, indicating that plagiochiline C possessed the same secoaromadendrane-type sesquiterpene hemiacetal as **1** and an exomethylene group might be located at C-10 of the seven-membered ring. Treatment of **3** with *m*-chloroperbenzoic acid afforded a pungent epoxide whose spectral data and chromatographic behavior were identical to those of the natural plagiochiline A. Thus, the structure of plagiochiline C was established to be **3** [3, 4].

Plagiochiline D (**4**)

The molecular ions of the highly acetylated plagiochilines D (**4**), E (**5**) and F (**6**) could not be detected even by CI–MS using *iso*-butane, methane or ammonia as the reaction gases. However, their structures were confirmed by the following arguments. Plagiochiline D (**4**), $C_{23}H_{30}O_{10}$ ($M^+ - 2$ Ac, *m/e* 346), exhibited the presence of the four acetoxyl groups [1740, 1235 cm^{-1}; δ 2.12 (9H, *s*), 2.23 (3H, *s*)] which was further confirmed by the fragment ion at *m/e* 226 ($M^+ - 4$ AcOH). In the ^1H NMR spectrum of **4**, neither a methyl group on a sp^3 carbon nor a vinylic methyl group was observed. However, the signal pattern between 2 and 7 ppm was almost identical to that of plagiochiline B (**2**), except for the presence of an additional acetoxyl group and one methylene group placed between an acetoxyl group and a quaternary sp^3 carbon. These ^1H NMR spectral data indicated that the additional acetoxyl group was further attached to a quaternary methyl group on a cyclopropane ring of plagiochiline B (**2**). This assumption was further supported by the absence of the well separated IR bands in the region 1370 cm^{-1}, characteristic of the gem-dimethyl group of the aromadendrane-type sesquiterpenes. The above spectral evidence, coupled with biogenetic consideration and coexistence of plagiochiline C, led to structure **4** for plagiochiline D.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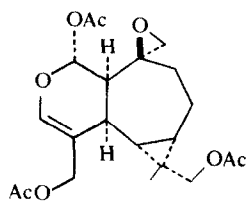
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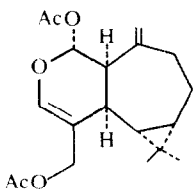
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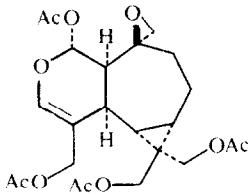
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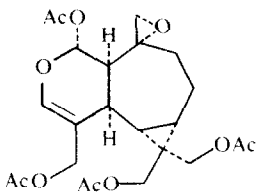
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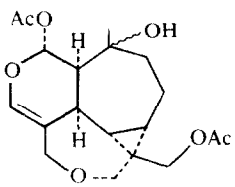
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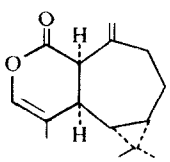
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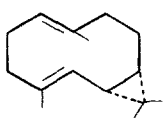
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Plagiochiline E (**5**)

The IR and MS of the third hemiacetal, $C_{23}H_{30}O_{10}$ ($M^+ - 2$ AcOH, *m/e* 346), were almost identical to those of plagiochiline D (**4**), however, the chromatographic behavior and ^1H NMR signal pattern of the epoxide methylene protons were different, indicating that plagiochiline E was the stereoisomer of the epoxide of plagiochiline D. This evidence led to structure **5** for plagiochiline E.

Plagiochiline F (**6**)

The fourth new hemiacetal, $C_{19}H_{26}O_7$ ($M^+ - 18 -$ AcOH, *m/e* 288) was isolated as a minor component. Its IR spectrum indicated the presence of a hydroxyl group (3450 cm^{-1}) and an acetoxyl group (1740, 1235 cm^{-1}). The ^1H NMR spectrum contained signals attributable to one tertiary methyl group on a carbon bearing a hydroxyl group (δ 1.10) and two acetoxyl groups (2.03, 2.13, each *s*) and the lower field signal pattern was closely similar to that of plagiochilines A–E, except for the absence of epoxide methylene protons, indicating that plagiochiline F might be a secoaromadendrane-type sesquiterpene hemiacetal. Part of the signal pattern was identical to that of plagiochilines A–E. The ^1H NMR spectrum of **6** further included the signal of a methylene group (δ 2.13–2.35) located between a quaternary sp^3 carbon and an ether oxygen, a methylene group (4.24, 4.56, 2H, each *d*, J = 12 Hz) placed between a double bond and an ether oxygen and an acetoxymethyl group (3.60, 3.98, 2H, each *d*, J = 12 Hz). The latter signal pattern was identical to that of the acetoxymethyl group on a cyclopropane ring of plagiochilines D and E. These results together with the molecular formula, biogenetic considerations and coexistence of the other plagiochilines showed that plagiochiline F was most favourably represented by formula **6**. The stereochemistry at C-10 remains to be established.

From the hydrocarbon fraction of *P. semidecurrens*, (−)-bicyclogermacrene (**8**), which might be a precursor of the present secoaromadendrane-type sesquiterpene hemiacetals, has been obtained, together with calamenene and cuparene. An unknown secoaromadendrane-type sesquiterpene hemiacetal possessing one aldehyde group and two hydroxyl groups has also been isolated from the same species. *P. asplenioides* also contained (−)-bicyclogermacrene, longifolene, calamenene and camphene. *P. semidecurrens* has a slightly pungent taste. This is due to the presence of a small amount of plagiochiline A (**1**). In spite of careful examination using TLC and spectroscopic methods, plagiochilines A and B have not been detected in *P. asplenioides*. The crude extract of the present two species showed the inhibitory activity against the germination of rice in the husk at *ca* 1000 ppm. One of the active substances is plagiochiline A which inhibited the germination of rice at 200 ppm. The details of the bioassay of the hemiacetals (**1–6**) will be reported elsewhere.

EXPERIMENTAL

TLC and PLC were on Si gel F_{254} (Merk) using 50% H_2SO_4 and UV light (254 and 360 nm) as detector. GLC was on a column (3 m × 2 mm) containing 5% SE-30 with N_2 30 ml/min at 50–250°/min. GC–MS were obtained at 70 eV, column OV-1 or OV-17 5%, 3 m × 2 mm, temp. programme 50–250° at 5°/min, He 30 ml/min. CI–MS were obtained at 500 eV, direct inlet method, the reaction gases: *iso*-butane, NH_3 and CH_4. The solvents used for spectral determinations were: TMS–$CDCl_3$ (^{1}H NMR 90 and 60 MHz); $CHCl_3$($[\alpha]_D$ and IR) unless otherwise stated.

Extraction and isolation. Plagiochila semidecurrens collected in Kamikatsu-cho, Tokushima, Japan, in June, 1977 and *Plagiochila asplenioides* collected in Puymartin, Dordogne, France in April, 1978 were ground, after being air-dried for 1 week. Each ground material (40 g, *P. semidecurrens*; 116 g, *P. asplenioides*) was extracted with Et_2O for 2 weeks. The crude extract (1.220 g) of *P. semidecurrens* was directly chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane 100%) contained sesquiterpene hydrocarbons (85 mg) in which calamenene, cuparene and bicyclogermacrene were detected by GC–MS analysis. The main hydrocarbon, (−)-bicyclogermacrene, was isolated by prep GLC. (−)-Bicyclogermacrene (**8**): $[\alpha]_D$ −56° (*c*, 0.7) (lit. [5] +61°). The second fraction (*n*-hexane–EtOAc, 19:1) gave a dark yellow fragrant oil (30 mg), not identified. The third fraction (9:1) contained the characteristic sesquiterpene hemiacetals and was purified by PLC to give plagiochiline A (**1**) (20 mg) [1] and plagiochiline C (**3**) (180 mg). Plagiochiline C (**3**): $[\alpha]_D$ +28° (*c*, 1.5); IR ν_{max}^{Nujol} cm^{-1}: 1757, 1740, 1230 (OAc), 1672 (—O—C=C), 1638, 1190, 1160, 1120, 1105, 1080, 1060, 1020, 960, 915, 905, 850; ^{1}H NMR: δ 4.40 (2H, *bs*, C=C—C$\underline{H}_2$—OCO—), 4.83 (2H, *bs*, —C=CH_2), 6.35 (1H, *bs*, O—C$\underline{H}$=C), 6.61 (1H, *d*, *J* = 10, —O—C$\underline{H}$—OCO—); CI–MS *m/e* (rel. int.): 335 (M^+ + 1), 275 (M^+ + 1 − AcOH, 4), 232 (M^+ + 1 − 43 − AcOH, 11), 215 (M^+ + 1 − 2 AcOH, base): EI–MS: no parent peak, 291 (M^+ − 43, 2), 274 (M^+ − AcOH, 10), 231 (M^+ − 43 − AcOH, 50), 214 (M^+ − 2 AcOH, base), 199 (35), 189 (55), 171 (55), 43 (70). The fourth fraction (4:1) gave a sterol mixture (5 mg). The fifth fraction (1:1) contained a fatty acid and an unknown hemiacetal which was purified by PLC to afford pure hemiacetal (6 mg). 2,4DNP- test (+); IR ν_{max} cm^{-1}: 3200 (OH), 1740, 2130 (OAc), 2750, 1705 (CHO), 1670 (—O—C=C), 1190, 1180, 1155, 1140, 1120, 1110, 1090, 1055, 1020, 960, 900, 870, 830; ^{1}H NMR δ 1.10 (3H, *s*), 1.27 (3H, *s*), 2.03 (3H, *s*, OAc). 4.47 (2H, *bs*, C=C—C$\underline{H}_2$—OCO), 6.43 (1H, *bs*, —O—C$\underline{H}$=C), 6.85 (1H, *dd*, *J* = 6, 3), 9.45 (1H, *s*, C$\underline{H}$O); EI–MS *m/e* (rel. int.): 306 (M^+ − 18, 2), 288 (M^+ − 18 − 18, 28), 246 (M^+ − 18 − AcOH, 16), 228 (M^+ − 18 − 18 − AcOH, 32), 136 (52), 43 (base); CI-MS: 289 (20), 231 (45), 229 (base), 61 (38).

The crude extract (3.670 g) of *P. asplenioides* was treated in the same manner to that described above. From the fraction eluted with *n*-hexane, mono- and sesquiterpene hydrocarbons (1.151 g) were obtained. GC–MS analysis indicated the presence of camphene, calamenene, longifolene and bicyclogermacrene (**8**). The second fraction (*n*-hexane–EtOAc, 19:1) gave an intense fragrant oil (20 mg), not identified. The third fraction (9:1) contained sesquiterpene hemiacetals in which plagiochiline C (**3**) was detected by GC–MS analysis. The fourth fraction (3:2) gave fatty acids and hemiacetals (228 mg) which were rechromatographed on Si gel to afford plagiochiline F (**6**) (34 mg). $[\alpha]_D$ +30.4° (*c*, 1.2); IR ν_{max} cm^{-1}: 3450 (OH), 1740, 1235 (OAc), 1670 (—O—C=C), 1190, 1165, 1120, 1080, 1060, 1005, 890, 845, 760, 670; EI–MS *m/e* (rel. int.): $C_{19}H_{26}O_7$ (no parent peak), 288 (M^+ − 18 − AcOH, 10), 246 (M^+ − 2 AcOH, 30), 228 (M^+ − 18 − 2 AcOH, base), 199 (76), 171 (60), 131, (57), 105 (58), 91 (65), 43 (91); CI–MS: no parent peak, 289 (M^+ + 1 − 18 − AcOH, 14), 247 (M^+ + 1 − 2 AcOH, 41), 229 (M^+ + 1 − 18 − 2 AcOH, base), 201 (16). The fifth fraction (1:1) gave sesquiterpene hemiacetals (327 mg), purified by PLC to afford plagiochiline D (**4**) (41 mg) and plagiochiline E (**5**) (32 mg). Plagiochiline D (**4**): $[\alpha]_D$ + 21° (*c*, 1.4); IR ν_{max} cm^{-1}: 1740, 1235 (OAc), 1680 (—O—C=C), 1190, 1187, 1160, 1150, 1120, 1090, 1030, 920, 885, 750, 665, 600; ^{1}H NMR: δ 2.12 (9H, *s*, 3 × OAc), 2.23 (3H, *s*, OAc), 2.50 (2H, *bs*, C—C$\underline{H}_2$—O—), 3.82, 4.19 (2H, each *d*, *J* = 12, —C—C$\underline{H}_2$—OAc), 4.30 (2H, *bs*, —C—C$\underline{H}_2$—OAc), 4.57 (2H, *bs*, C=C—C$\underline{H}_2$—OAc), 6.43 (1H, *bs*, —O—C$\underline{H}$=C), 6.85 (1H, *d*, *J* = 10, —O—C$\underline{H}$—OAc); MS *m/e* (rel. int.) $C_{23}H_{30}O_{10}$ (no parent peak), 346 (M^+ − 2 AcOH, 9), 286 (M^+ − 3 AcOH, 23), 226 (M^+ − 4 AcOH, 26), 91 (32), 43 (base); CI–MS: no parent peak, 407 (M^+ + 1 − AcOH, 46), 347 (M^+ + 1 − 2 AcOH, 57), 287 (M^+ + 1 − 3 AcOH, 24), 227 (M^+ + 1 − 4 AcOH, base). Plagiochiline E (**5**): $[\alpha]_D$ +22° (*c*, 1.1); IR ν_{max} cm^{-1}: 1745, 1240 (OAc), 1675 (—O—C=C), 1192, 1180, 1117, 1105, 1095, 1030, 920, 885, 845, 760, 670, 605; ^{1}H NMR δ 2.08 (3H, *s*, OAc), 2.10 (6H, *s*, 2 × OAc), 2.23 (3H, *s*, OAc), 2.37–2.50 (2H, —C—O—C$\underline{H}_2$—C), 3.90, 4.18 (2H, each *d*, *J* = 10, —C—C$\underline{H}_2$—OAc), 4.30 (*bs*, $W_{\frac{1}{2}}$ = 4 Hz, C—C$\underline{H}_2$—OAc), 4.50 (2H, *bs*, $W_{\frac{1}{2}}$ = 4 Hz, C=C—C$\underline{H}_2$—OAc), 6.43 (1H, *bs*, —O—C$\underline{H}$=C), 6.86 (1H, *d*, *J* = 10, —O—C$\underline{H}$—OAc); MS *m/e* (rel. int.) $C_{23}H_{30}O_{10}$ (no parent peak), 346 (M^+ − 2 AcOH, 4), 286 (M^+ − 3 AcOH, 31), 226 (M^+ − 4 AcOH, 77) 91 (43), 43 (base), CI–MS: no parent peak, 407 (M^+ + 1 − AcOH, 1), 347 (M^+ + 1 − 2 AcOH, 7), 287 (M^+ + 1 − 3 AcOH, 47), 227 (M^+ + 1 − 4 AcOH, base).

Epoxidation of plagiochiline C (**3**). Compound **3** (40 mg) in $CHCl_3$ (2 ml) was treated with *m*-chloroperbenzoic acid (50 mg) at 0° for 1 hr. Work-up as usual gave the epoxide mixture which was chromatographed on Si gel using a C_6H_6–EtOAc gradient to afford the pungent plagiochiline A (**1**) (12 mg) whose spectral data and chromatographic behaviour were completely identical to those of the natural plagiochiline A.*

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* The stereochemistry of the C-10 epoxide of **1** and **2** and the acetoxymethyl group of **2** have been determined by ^{1}H NMR, ^{1}H NMDR and ^{13}C NMR techniques of PND, selective decoupling, partial relaxed Fourier transform (PRFT) and gated decoupling spectra (Dr. Kubo and Prof. K. Nakanishi, Columbia University, New York; private communication).