Pergamon

PII: S0031-9422(96)00407-4

Phytochemistry, Vol. 44, No. 1, pp. 89–99, 1997

0031-9422/97 $17.00 + 0.00

PLAGIOCHILINES AND OTHER SESQUITERPENOIDS FROM *PLAGIOCHILA* (HEPATICAE)*

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(*Received in revised form* 21 *May* 1996)

Key Word Index—*Plagiochila cristata*; *P. ericicola*; *P. squamulosa* var. *sinuosa*; *P. adianthoides*; Jungermanniales; Hepaticae; structure elucidation; sesquiterpenoids; *ent*-2,3-secoaromadendranes; bicyclogermacranes; phenyldihydroisocoumarin.

Abstract—Three new plagiochilines, O, P and Q, together with the known plagiochilines C and H, 3α-acetoxybicyclogermacrene, (−)-maaliol, (−)-spathulenol, and riccardin D were isolated from *Plagiochila cristata*. The new, plagiochiline R and (+)-3α-acetoxy-2α-[3-(4-hydroxy-3-methoxyphenyl)propanoyloxy]bicyclo-germacra-*E*1(10), 4(12)-dien-5β-ol, as well as the known plagiochilines C and H, 3α-acetoxybicyclo-germacrene and (−)-spathulenol were isolated from *P. ericicola*. Isoplagiochilide was isolated from *P. sinuosa* var. *squamulosa* Plant material of these *Plagiochila* species was collected in the wild. In contrast, *P. adianthoides* grown in axenic culture afforded the new plagiochiline S and 9,10-dihydroovalifolienal, the known plagiochilines A and H, and for the first time the (+)-enantiomer of the previously known (±)-hydrangenol-monomethyl ether. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Within the Hepaticae, the genus *Plagiochila* with its several hundred species, mainly distributed in the tropics, is a rich source of various types of secondary metabolites. So far, mostly terpenoids have been isolated from this genus [1–8]. The majority of *Plagiochila* species, however, have not yet been investigated, due to the difficulties in obtaining sufficient amounts of plant material for chemical studies. The use of axenic cultures is one way to overcome this obstacle [9]. We recently reported the isolation of fusicoccane- and 13-epi-homoverrucosane-type diterpenes from axenic cultures of *P. adianthoides* (Sw.) Dum. and from *P. cristata* (Sw.) Lindenb. collected in the wild [8]. In continuation of our phytochemical investigations of *Plagiochila* species, we now report the isolation and chemical characterization of additional lipophilic compounds from these two species. We also included in this study *P. ericicola* Steph. and *P. squamulosa* var. *sinuosa* (Mitt.) Vandenb., both collected in the wild.

RESULTS AND DISCUSSION

The diethyl ether extract of the Colombian liverwort *P. cristata* was subjected to vacuum liquid chromatography (VLC) [10, 11] on silica gel. Six fractions of increasing polarity were further purified by a combination of VLC, Sephadex LH-20 chromatography, and HPLC, which resulted in the isolation of three new *ent*-2,3-secoaromadendrane-type sesquiterpenoids, named plagiochiline O (**1**), P (**2**) and Q (**3**). Five known sesquiterpenoids, plagiochiline C (**4**), and H (**5**), 3α-acetoxybicyclo-germacrene (**6**), (−)-maaliol (**7**), (−)-spathulenol (**8**), as well as the bisbibenzyl riccardin D (**9**) were also isolated. The identities of **4**, **5**, **6**, **7**, **8** and **9** were established by comparison of their spectral data with those reported in the literature [12–17].

Compound **1** was an oil. The CI mass spectrometry $[M+H]^+$ ion at *m/z* 409 was in agreement with the molecular formula $C_{21}H_{28}O_8$. The IR spectrum indicated the presence of a hydroxyl (3446 cm^{-1}), an exomethylene group (3078 cm^{-1}), and an ester carbonyl (1733 cm^{-1}). The ^1H NMR (C_6D_6) spectrum showed signals indicating the presence of three acetoxy groups at $\delta_H = 1.86$, 1.67, and 1.64, as well as a signal at $\delta_H = 3.18$ typical for a proton geminal to a hydroxyl, and two singlets corresponding to exomethylene protons ($\delta_H = 4.08$, 4.72) indicating an *ent*-2,3-seco-aromadendrane skeleton for **1**. The ^1H NMR and ^{13}C NMR spectral data of **1** were similar to those obtained

*Publication No. 100 of 'Arbeitskreis Chemie und Biologie der Moose'.

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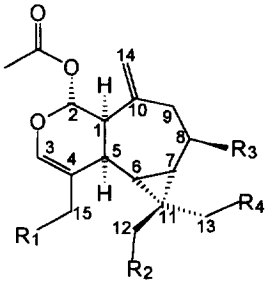
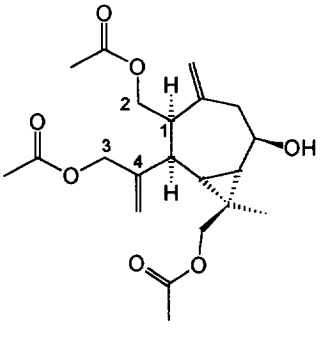
for plagiochiline C (**4**) [12, 15]. The coupling constants of H-1 ($\delta_H = 2.80$, *dd*, $J = 10.1, 3.6$), H-2 ($\delta_H = 6.78$, *d*, $J = 10.1$), H-5 ($\delta_H = 1.97$, *dd*, $J = 10.1, 3.6$), and H-6 ($\delta_H = 0.45$, *t*, $J = 10.1$) indicated *trans* positions for H-1/H-2 and for H-5/H-6, as well as a *cis* position for H-1/H-5, which are in agreement with the proposed skeleton. The ^{1}H NMR spectrum of **1**, when compared with that of **4**, displayed signals for an additional methylene group at $\delta_H = 3.98$ and 3.39, as well as an additional acetoxy group and only one methyl group ($\delta_H = 0.93$, *s*) located at the cyclopropane ring. From these spectral data, two acetoxy groups were positioned at C-2 and C-15, whereas the third acetoxy group could be located at either α-oriented C-13 or β-oriented C-12. According to published ^{13}C NMR data [15] for **4**, the signal of α-oriented methyl group C-13 occurred at $\delta_C = 15.6$ and the signal of the β-oriented methyl group C-12 was at $\delta_C = 28.8$. In the spectrum of **1**, however, the signal at $\delta_C = 28.8$ was not present, but the signals of a methyl group at $\delta_C = 11.4$ and a methylene group bearing an oxygen at $\delta_C = 73.2$ were observed. The spectroscopic data of **1**, compared with those of **4**, suggested a β-orientation of the acetoxymethylene group at the cyclopropane ring, which was confirmed by NOE experiments (Fig. 1). Irradiation of the α-configured H-5 gave enhancement (9%) of the methyl group at C-11. No NOE effect was detectable between H-5 and the methylene group located at C-11. Amplifications of H-6 (4%) and H-7 (7%), both β-configured, were caused by irradiation of the methylene at C-11. No NOE effect was observed between the methylene protons at C-11 and H-5α. On the basis of these observations, the acetoxymethylene group located at the cyclopropane ring had to be β-oriented. The position of the hydroxyl at C-8 was determined by close examination of the ^{1}H–^{1}H COSY spectrum. The signal for H-8 ($\delta_H = 3.18$, *brt*, $J = 10.7$), geminal to the hydroxyl, showed couplings to H-7 ($\delta_H = 0.69$, *m*), and H-9β



1: $R_1 = R_2 = OAc$, $R_3 = OH$, $R_4 = H$
4: $R_1 = OAc$, $R_2 = R_3 = R_4 = H$
5: $R_1 = R_2 = R_3 = R_4 = H$
13: $R_1 = R_2 = R_3 = H$, $R_4 = OAc$



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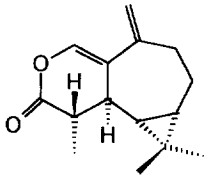

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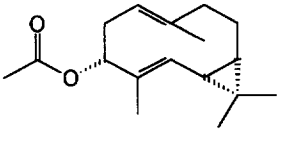

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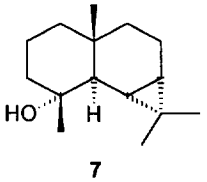

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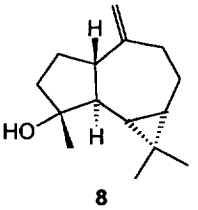

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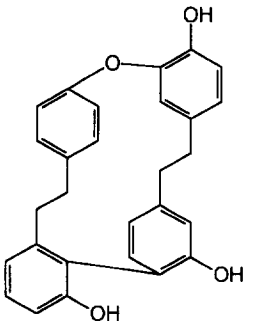

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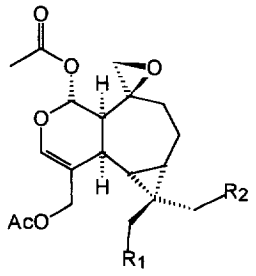
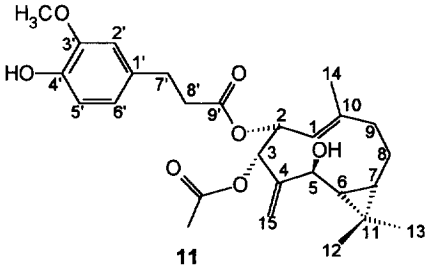


14: $R_1 = R_2 = H$
10: $R_1 = OAc$, $R_2 = H$
plagiochiline B: $R_1 = H$, $R_2 = OAc$



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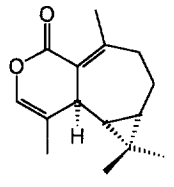

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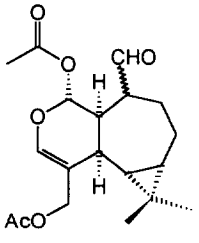

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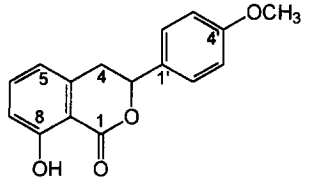

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($\delta_H = 2.52$, *dd*, $J = 12.0$, 11.4), and a weak coupling to H-9α ($\delta_H = 2.36$, *brd*, $J = 12.4$). The coupling constant of H-8 indicated a diaxial coupling to H-7 and H-9β, respectively. The proposed α-configuration of H-8 was established by NOE effects between H-8 and H-13 (9%), H-8 and H-5 (4%), and H-8 and H-9α (3%), respectively. On the basis of these results, we established the structure of **1** and suggest the name plagiochiline O.

Compound **2** was an oil. The ^{1}H NMR and ^{13}C NMR spectra (both in $CDCl_3$) of **2**, when compared with those of **1**, indicated a similarity between **1** and **2**. However, the typical signals for the enol-acylal group (H-2, H-3, C-2) of most known plagiochilines [1, 5, 7] including **1**, **4**, **5**, **10** and **13**–**15** were missing in the spectra of **2**. The ^{1}H NMR spectrum of **2**, compared with that of **1**, showed two additional double doublets at $\delta_H = 4.32$ and 4.17 (H-2a + b) and two singlets at $\delta_H = 5.17$ and 5.20 (H-15a + b) suggesting the presence of an additional exomethylene in **2**. The proton H-5 in **2**, compared with that of **1**, showed a reduction of its multiplicity from a double doublet to a broad doublet. In the ^{13}C NMR of **2**, compared with that of **1**, the signals of two methine groups were replaced by those of two methylenes. All other NMR data of **2** corresponded with those of **1** indicating a seven-membered ring bearing an exomethylene, an 8β-hydroxyl, and a cyclopropane ring with an α-oriented methyl group and a β-oriented acetoxymethylene. The ^{1}H–^{1}H COSY spectrum of **2** showed the same ^2J and ^3J connectivities for the seven- and the three-membered rings similar to those in **1**. The CI-mass spectrum showed a $[M + H]^+$ ion peak at m/z 395, which, together with the ^{13}C NMR data corresponded to the molecular composition $C_{21}H_{30}O_7$. Compared with **1**, compound **2** showed one less oxygen and only seven double bond equivalents. Due to the presence of the same numbers of double bonds and carbonyls in **1** and **2**, compound **2** was assumed to be a bicyclic sesquiterpenoid with three acetoxy groups. This observation suggested that the

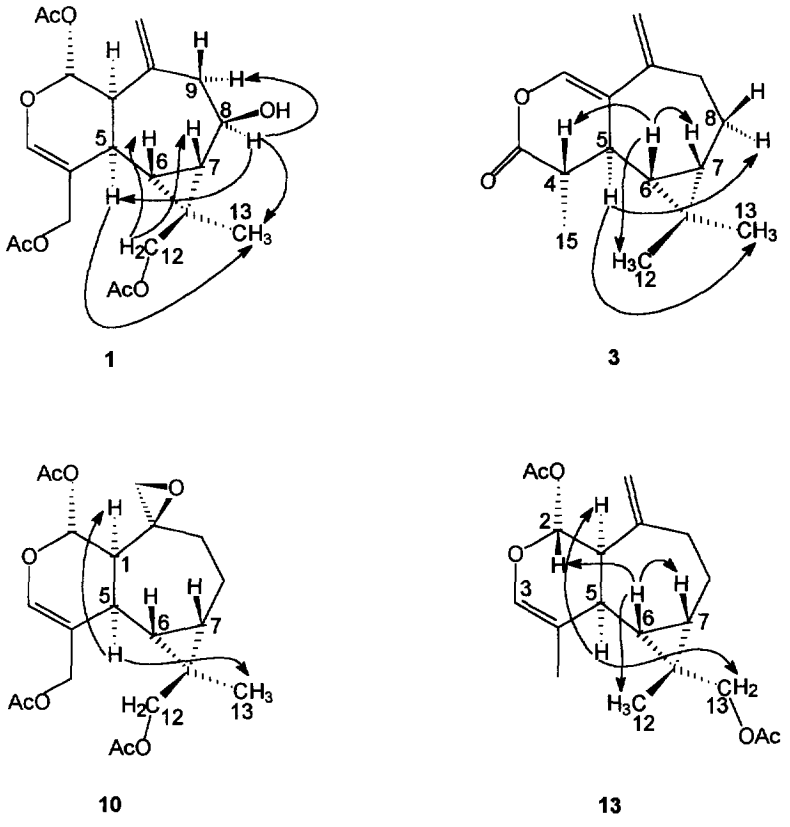


Fig. 1. NOE interactions of compounds **1**, **3**, **10** and **13**.

heterocyclic six-membered ring, typical for plagiochilines, was open in **2**. In the ^{1}H–^{1}H COSY spectrum of **2**, H-5 showed a vicinal coupling to H-6 and an allylic long range coupling to exomethylene protons H-15a + b, which were in turn coupled allylic to the acetoxymethylene protons H3a + b ($\delta_H = 4.67$, 4.55). The ^{1}H–^{1}H COSY spectrum also displayed a ^3J connectivity between H-1 and another acetoxymethylene (H-2a + b). No ^3J connectivity was detected between H-1 and H-5. This can be explained with the increased flexibility of the seven-membered ring in **2**, when compared with the more rigid skeleton of **1** and other plagiochilines. The more flexible ring of **2** allows the torsion angle of the system H-5–C-5–C-1–H-1 to come close to a value of 90°. On the basis of these investigations, we established the structure **2** for plagiochiline P.

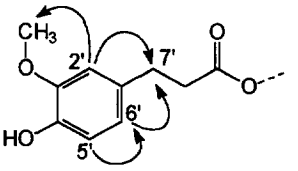
The ^{13}C NMR of compound **3**, an oil, revealed the signals of 15 carbons including those corresponding to three methyls (C-12, C-13, C-15), two methylenes (C-8, C-9), four methines (C-4, C-5, C-6, C-7), one quaternary carbon (C-11), four signals of olefinic carbons (C-1, C-2, C-10, C-14) including one exomethylene (C-14), and one ester or lactone carbonyl (C-3). The carbonyl absorption at 1771 cm^{-1} in the IR spectrum corresponded to the presence of an ester or a lactone group. The GC-MS spectrum showed the molecular ion peak of *m/z* 232, which was in agreement with the molecular formula $C_{15}H_{20}O_2$. The resulting six double bond equivalents indicated a tricyclic sesquiterpenoid with a ring double bond, an exomethylene group and an ester function. The ^{1}H NMR confirmed the presence of an exomethylene group ($\delta_H = 4.87$, 4.80, both *brs*, H-14a + b). The signals at $\delta_H = 0.42$ (*dd*, $J = 10.5$, 9.4, H-6) and $\delta_H = 0.72$ (*m*, H-7) indicated a cyclopropane ring substituted with a geminal dimethyl group ($\delta_H = 1.00$, 1.03, both *s*, 3H, H-12/13). The doublet at $\delta_H = 1.25$ corresponded to a third methyl group (H-15, *d*, $J = 7.0$). The vinylic proton H-2 experienced a downfield shift caused by a nearby oxygen. The observed spectral data in **3** suggested an *ent*-2,3-secoaromadendrane skeleton. The ^2J and ^3J connectivities for the protons H-6, H-7, H-8, and H-9 in **3** were similar to those observed in plagiochiline C (**4**). In the ^{1}H–^{1}H COSY spectrum of **3**, H-5 showed vicinal coupling to H-4 and H-6, respectively. H-4 ($\delta = 2.44$, *m*) showed coupling to methyl H-15. The ^{1}H–^{1}H COSY spectrum also revealed a long range coupling between H-5 and vinylic H-2. The coupling constants of H-5 ($\delta_H = 2.14$, *ddd*, $J = 10.6$, 10.5, 1.5) indicated a diaxial coupling to H-4 and H-6, respectively, which was further confirmed by NOE examinations (Fig. 1). Irradiation of H-6β produced enhancements of H-7, H-4, and H-12. NOE effects were also observed between H-5, H-13 and H-8α. Based on these data, the structure **3** was established for plagiochiline Q.

The diethyl ether extract of the African–Alpine

liverwort *P. ericicola* was separated into six fractions of different polarity by VLC. These fractions were treated as described for those of *P. cristata*, which resulted in the isolation of a new *ent*-2,3-secoaromadendrane sesquiterpenoid, named plagiochiline R (**10**), and the new (+) - 3α - acetoxy - 2α - [3 - (4 - hydroxy - 3 - methoxyphenyl)propanoyloxy]bicyclogermacra-*E*1(10), 4(12)-dien-5β-ol (**11**). The previously known sesquiterpenoids, plagiochilines C (**4**) and H (**5**), 3α-acetoxybicyclogermacrene (**6**), and (−)-spathulenol (**8**), were also isolated.

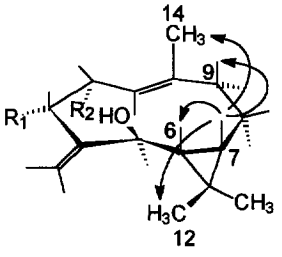
The spectral data of **10** indicated an *ent*-2,3-seco-aromadendrane-type sesquiterpenoid. The CI-mass spectrometry $[M+H]^+$ ion at *m/z* 409 was in agreement with the molecular composition $C_{21}H_{28}O_8$. The ^1H NMR and ^{13}C NMR spectra of **10** were similar to those reported for the known plagiochiline B [18, 19]. In the ^{13}C NMR spectrum of **10**, however, the shift values observed for the methyl and the acetoxy-methylene groups, both located at C-11, were different from those reported for plagiochiline B. In the ^{13}C NMR spectrum of plagiochiline B, the signal for the methyl group located at C-11 was reported at $\delta_c = 24.3$ and that for acetoxymethylene at $\delta_c = 65.4$. In the spectrum of **10**, however, the signal of the methyl located at C-11 appeared at $\delta_c = 11.7$ and that of acetoxy-methylene at $\delta_c = 73.8$. This observation suggested a reversed stereochemistry for the acetoxy-methylene group on the cyclopropane ring in **10**. The suggested β-orientation of the acetoxymethylene group at C-11 was confirmed by NOE experiments (Fig. 1). Irradiation of the α-oriented H-5 gave enhancements of the methyl group H-13 (9%) and the α-oriented H-1 (10%). Since the epoxide rings of all known epoxide bearing plagiochilines [1, 4, 5, 13, 15, 19–21] were reported as β-oriented, it was very likely that the epoxide ring of **10** also had a β-orientation. This suggestion was confirmed by comparison of ^1H and ^{13}C NMR shift values of **10** with literature data of plagiochiline B and those of synthesized epoxyovalifoliene, which has an α-oriented epoxide ring [15, 21]. On the basis of these data, structure **10** was established for plagiochiline R.

The ^{13}C NMR spectrum of the oily compound **11** revealed 27 C atoms comprising eight tetrasubstituted, nine trisubstituted, five bisubstituted and five monosubstituted carbons. The EI-mass spectrum showed the molecular ion peak $[M]^+$ at *m/z* 472, which was in agreement with the molecular compositions $C_{27}H_{36}O_7$. From the ^1H and ^{13}C NMR spectra, the structure of **11** was elucidated as an ester of a substituted phenylpropanoic acid and an acetoxylated sesquiterpene alcohol. In the ^1H NMR, the singlet at $\delta_H = 3.84$ which showed C–H correlation to a signal at $\delta_c = 55.9$ in the ^{13}C NMR, was assigned to a methyl group of a phenyl methyl ether. Two ^1H NMR triplets, integrated for two protons each ($\delta_H = 2.83$, 2.56, both *t*, $J = 7.8$), corresponded to the ethylene protons H-7′ and H-8′. The shift values of these protons indicated a phenyl substituent for methylene CH_2-7′ and a carboxyl substituent for methylene CH_2-8′, which were further confirmed by long range C–H correlation between H-7′ and C-1′ as well as between H-8′ and C-9′. From the coupling pattern of the phenyl protons ($\delta_H = 6.66$, *d*, $J = 1.8$ Hz, H-2′; $\delta_H = 6.80$, *d*, $J = 8.0$ Hz, H-5′; $\delta_H = 6.65$, *dd*, $J = 8.0$, 1.8, H-6′), the benzene ring had to be connected to an additional substituent. In the EI-mass spectrum of **11**, the peaks of *m/z* 179 (62%), 137 (100%), and 123 (32%) corresponded to the cleavage of hydroxy-methoxy substituted phenylpropanoyl, benzyl, and phenyl fragments. Based on this evidence, the additional substituent on the aromatic ring was determined to be a hydroxyl group. NOE experiments allowed the assignment of the aromatic protons and the position of the hydroxyl and methoxy groups in **11** (Fig. 2). NOE effects could be observed between H-2′ and OMe (10%), H-2′ and H-7′ (3%), H-6′ and H-7′ (3%), and between H-5′ and H-6′ (3%). After the elucidation of the phenylpropanoyloxy moiety of **11**, for the sesquiterpenoid part of the molecule 17 carbons, 25 hydrogens and three oxygens remained. The ^1H NMR showed the typical signals of a condensed three-membered ring with a geminal dimethyl group (δ_H = 1.06, 1.02, both 3H, *s*, H-12/13; $\delta_H = 0.78$, *dd*, $J = 9.1$, 8.7, H-6; $\delta_H = 0.51$, *m*, H-7). The proton spectrum also revealed an exomethylene group ($\delta_H = 5.52$, 5.61, both *s*, H-15a + b), a vinylic proton ($\delta_H = 5.34$, *brd*, 10.4, H-1), and a vinylic methyl group ($\delta_H = 1.77$, *s*, H-14). The signals at $\delta_H = 5.55$ (*dd*, $J = 10.4$, 3.5, H-2) and $\delta_H = 5.75$ (*d*, $J = 3.5$, H-3) corresponded to two protons geminal to ester functions. The proton spectrum also exhibited the signals of an acetoxymethyl group ($\delta_H = 2.06$, *s*) and a proton geminal to a hydroxyl ($\delta_H = 4.12$, *d*, $J = 9.1$, H-5). Of the five double bond equivalents calculated for the sesquiterpenoid part of **11**, four resulted from the above-mentioned functionalities; thus a C_{10}-macrocycle bearing these groups was postulated. The remaining possibilities were either a bicyclogermacrane with a *cis*-linked cyclopropane ring or a lepidozane skeleton with a *trans*-linked cyclopropane ring. NOE effects between cyclopropane protons H-7 and H-6 (7%) confirmed a bicyclogermacrane skeleton for **11**. Since all bicyclogermacrane components isolated from liverworts show the cyclopropane ring in α-orientation of the C_{10}-macrocycle, we assumed the cyclopropane ring in **11** to have an α-orientation. All conclusions drawn from NOE experiments (Fig. 2) were based on this assumption. The positions of the two esters and the hydroxyl as well as the location of the double bond and the exomethylene group were determined by close examination of ^1H–^1H COSY, ^{13}C–^1H COSY, and long range ^{13}C–^1H correlation experiments. The ^1H–^1H COSY spectrum indicated a ^3J coupling between the cyclopropane protons H-6 and H-7. H-6 also coupled to a vicinal proton which itself was in a geminal position to a hydroxyl at C-5. The spectrum also revealed a sequence of two methylene groups (H-8α + β, H-9α + β) next to H-7. Protons H-2 and H-3, both geminal to ester functions, showed ^3J connectivities to each other and H-2 also to



11A: 4'-hydroxy-3'-methoxyphenyl-propanoic acid moiety of **11**

11 A



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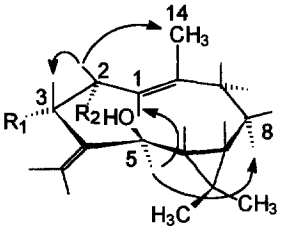

11*

*** free ending bonds refer to protons, R_1: OAc, R_2: 11A**

Fig. 2. NOE interactions of compound **11**.

vinylic proton H-1. The ^{13}C–^{1}H COSY spectrum exhibited a long range correlation between H-9α + β and the vinylic methyl group C-14. The positions of the acetoxy group at C-3 and the phenylpropanoyloxy at C-2 were determined by long range C–H correlations between H-3 and the carboxyl carbon of the acetoxy group as well as between H-2 and carboxyl C-9′. The relative stereochemistry of the substituents were ascertained by NOE experiments based on the assumption of an α-configured cyclopropane ring (Fig. 2). These experiments also indicated the conformation of the C_{10}-macrocycle. Irradiation of β-standing H-7 revealed an effect on H-6, H-12, H-9β, and H-14. The NOE effects between H-5 and H-8α, as well as between H-5 and H-1 were due to an α-orientation of H-5. The *trans*-configuration of CH_3-14 and H-1 at the double bond was confirmed by the irradiation of H-1 which caused no enhancement of the CH_3-14 signal. Irradiation of H-2 gave a clear enhancement of H-3 (11%) and H-14 (4%), but no effect on H-1, indicating a β-configuration of H-2 and H-3 and an α-orientation for H-1. Thus, the structure of **11** was elucidated as (+) - 3α - acetoxy - 2α - [3 - (4 - hydroxy - 3 - methoxyphenyl)-propanoyloxy]bicyclo - germacra - *E*1(10),4(12) - dien - 5β - ol.

The diethyl ether extract of the Rwandan liverwort *P. squamulosa* var. *sinuosa* was processed in a similar manner as described for the extract of *P. cristata*. The six fractions obtained were compared by TLC on silica-gel (CH_2Cl_2–EtOAc, 98:2) with those obtained from *P. cristata* and *P. ericicola*. After developing and visualization with anisaldehyde-sulphuric acid, one red-coloured spot could be seen in fraction 2 of *P. squamulosa* var. *sinuosa* that was not detected in fractions of *P. cristata* and *P. ericicola*. This compound was isolated and identified as isoplagiochilide (**12**), which was recently reported from *P. elegans* [22].

For the continuing study of *P. adianthoides*, plant material from axenic cultures was used [8]. The dichloromethane extract of *P. adianthoides* was treated as described for the diethyl ether extract of *P. cristata* resulting in the isolation of two new *ent*-2,3-seco-aromadendrane sesquiterpenoids, plagiochiline S (**13**) and 9,10-dihydroovalifolienal (**15**), together with the previously known plagiochilines H (**5**) and A (**14**), and 3α-acetoxybicyclogermacrene (**6**) [13, 18]. The (+)-enantiomer (**16**) of the previously known (±)-3,4-dihydro-3-(4-methoxyphenyl)isocoumarin-8-ol [23] was isolated for the first time from a natural source.

The ^{1}H NMR spectrum of **13** indicated an *ent*-2,3-secoaromadendrane-type sesquiterpenoid with two acetoxy groups. In the EI-mass spectra and CI-mass spectra, the molecular ion peak could not be observed. The highest *m/z* ratio found in the EI-mass spectrum was *m/z* 274 (9%). The cleavage of only one CH_3COOH fragment gave a peak at *m/z* 214 (63%). This indicated that the peak at *m/z* 274 corresponded to a fragment resulting from the cleavage of an additional acetoxy group and that **13** had a molecular weight of 334. The ^{1}H NMR spectra of **13** was similar to those of the plagiochilines C (**4**) and H (**5**) and revealed the presence of an exomethylene group (δ_H = 4.76, 4.75, both *s*, H-14a + b), two acetoxy groups (δ_H = 2.06, 2.05, both *s*, 3H), and one acetoxymethylene group (δ_H = 4.15, 4.07, both *d*, *J* = 11.7, H-13a + b). However, the proton spectrum of **13** showed an additional signal for a vinylic methyl group (δ_H = 1.59, *d*, *J* = 1.2, H-15), whose protons showed a long range coupling to

the vinylic proton H-3 ($\delta_H = 5.96$, *d*, $J = 1.2$), also found in **5**. In contrast to the spectrum of **5**, the spectrum of **13** displayed only one methyl group located at the cyclopropane ring. This observation suggested that the cyclopropane ring in **13** was substituted with a methyl group and an acetoxymethylene group as in the case of **1** and **2**. The configuration of the acetoxymethylene was determined by NOE experiments (Fig. 1). Irradiation of β-configurated H-6 gave a clear enhancement of β-configurated H-7, H-2 and the methyl group located at the three-membered ring. Amplification of the α-oriented H-1 and the acetoxymethylene protons were caused by irradiation of the α-oriented H-5. Thus, structure **13** was elucidated for plagiochiline S.

The ^{1}H and ^{13}C NMR spectral data of **15** revealed another *ent*-2,3-secoaromadendrane-type sesquiterpenoid. In the ^{1}H NMR spectrum the signals at $\delta_H = 2.07$ and 2.04 (both *s*, 3H) were assigned to two acetoxy groups confirmed by ^{13}C NMR spectral data. The ^{1}H NMR signal at $\delta_H = 9.55$ (*d*, $J = 2.5$) and the ^{13}C NMR signal at $\delta_c = 202.8$ corresponded to an aldehyde function in the molecule. Two singlets at $\delta_H = 1.03$ and 0.97 (both *s*, 3H) indicated a geminal dimethyl substituent at the cyclopropane ring. With the information of the NMR spectral data, the peak at *m/z* 291 in the Cl-mass spectrum was assigned to a fragment $[M + H - CH_3COOH]^+$ resulting from the cleavage of one acetoxy group. In the ^{1}H–^{1}H COSY spectrum, the acetoxymethylene protons (15a + b) showed an allylic long range coupling to the vinylic proton H-3 indicating the position of an acetoxy group at C-15. The shift value of proton H-2 ($\delta_H = 6.15$, *d*, $J = 9.4$) corresponded to a geminal position to another acetoxy group. The coupling patterns of the sequence H-5, H-6, H-7, H-8, and H-9 in **15** were similar to those observed for **3**. The methylene protons H-9a + b, however, were coupled further to a proton at $\delta_H = 2.60$ (*m*, H-10). Both, H-5 and H-2 showed ^3J connectivities to H-1 ($\delta_H = 2.60$, *m*). The signal of H-1 in **15**, when compared to that in the plagiochilines **1**, **2**, **4**, **5**, **10** and **14** reported here, showed an increase of its multiplicity from a double doublet to a multiplet indicating coupling to an additional neighbour (H-10). The signals of H-1 and H-10 overlapped. On the basis of these data, the structure of **15** was determined as the 9,10-dihydroderivative of the previously reported ovalifolienal [15, 20]. The stereochemistry of the aldehyde group could not be determined due to the instability of this compound.

Compound **16**, isolated as a powder with a melting point of 105°, showed a violet fluorescence under UV 366 nm. The optical rotation was $[\alpha]_D = +90.6°$ (*c*, 0.2, $CHCl_3$). On the basis of its ^{1}H NMR, ^{13}C NMR, MS and IR spectroscopic data, as well as the optical rotation value, **16** was identified as the (+)-enantiomer of 3,4-dihydro-3-(4-methoxyphenyl)isocoumarin-8-ol also known as hydrangenol-monomethyl ether. Hydrangenol-monomethyl ether was reported by Kaneko *et al.* [23] from *Hydrangea macrophylla* var. *thunbergii* with a melting point of 124° but no value was given for its optical rotation. The melting point of the racemate (±)-hydrangenol-monomethyl ether synthesized by Asahina and Asano [24] was 122–123°. The similar melting points of the synthetic product and the hydrangenol-monomethyl ether isolated by Kaneko *et al.* indicate that the naturally occurring compound was also racemic. This assumption was supported by the fact that hydrangenol-8-β-glucoside occurs as a racemate in *Hydrangea* species [25]. It is also interesting to note that hydrangenol isolated from this genus was shown to be racemic by converting it to its monomethyl ether with diazomethane and proving the identity of the product with the synthesized (±)- hydrangenol-monomethyl ether by mixed melting point [26]. These data show that the (+)-enantiomer **16** is not identical with the previously reported hydrangenol monomethyl ether and, thus, isolated for the first time from a natural source.

EXPERIMENTAL

General. Mps: uncorr., solvents used for spectral measurements: $CDCl_3$, C_6D_6. The measurements of the NMR spectra were carried out on a Bruker AM 400 NMR spectrometer [^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz)]. The ^{13}C–^{1}H correlation experiments of compound **11** were carried out on a Bruker DRX 500 NMR spectrometer.

The assignments of ^{1}H NMR were confirmed by ^{1}H–^{1}H COSY and NOE examinations. The assignments of ^{13}C NMR were confirmed by DEPT-spectra, ^{13}C–^{1}H correlation experiments in the case of **11**, as well as by comparison with literature data of similar compounds. Previously known compounds were identified by comparison of their spectral data with published data. IR: KBr (solids) or neat, Perkin Elmer 2000. CI-MS: 120 eV isobutane, Finnigan MAT TSQ 70. EI-MS: 70 eV, Finnigan MAT 90. GC-MS: Hewlett Packard 5790A.

Plant material. Plagiochila cristata was collected in May 1991 in Colombia (Parque Montañes de Chicaque, departamento Cundinamarca: San Antonio de Tena). *Plagiochila ericicola* was collected in September 1991 in Zaire (Province of Kivu, Kahuzi-Biega Natl Park, Mt Kahuzi). *Plagiochila squamulosa* var. *sinuosa* was collected in August 1991 in Rwanda (Pref. de Gikongoro Foret de Nyungwe, Rwasenoko). All voucher specimens (3517, 7896, 6187, respectively) are deposited in the herbarium of the 'Fachbereich Botanik der Universität des Saarlandes', Saarbrücken, Germany. *Plagiochila adianthoides* was collected in Jan. 1988 in Panamá (summit area of 'Cerro Jefe', Parque Nacional Chargres, Provincia Panamá). Voucher specimens (051) are deposited in the herbaria of the 'Fachbereich Pharmakognosie und Analytische Phytochemie der Universität des Saarlandes', Saarbrücken, and 'Departamento de Botánica, Escuela de Biologia, Universidad de Panamá', Panamá.

Axenic culture. The culture was performed as described in ref. [8]. One unit volume of axenic culture

yielded 8 g fresh/1.3 g dried plant material. A voucher specimen (052) is deposited in the herbarium of the 'Fachbereich Pharmakognosie und Analytische Phytochemie der Universität des Saarlandes', Saarbrücken.

Extraction and isolation. Air dried and powdered plant material (120 g) of *P. cristata* was treated as described in [8] to yield 6 frs of increasing polarity (0, 2, 5, 8, 20 and 100% EtOAc, respectively). Fr. 5 was sepd with VLC (RP-18, 20-45 μm, MeOH–H_2O, 4:1) followed by HPLC (LiChrospher RP-18, 5 μm, MeOH–H_2O, 85:15) yielding compounds reported in [8] and a mixture of **1**, **2** and **9**. From this mixt., riccardin D (35 mg) (**9**) was isolated by HPLC (LiChrospher RP-18, 5 μm, MeOH–H_2O, 3:1) and plagiochiline O (9 mg), (**1**) and P (**2**) (3 mg), were obtained using HPLC (LiChrospher RP-18, 5 μm, MeOH–H_2O, 7:3) followed by HPLC (LiChrospher Diol, 5 μm, *n*-hexane–EtOAc, 7:3). Fr. 2 was chromatographed on Sephadex LH-20 as reported in [8], followed by HPLC (LiChrospher Si 60, 5 μm, *n*-hexane–EtOAc, 98:2 and LiChrospher Diol, 5 μm, *n*-hexane–EtOAc–HOAc, 99.5:0.5:0.5) yielding plagiochiline Q (3.5 mg) (**3**) and a mixt. of plagiochiline H (**5**) and 3α-acetoxybicyclogermacrene (**6**). This mixt was sepd using HPLC (LiChrospher RP-18, 5 μm, MeOH–H_2O, 85:15) yielding **5** (1 mg) and **6** (1.5 mg). HPLC (LiChrospher Diol, 5 μm, *n*-hexane–EtOAc, 93:7) of fr. 3 resulted in the isolation of (−)-spathulenol (150 mg) (**8**), and in a mixture of plagiochiline C (**4**) and (−)-maaliol (**7**). VLC (RP-18, 20–45 μm, MeOH–H_2O gradient) yielded **7** (35 mg) and further HPLC (LiChrospher RP-18, 5 μm, MeOH–H_2O, 3:1) yielded **4** (91 mg).

Air dried and powdered plant material (50 g) of *P. ericicola* was treated as described for *P. cristata* in [8] yielding 6 frs of increasing polarity. Compounds **5** (3 mg) and **6** (4 mg) were obtained from fr. 2 by VLC (silica gel Si 60, 15 μm, *n*-hexane–EtOAc gradient) followed by HPLC (LiChrospher RP-18, 5 μm, MeOH–H_2O, 85:15). HPLC (LiChrospher Si 60, 5 μm, *n*-hexane–EtOAc–HOAc, 92:8:1) of fr. 3 resulted in the isolation of **4** (12 mg) and **8** (12.5 mg). Fr. 6 was applied to VLC (RP-18, 20–45 μm, MeOH–H_2O gradient) and VLC (silica gel Si 60, 15 μm, *n*-hexane–EtOAc gradient) resulting in the isolation of plagiochiline R (7 mg) (**10**). From fr. 5, (+) - 3α - acetoxy - 2α - [3 - (4 - hydroxy - 3 - methoxyphenyl) - propanoyloxy]bicyclogermacra - *E*1(10), 4(12)-dien-5β-ol (17 mg) (**11**) was isolated by VLC (RP-18, 20–45 μm, MeOH–H_2O gradient) followed by HPLC (LiChrospher RP-18, 5 μm, MeOH–H_2O, 3:1).

Air dried and powdered plant material (50 g) of *P. squamulosa* var. *sinuosa* was treated as described for *P. cristata* in [8] yielding 6 frs of increasing polarity. Isoplagiochilide (5.5 mg) (**12**) was isolated from fr. 2 by HPLC (LiChrospher Si 60, 5 μm, *n*-hexane–EtOAc, 97.5:2.5) followed by Sephadex LH-20 chromatography (CH_2Cl_2–MeOH, 1:1).

Air dried and powdered plant material (70 g) of *P. adianthoides* was treated as described in [8] to yield 7 frs of increasing polarity. Compounds **5** (1 mg) and **6** (1.5 mg) were isolated from fr. 2 by Sephadex LH-20 chromatography (CH_2Cl_2–MeOH, 1:1) followed by HPLC (LiChrospher Si 60, 5 μm, *n*-hexane–EtOAc, 97:3 and CH_2Cl_2-*n*-hexane, 65:35). Fr. 7 was chromatographed on Sephadex LH-20 (CH_2Cl_2–MeOH, 1:1) and applied to HPLC (LiChrospher Diol, 5 μm, *n*-hexane–EtOAc, 70:30 and LiChrospher Si 60, 5 μm, *n*-hexane–EtOAc, 70:30) yielding plagiochiline A (30 mg) (**14**) and 9,10-dihydroovalifolienal (1 mg) (**15**). Fr. 4 was sepd by MPLC (LiChroprep Si 60, Lobar C, 40–63 μm, *n*-hexane–EtOAc gradient), yielding a mixt. of **13** and **16**. HPLC (LiChrospher Si 60, 5 μm, *n*-hexane–EtOAc, 97.5:2.5 and LiChrospher RP-18, 5 μm, MeOH–H_2O, 3:1) resulted in the isolation of plagiochiline S (6.8 mg) (**13**). (+)-3,4-dihydro-3-(4-methoxyphenyl)isocoumarin-8-ol (1 mg) (**161**) was isolated by HPLC (LiChrospher Diol, 5 μm, *n*-hexane–EtOAc, 97.5:2.5). For structure elucidation, an additional 6 mg of **16** was isolated from 120 g air dried and powdered plant material of *P. adianthoides*.

Plagiochiline O (**1**). $[\alpha]_D = +22.1°$ (*c* 0.2, $CHCl_3$); CI-MS *m/z* 409 $[M+H]^+$, IR ν_{max} cm^{-1}: 3446, 3078, 2954, 1733, 1674, 1640, 1434, 1379, 1228, 1192, 1148, 1045, 925, 857, 760, 714, 628, 611, 560; ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$) δ: 49.7 (*d*, C-1), 90.6 (*d*, C-2), 140.5 (*d*, C-3), 115.5 (*s*, C-4), 33.3 (*d*, C-5), 25.0 (*d*, C-6), 33.3 (*d*, C-7), 64.4 (*d*, C-8), 45.4 (*t*, C-9), 143.0 (*s*, C-10), 23.3 (*s*, C-11), 73.2 (*t*, C-12), 11.4 (*q*, C-13), 119.9 (*t*, C-14), 62.7 (*t*, C-15), 20.8 (*q*, OAc), 169.4 (*s*, OAc), 20.8 (*q*, OAc), 170.7 (*s*, OAc), 20.9 (*q*, OAc), 171.0 (*s*, OAc).

Plagiochiline P (**2**). $[\alpha]_D = -15.4°$ (*c* 0.3, $CHCl_3$); CI-MS *m/z* 395 $[M+H]^+$; IR ν_{max} cm^{-1}: 3453, 3081, 2907, 1735, 1639, 1460, 1371, 1223, 1027, 906, 794, 594, 543; ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$) δ: 47.7 (*d*, C-1), 62.2 (*t*, C-2[a]), 66.4 (*t*, C-3[a]), 146.4 (*s*, C-4[b]), 40.6 (*d*, C-5), 22.0 (*d*, C-6), 31.8 (*d*, C-7), 69.1 (*d*, C-8), 44.0 (*t*, C-9), 144.8 (*s*, C-10[b]), 23.2 (*s*, C-11), 73.3 (*t*, C-12), 10.2 (*q*, C-13), 116.9 (*t*, C-14[c]), 114.2 (*t*, C-15[c]), 20.8 (*q*, OAc), 171.1 (*s*, OAc), 20.8 (*q*, OAc), 171.2 (*s*, OAc), 20.8 (*q*, OAc), 171.2 (*s*, OAc) ([a,b,c]assignments interchangeable).

Plagiochiline Q (**3**). $[\alpha]_D = -136°$ (*c* 0.35, $CHCl_3$); GC-MS *m/z* (rel. int.): 232 (31), 217 (5), 203 (5), 189 (24), 176 (38), 161 (45), 147 (44), 133 (90), 119 (47), 105 (91), 91 (100), 77 (70), 65 (36), 55 (52); IR ν_{max} cm^{-1}: 3512, 3082, 2919, 2372, 1771, 1645, 1620, 1458, 1380, 1342, 1161, 1077, 897, 864, 839, 781, 737, 712, 631, 469; UV $\lambda_{max}^{CHCl_3}$ nm: 239; ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$) δ: 126.6 (*s*, C-1), 134.9 (*d*, C-2), 171.6 (*s*, C-3), 40.1 (*d*, C-4[a]), 37.0 (*d*, C-5[a]), 29.4 (*d*, C-6[b]), 25.6 (*d*, C-7[b]), 21.7 (*t*, C-8), 36.4 (*t*, C-9), 145.2 (*s*, C-10), 16.7 (*s*, C-11), 28.4 (*q*, C-12), 15.4 (*q*, C-13), 113.3 (*t*, C-14), 14.2 (*q*, C-15) ([a,b]assignments interchangeable).

Plagiochiline R (**10**). $[\alpha]_D = +25.4°$ (*c* 0.3, $CHCl_3$); CI-MS *m/z* 409 $[M+H]^+$; IR ν_{max} cm^{-1}: 3488, 2918, 1741, 1670, 1447, 1365, 1228, 1177, 1114, 1022, 880, 840, 788, 754, 657, 594, 537, 457; ^1H NMR: see Table

Table 1. ^{1}H NMR spectral data of compounds **1**, **2**, **3**, **10**, **13** and **15**

H	**1** (C_6D_6)	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**10** ($CDCl_3$)	**13** ($CDCl_3$)	**15** ($CDCl_3$)
1	2.80 *dd* (10.1, 3.6)	2.76	2.82 *dd* (10.0, 4.8)		1.73 *dd* (10.1, 3.3)	2.79 *dd* (9.7, 3.9)	2.60 *m*
2	6.78 *d* (10.1)	6.49	4.32 *dd* (10.9, 10.0) 4.17 *dd* (10.9, 4.8)	6.49 *d* (1.5)	6.75 *d* (10.1)	6.49 *d* (9.7)	6.15 *d* (9.4)
3	6.14 *s*	6.31	4.67 *brd* (13.3) 4.55 *brd* (13.3)		6.29 *s*	5.96 *d* (1.2)	6.38 *s*
4				2.44 *m*			
5	1.97 *dd* (10.1, 3.6)	2.02	1.96 *brd* (11.0)	2.14 *ddd* (10.6, 10.5, 1.5)	2.11 *dd* (9.2, 3.3)	1.87 *dd* (9.8, 3.9)	2.23 *dd* (4.7, 10.9)
6	0.45 *t* (10.1)	0.81	0.95 *dd* (11.0, 10.1)	0.42 *dd* (10.5, 9.4)	0.78 *t* (9.2)	0.75 *t* (9.3)	0.52 *dd* (9.0, 8.9)
7	0.69 *m*	1.11	1.02–1.11 *m*	0.72 *m*	1.07–1.17 *m*	0.98 *m*	0.72 *m*
8	3.18 *brt* (10.7)	3.38	3.47 *brt* (9.6)	1.16 *m* (α)	1.07–2.11 *m*	0.93–2.32 *m*	2.01 *m*
				1.86 *m* (β)	1.07–2.11 *m*	0.93–2.32 *m*	1.31 *m*
9	2.36 *brd* (12.4) (α)	2.55–2.43	2.46 *brd* (12.1) (α)	2.49[a] *m* (α)	1.07–2.11 *m*	0.93–2.32 *m*	1.79 *m*
	2.52 *dd* (12.0, 11.4) (β)	2.55–2.43	2.66 *dd* (12.1, 11.0) (β)	2.42[a] *m* (β)	1.07–2.11 *m*	0.93–2.32 *m*	1.70 *m*
10							2.60 *m*
12	3.98 *d* (11.1)	4.02	3.97 *d* (11.2)	1.03[b] *s*	4.01 *d* (11.1)	1.11 *s*	1.03[a] *s*
	3.39 *d* (11.1)	3.68	3.81 *d* (11.2)		3.62 *d* (11.1)		
13	0.93 *s*	1.12	1.07 *s*	1.00[b] *s*	1.09 *s*	4.15 *d* (11.7) 4.07 *d* (11.7)	0.97[a] *s*
14	4.80 *brs*	5.00	4.88 *brs*	4.80 *brs*	2.42 *d* (4.8)	4.76 *s*	9.55 *d* (2.5)
	4.72 *brs*	4.95	4.86 *brs*	4.87 *brs*	2.50 *dd* (4.8, 2.0)	4.75 *s*	
15	4.62 *d* (12.3)	4.55	5.20 *s*	1.25 *d* (7.0)	4.59 *d* (12.4)	1.59 *d* (1.2)	4.41 *d* (11.3)
	4.19 *d* (12.3)	4.35	5.17 *s*		4.34 *d* (12.4)		4.39 *d* (11.3)
OAc	1.64 *s*	2.03	1.98 *s*		2.04 *s*	2.05 *s*	2.07 *s*
	1.67 *s*	2.06	2.05 *s*		2.06 *s*	2.06 *s*	2.04 *s*
	1.86 *s*	2.07	2.06 *s*		2.19 *s*		

[a,b]Assignments may be interchanged.

Table 2. ^{1}H NMR and ^{13}C NMR spectral data of compound **11** ($CDCl_3$)

H	δ (ppm)	C	δ (ppm)
1	5.34 *br d* (10.4)	1	117.1 *d*
2	5.55 *dd* (10.4, 3.5)	2	72.5 *d*
3	5.75 *d* (3.5)	3	77.7 *d*
5	4.12 *d* (9.1)	4	145.3 *s*
6	0.78 *dd* (9.1, 8.7)	5	78.7 *d*
7	0.51 *m*	6	29.3 *d*
8	1.24 *m* (α)	7	29.2 *d*
	2.02 *m* (β)	8	27.4 *t*
9	2.47 *m* (α)	9	36.7 *t*
	1.71 *m* (β)	10	143.8 *s*
12	1.06 *s*	11	18.6 *s*
13	1.02 *s*	12	28.9 *q*
14	1.77 *s*	13	15.9 *q*
15	5.52 *s*	14	21.6 *q*
	5.61 *s*	15	119.3 *t*
2′	6.66 *d* (1.8)	1′	132.4 *s*
5′	6.80 *d* (8.0)	2′	111.0 *d*
6′	6.65 *dd* (8.0, 1.8)	3′	146.5 *s*
7′	2.83 *t* (7.8)	4′	144.1 *s*
8′	2.56 *t* (7.8)	5′	114.4 *d*
OCH_3	3.84 *s*	6′	120.9 *d*
OAc	2.06 *s*	7′	30.7 *t*
		8′	36.3 *t*
		9′	172.1 *s*
		OCH_3	55.9 *q*
		OAc	21.1 *q*

1; ^{13}C NMR ($CDCl_3$) δ: 49.6 (*d*, C-1), 91.6 (*d*, C-2), 140.4 (*d*, C-3), 116.0 (*s*, C-4), 30.7 (*d*, C-5[a]), 27.3 (*d*, C-6[a]), 24.6 (*d*, C-7[a]), 21.2 (*t*, C-8), 33.6 (*t*, C-9), 59.9 (*s*, C-10), 22.9 (*s*, C-11), 73.8 (*t*, C-12), 11.7 (*q*, C-13), 51.4 (*t*, C-14), 62.5 (*t*, C-15), 20.8 (*q*, OAc), 169.5 (*s*, OAc), 20.9 (*q*, OAc), 170.7 (*s*, OAc), 21.2 (*q*, OAc), 171.0 (*s*, OAc) ([a]assignments interchangeable).

Compound **11**. $[\alpha]_D = +24.8°$ (*c* 0.3, $CHCl_3$); EI-MS *m/z* (rel. int): 472 (34), 470 (29), 455 (24), 412 (8), 395 (7), 293 (12), 277 (17), 259 (88), 233 (21), 217 (47), 199 (65), 195 (62), 179 (62), 137 (100), 123 (32), 95 (30); ^{1}H NMR and ^{13}C NMR: see Table 2.

Isoplagiochilide (**12**). $[\alpha]_D = +82.5°$ (*c* 0.3, $CHCl_3$); GC-MS *m/z* (rel. int.): 232 (4), 217 (3), 203 (20), 189 (13), 164 (12), 161 (22), 149 (21), 135 (28), 121 (30), 105 (20), 91 (42), 79 (39), 77 (38), 69 (100); IR ν_{max} cm^{-1}: 3091, 2919, 2859, 2744, 2369, 2345, 1717, 1694, 1647, 1607, 1459, 1377, 1326, 1268, 1211, 1176, 1137, 1103, 1040, 982, 953, 918, 865, 829, 806, 765, 665, 541, 486; UV $\lambda_{max}^{CHCl_3}$ nm: 243, 280 sh; ^{1}H NMR (C_6D_6) δ: 5.96 (*brs*, H-3), 2.52 (*brd*, *J* = 10.2, H-5), 0.26 (*dd*, *J* = 10.2, 9.6, H-6), 0.16 (*ddd*, *J* = 9.6, 7.5, 1.9, H-7), 1.35 (*m*, H-8α), 1.40 (*m*, H-8β), 1.79 (*dt*, *J* = 13.2, 5.7, H-9α), 1.53 (*m*, H-9β), 0.77 (*s*, H-12), 0.77 (*s*, H-13), 2.35 (*s*, H-14), 1.26 (*s*, H-15); ^{13}C NMR ($CDCl_3$) δ: 115.7 (*s*, C-1[a]), 161.5 (*s*, C-2[b]), 133.1 (*d*, C-3), 122.4 (*s*, C-4[a]), 37.1 (*d*, C-5), 20.8 (*d*, C-6[c]), 31.3 (*d*, C-7[c]), 16.8 (*t*, C-8), 37.1 (*t*, C-9), 160.0 (*s*, C-10[b]), 19.5 (*s*, C-11), 28.8 (*q*, C-12), 15.6 (*q*, C-13[d]), 24.1 (*q*, C-14), 15.5 (*q*, C-15[d]) ([a,b,c,d]assignments interchangeable).

Plagiochiline S (**13**). CI-MS *m/z* 274 $[M-CH_3COOH]^+$, EI-MS (rel. int.) *m/z* 274 (9), 214 (63), 173 (43); ^{1}H NMR: see Table 1.

9,10-*Dihydroovalifolienal* (**15**). CI-MS *m/z* 291 $[M+H-CH_3COOH]^+$; ^{1}H NMR: see Table 1; ^{13}C NMR ($CDCl_3$) δ: 202.8 (CHO), 170.9 (OAc), 169.3 (OAc), 141.0 (C-3), 114.1 (C-4), 100.7, 92.1, 63.3, 49.3, 40.9, 31.3, 29.4, 31.3, 29.4, 28.7, 24.3, 21.2, 21.0, 20.8, 19.8, 15.6.

(+) - 3,4 - *Dihydro* - 3 - (*4* - *methoxyphenyl*)-*isocoumarin* - 8 - *ol* (**16**). Mp = 105°; $[\alpha]_D = +90.6°$ (*c* 0.2, $CHCl_3$); EI-MS *m/z* (rel. int.): 270 (100), 252 (77), 237 (6), 224 (27), 209 (15), 196 (6), 181 (26), 165 (13), 153 (20), 134 (21), 121 (6), 105 (9), 92 (4), 78 (14), 65 (7), 63 (9), 51 (11); IR ν_{max} cm^{-1}: 3040, 2920, 1650, 1615, 1583, 1520, 1455, 1360, 1330, 1300, 1250, 1230, 1190, 1110, 1060, 1020, 970, 910, 833, 809, 788, 720; UV $\lambda_{max}^{CHCl_3}$ nm: 246, 274, 281, 316; ^{1}H NMR ($CDCl_3$) δ: 5.53 (*dd*, *J* = 12.2, 3.1, H-3), 3.08 (*dd*, *J* = 16.4, 3.1, H-4a), 3.32 (*dd*, *J* = 16.4, 12.2, H-4b), 6.72 (*d*, *J* = 7.4, H-5), 7.42 (*t*, *J* = 7.4, H-6), 6.91 (*d*, *J* = 7.4, H-7), 7.37 (*d*, *J* = 8.6, 2H, H-2′/6′), 6.92 (*d*, *J* = 8.6, 2H, H-3′/5′), 10.99 (*s*, OH), 3.81 (*s*, OCH_3), ^{13}C NMR ($CDCl_3$) δ: 169.8 (*s*, C-1), 80.7 (*d*, C-3), 35.0 (*t*, C-4), 117.9 (*d*, C-5), 136.2 (*d*, C-6), 116.4 (*d*, C-7), 160.1 (*s*, C-8), 108.6 (*s*, C-9), 139.4 (*s*, C-10), 130.1 (*s*, C-1′), 127.7 (*d*, C-2′), 114.2 (*d*, C-3′), 162.4 (*s*, C-4′), 114.2 (*d*, C-5′), 127.7 (*d*, C-6′), 55.3 (*q*, OCH_3).

Acknowledgements—The authors thank Dr S. R. Gradstein (Utrecht), Dr E. Linares (Colombia), Dr J.-P. Frahm (Duisburg), Dr T. Pócs (Eger), Dr J. Spörle (Saarbrücken), Dr N. Salazar Allen (Panamá) and Dr H. Inoue (Tokyo) for the collection and identification of the plant material. The fieldwork in Africa was supported by Dr Bernd Steinhauer Burkart IZCN/GTZ, Bukavu, Directeur Régional de l'Institut Zairois pour la Conservation de la Nature (IZCN), Bakinahe NT Stanilas, Bukavu, Conservateur Principal IZCN Parc National Kahuzi-Biega, Mankoto Ma Oyisenzoo, Bukavu, Directeur General de l'Institut Zarois pour la Recherche en Sciences Naturelles, Lwiro, Directeur de l'Office Rwandaise du Tourisme et des Parcs Nationaux (O.R.T.P.N.) Kigali, Nyamacumu Anthanase, Chef de Section des Parcs Nationaux, Sites et Monuments Touristiques, O.R.T.P.N., Nyangezi Etienne, Chef de Service des Parcs Nationaux, Tourisme et Agence des Voyages, O.R.T.P.N., and Dr Joseph Mukyumwami, l'Institut de Recherche Scientifique et Technologique (I.R.S.T.), Butare. The fieldwork in Panamá was supported by Dr S. Heckadon Moreno, Director of the Environmental protection Agency of Panama (Inrenare). The field work in Colombia was supported by Dr E. Linares, Jardín Botánico de Bogotá, Bogotá. The authors also thank Dr J. Spörle (Saarbrücken) for establishing the axenic culture of *P. adianthoides*, and Dr G. Wächter and Dr B. Timmermann for fruitful discussions. S. V. gratefully acknowledges the financial support from the 'Graduiertenstipendium des Saarlandes'.

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