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Sesquiterpenoid constituents of the liverwort *Marsupella aquatica*

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Dedicated to Professor Dr. Dr. h-c. W. Francke on the occasion of his 65th birthday

Abstract—Nine new amorphane sesquiterpenoids, (+)-7β-hydroxyamorpha-4,11-diene, (−)-9α-hydroxyamorpha-4,7(11)-diene, (−)-3α-hydroxyamorpha-4,7(11)-diene, (−)-3α-acetoxyamorpha-4,7(11)-diene, (−)-amorpha-4,7(11)-dien-3-one, (+)-2,8-epoxyamorpha-4,7(11)-diene, (+)-5,9-epoxyamorpha-3,7(11)-diene, (−)-2α-hydroxyamorpha-4,7(11)-diene and (−)-2β-acetoxyamorpha-4,7(11)-diene, were isolated from the essential oil of the liverwort *Marsupella aquatica*, collected near Gaschurn/Montafon, Austria. The isolated compounds and their chemical transformations were investigated using enantioselective GC and extensive spectroscopic studies (HRMS, ^{1}H, ^{13}C and 2D NMR). The absolute configuration of most of the isolated compounds were established by conversions to known compounds. In addition, ^{1}H, and ^{13}C NMR data of (−)-myltayl-4-ene are reported for the first time.

1. Introduction

The liverwort *Marsupella emarginata* var. *aquatica* is closely related to *M. emarginata*, as indicated by the presence of longipinane type sesquiterpenoids. However, the two species clearly differ in their morphological properties[1] and chemical composition.[2] Oxygenated longipinane and gymnomitrane derivatives have been isolated from the ether extract of the French *M. aquatica*,[3,4] while four oxygenated amorphane derivatives have been isolated from the Scottish liverwort *M. emarginata* var. *aquatica*.[5] In our previous report, we have isolated (+)-amorpha-4,11-diene **1**, (−)-amorpha-4,7(11)-diene **2**, (−)-2α-acetoxyamorpha-4,7(11)-diene **3**, and (−)-myltayl-4(12)-ene **4** from the essential oil of the Austrian *M. aquatica*.[6] In continuation of our work on the liverworts, we herein report our results on the investigation of the constituents of the essential oil of the Austrian *M. aquatica.*

2. Results and discussion

2.1. Composition of the essential oil of *M. aquatica*

The essential oil of the liverwort *M. aquatica* was prepared by hydrodistillation and analysed by GC and GC–MS. The following known sesquiterpene hydrocarbons could be identified by comparison of their mass spectra and retention indices with a spectra library established under identical experimental conditions:[7,8] β-elemene (0.4%), α-barbatene (0.4%), isobazzanene (0.5%), β-barbatene (1.5%), (−)-myltayl-4(12)-ene **4** (3.2%), β-acoradiene (1.3%), (+)-amorpha-4,11-diene **1** (9.6%), (−)-amorpha-4,7(11)-diene **2** (25.2%) and traces of α-longipinene, cyclomyltaylane **5**, anastreptene, α-copaene, β-copaene, calarene, α-chamigrene, cadina-1,4-diene and δ-cuprenene.

2.2. Structure elucidation of (−)-myltayl-4-ene 6

(−)-Myltayl-4-ene **6** (0.5%), an irregular sesquiterpene hydrocarbon, elutes before β-elemene from a non-polar dimethylpolysiloxane column. Myltayl-4(12)-ene **4**, the double bond isomer of **6**, has previously been isolated from the same *M. aquatica* oil.[6] The mass spectrum of **6** exhibits a molecular ion signal at *m*/*z* 204 corresponding to the

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✠ Passed away on November 19, 2004. His scientific achievements keep him among us.

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molecular formula of $C_{15}H_{24}$ with four units of unsaturation, as determined by HR-EIMS ([M^+] *m*/*z* 204.1891). The ^{1}H NMR (C_6D_6) showed three methyl singlets at δ 0.88 (3H, s), 0.94 (3H, s), 0.99 (3H, s) and one downfield methyl at δ 1.62 (3H, s) indicating the attachment to an olefinic carbon. The 2D ^{1}H–^{1}H COSY, HMQC and HMBC spectra confirmed the structure of **6**. The relative configuration of **6** was deduced from the NOESY spectrum which showed spatial interactions for the methyl group protons (H-15) with methine H-5, and H-10b/H-9a. Spatial interactions of H-13 protons with H-3, H-1b, H-2b, H-8a and H-14 protons were observed. In addition, proton H-5 also interacts with H-15 and H-9a/10b (Fig. 1). A biogenetic pathway from the chamigrenyl cation via a Wagner–Meerwein rearrangement following Markownikoff's rule is proposed (Scheme 1)[9] as an alternative to the *anti*-Markownikoff route.[10] The co-occurrence of **4**, **6** and cyclomyltaylane **5**[11] support the myltaylanyl cation as an intermediate in both routes. We have also identified compounds **4**–**6** in the essential oils of the liverworts *Mylia taylorii* and *Kurzia trichoclados*.[12]

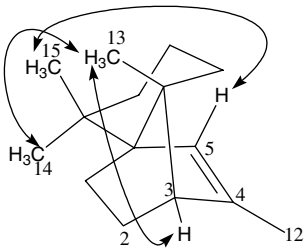
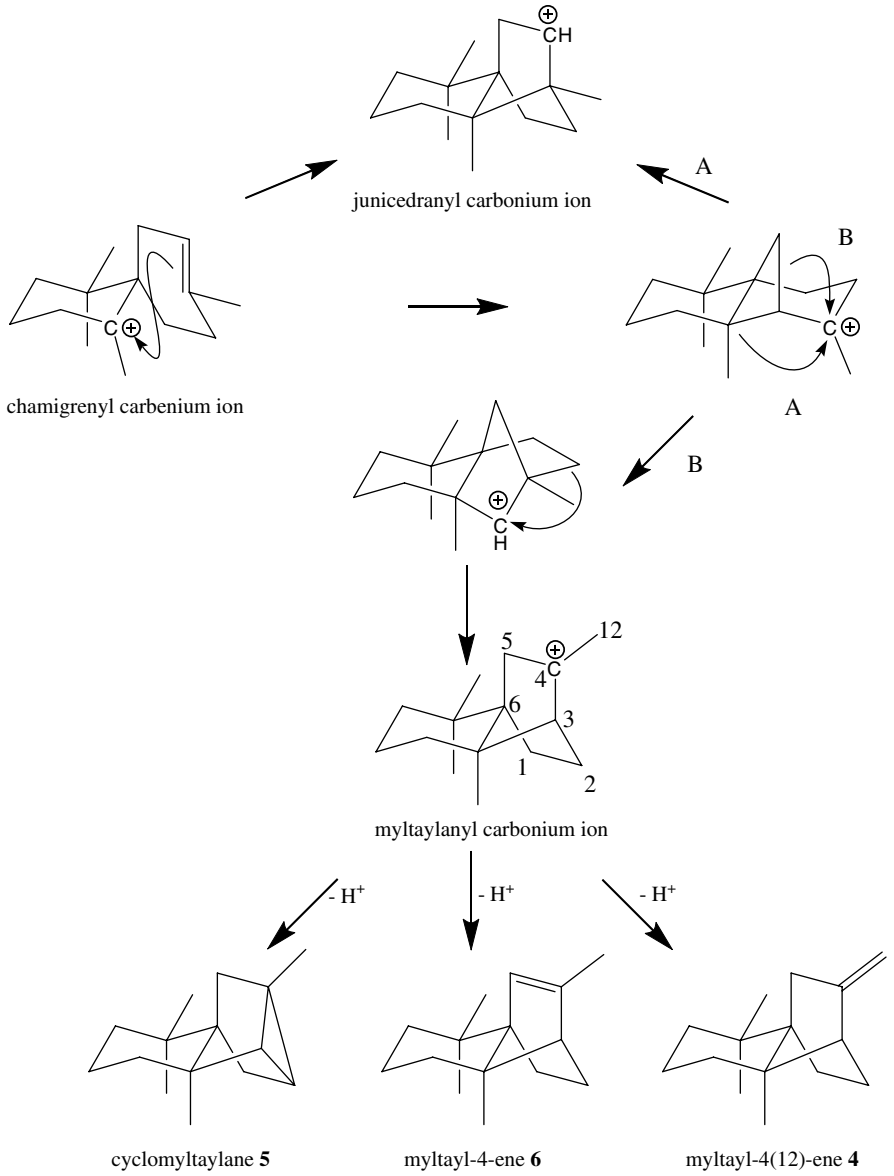


Figure 1. NOE correlations of **6**.

2.3. Structure elucidation of (+)-(1*R*,6*S*,10*S*)-7β-hydroxyamorpha-4,11-diene 7

Compound **7** (0.7%), showed a molecular ion signal at *m*/*z* 220, while the molecular formula was determined as



Scheme 1. Proposed biogenetic pathway for **4**–**6**.

Table 1. ^{13}C NMR (125.7 MHz, C_6D_6) δ (ppm) data of amorphane derivatives

	Compd no.							
	7	**9**	**10**	**12**	**13**	**14**	**16**	**17**
1	36.4	39.5	41.1	40.6	46.0	37.3	48.6	45.3
2	25.5	26.0	34.8	31.8	43.0	71.7	73.3	70.7
3	26.5	25.5	67.4	69.2	197.8	39.0	36.0	31.8
4	134.4	134.4	135.6	134.9	136.0	130.1	132.7	130.5
5	121.8	125.7	129.3	132.2	147.9	122.7	125.9	126.0
6	43.4	40.4	41.2	40.7	41.7	32.9	41.4	34.9
7	75.6	132.7	135.4	134.9	133.8	134.5	134.5	134.1
8	32.1	37.0	27.5	27.6	27.6	66.8	26.6	27.1
9	30.5	76.0	37.1	36.8	36.1	36.8	37.1	36.4
10	27.8	37.0	30.4	30.0	29.8	29.6	28.1	30.2
11	150.9	123.2	121.5	121.8	122.3	120.8	121.0	122.2
12	18.3	20.3[a]	20.6[a]	20.6[a]	20.2[a]	20.1	19.9	20.0
13	111.5	20.7[a]	20.7[a]	20.8[a]	20.4[a]	19.1	20.3	20.6
14	19.8	15.5	20.2	20.3	19.8	20.9	22.3	20.0
15	23.9	23.9	21.1	21.9	16.2	23.9	23.0	23.5
16				170.1				170.0
17				21.0				21.3

All assignments were confirmed by HMBC and HMQC.
[a] Interchangeable.

$C_{15}H_{24}O$ by HR-EIMS ([M^+] m/z 220.1832). The ^1H NMR spectrum (C_6D_6) of compound **7** exhibited signals of three methyl groups at δ 0.92 (3H, d, H-14, $J = 6.3$ Hz), 1.57 (3H, br s, H-15) and 1.81 (3H, s, H-12). The signals at δ 4.85 (1H, s, H-13a), 4.90 (1H, s, H-13b) and 1.81 (3H, s, H-12) suggested the existence of an isopropenyl group. The olefinic carbon signals at δ 150.9 (s) and 111.5 (t) confirmed the exomethylene double bond. The signal at δ 75.6 was assigned to the tertiary carbon attached to the hydroxyl group. The 2D ^1H–^1H-COSY, HMQC and HMBC spectra, in addition to the ^{13}C NMR (Table 1) confirmed the structure. Its relative configuration resulted from the NOESY spectrum (Fig. 2). The absolute configuration was determined by a comparison of the fully hydrogenated products of (−)-amorpha-4,7(11)-diene **2** with those of (+)-**7** using enantioselective GC on a modified cyclodextrin stationary phase. The four fully hydrogenated derivatives of **7** are identical in GC–MS characteristics and retention times on achiral polysiloxane and chiral cyclodextrin derived GC phases with those of compound **2**. Therefore, the absolute configuration of **7** at C-1, C-6 and C-10 was concluded to be (1*R*,6*S*,10*S*). The (7*S*)-configuration was inferred from the four hydrogenation products which suggested that proton H-6 must have a *cis*-configuration

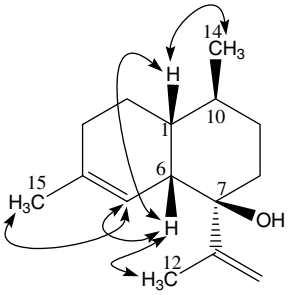


Figure 2. NOE correlations of **7**.

to the hydroxy group at C-7. In addition, the assigned (7*S*)-configuration is consistent with (+)-amorpha-4,11-diene **1** and (+)-1-*epi*-cubenol **8**[13] isolated and characterised in the same *M. aquatica* oil.

2.4. Structure elucidation of (+)-(1*R*,6*S*,7*S*,10*S*)-1-*epi*-cubenol 8

(+)-1-*epi*-Cubenol (**8**, 1.5%) was isolated and characterised. The spectral data of **8** were consistent with those reported in the literature.[13] Its absolute configuration was determined by the treatment of **8** with an acidic ion exchange resin (Amberlyst) for 2 h at room temperature to afford (+)-cadina-1,4-diene (85%), as well as the oxidation product (+)-*trans*-calamenene (5%), as determined by GC–MS and enantioselective GC with authentic standards.

2.5. Structure elucidation of (−)-(1*S*,6*S*,9*R*,10*R*)-9α-hydroxyamorpha-4,7(11)-diene 9

Compound **9** has the same bicyclic sesquiterpene skeleton as **7** and exhibits a molecular ion signal at m/z 220. Its molecular formula was determined to be $C_{15}H_{24}O$ by HR-EIMS ([M^+] m/z 220.1816). The ^1H NMR spectrum (C_6D_6) of **9** showed four methyl groups at δ 1.03 (3H, d, H-14, $J = 6.6$ Hz), 1.58 (3H, s, H-15) and 1.66 (6H, br s, H-12 and H-13). The signal at δ 2.98 (1H, dt, H-9, $J = 4.1$, 11.4 Hz) was assigned to the methine proton H-9. Additional useful structural information was obtained from the ^{13}C NMR (Table 1). The relative configuration of **9** was deduced from the NOESY spectrum (Fig. 3), and a (1*S*,6*S*,9*R*,10*R*)-configuration was assigned by using the same method as for **7**, that is, hydrogenation to yield four diastereomers that matched the hydrogenation products of (−)-**2** in GC–MS characteristics and coelute on achiral and chiral cyclodextrin derived GC-phases. The α-orientation of the secondary hydroxyl group was deduced from the spatial interactions of H-9 and H-14. Additionally, H-9

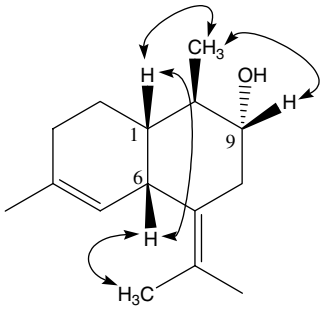


Figure 3. NOE correlations of **9**.

has two large couplings, which suggest that it must be axial with two antiperiplanar vicinal protons, hence it must be β in structure **9**.

2.6. Structure elucidation of (−)-(1*R*,6*S*,9*R*,10*R*)-3α-hydroxyamorpha-4,7(11)-diene 10

Compound **10** (0.5%), $C_{15}H_{24}O$ (*m*/*z* 220.1826 [M^+]) exhibits a similar ^1H NMR as **9** except that the α-oriented secondary hydroxyl group is now at C-3 at δ 67.4 (d). The 2D ^1H–^1H-COSY, HMQC and HMBC data of **10** were consistent with the assigned structure. For ^{13}C NMR (C_6D_6) data see Table 1. The relative configuration of **10** was determined from the NOESY spectrum (Fig. 4). Direct hydrogenation of **10** afforded identical products as in **7** and **9**. In addition, alcohol **10** dehydrates at the injector port (200 °C) of the preparative GC during purification to afford (+)-amorpha-2,4,7(11)-triene **11**. Compound **11** showed a molecular ion signal at *m*/*z* 202 corresponding to the molecular formula of $C_{15}H_{22}$. The ^1H NMR spectrum (C_6D_6) of **11** indicated the loss of the secondary hydroxyl at C-3 when compared to **10**, which was replaced by the formation of an endocyclic double bond at δ 5.85 (1H, d, $J = 9.5$ Hz) and 6.08 (1H, dd, $J = 6.0$, 9.4 Hz). The relative configuration of **11** was deduced from its NOESY spectrum, which is similar to that of **10** (1*R*,6*S*,9*R*,10*R*) (Fig. 4). The fully hydrogenated products of **11** were identical to those obtained from **2**, **7**, **9** and **10**. Hence, **11** was assigned a (1*S*,6*S*,10*S*)-configuration.

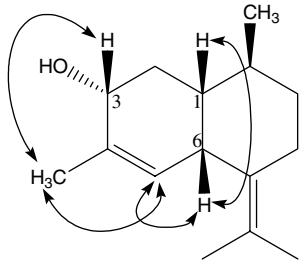


Figure 4. NOE correlations of **10**.

2.7. Structure elucidation of (−)-(1*R*,3*R*,6*S*,10*S*)-3α-acetoxyamorpha-4,7(11)-diene 12

Compound **12** (0.7%), the acetate of **10**, was isolated from the same essential oil. It showed a molecular ion signal at 262 corresponding to the molecular formula of $C_{17}H_{26}O_2$. The ^1H NMR spectrum of **12** showed three signals corresponding to five methyl groups at δ 0.86 (3H, d, H-14, $J = 6.3$ Hz), 1.65 (9H, br s, H-12, H-13 and H-15) and 1.74 (3H, s, H-17). The signal at δ 5.33 (1H, br d, H-3, $J = 5.0$ Hz) was assigned to the methine proton connected to the acetoxy group. Additional structural information was obtained through ^{13}C NMR (Table 1). The relative configuration of **12** resulted from its NOESY spectrum, which is similar to that of **10** (Fig. 4). The hydrolysis of **12** using K_2CO_3/MeOH afforded **10**. Therefore, **12** should have the same (1*R*,3*R*,6*S*,10*S*) configuration as **10**.

2.8. Structure elucidation of (−)-(1*R*,6*S*,10*S*)-amorpha-4,7(11)-dien-3-one 13

Compound **13** (1.5%), an α,β-unsaturated ketone with amorphane skeleton, showed a molecular ion signal at *m*/*z* 218 corresponding to the molecular formula of $C_{15}H_{22}O$, as determined by HR-EIMS ([M^+] *m*/*z* 218.1668). The ^1H NMR spectrum (C_6D_6) indicated signals for four methyl signals at δ 0.72 (3H, d, H-14, $J = 6.3$ Hz), 1.59 (6H, br s, H-12 and H-13) and 1.82 (3H, s, H-15). The proton signal at δ 5.91 was assigned to the methine proton H-5 with a downfield carbon signal at δ 147.9 (d). The 2D ^1H–^1H COSY, HMQC and HMBC in addition to the ^{13}C NMR (Table 1) spectra confirmed the structure of **13**. Its relative configuration was determined from the NOESY spectrum, again similar to that of **10** (Fig. 4). Treatment of **10** with pyridinium dichromate (PDC) in dry dichloromethane gave a product identical (same GC–MS characteristics and same retention times on achiral polysiloxane and cyclodextrin derived GC phases) to (−)-**13**. Consequently, identical to **10**, **13** shows a (1*R*,6*S*,10*S*)-configuration.

2.9. Structure elucidation of (+)-(1*R*,2*S*,6*R*,8*S*,10*S*)-2,8-epoxyamorpha-4,7(11)-diene 14

Compound **14** (0.7%), showed a molecular ion signal at *m*/*z* at 218 corresponding to $C_{15}H_{22}O$ as determined by HR-EIMS ([M^+] *m*/*z* 218.1680). The ^1H NMR spectrum (C_6D_6) showed signals of four methyl groups at δ 0.87 (3H, d, H-14, $J = 7.3$ Hz), 1.39 (3H, s, H-13), 1.58 (3H, s, H-12) and 1.61 (3H, br s, H-15). The signals at δ 4.04 (1H, s) and 4.49 (1H, s) are assigned to the two methine protons at the oxygenated C-2 and C-8, respectively. The 2D ^1H–^1H-COSY, HMQC and HMBC spectra in addition to the ^{13}C NMR extracted from the HMBC and HSQC data (Table 1) confirmed the structure. Its relative configuration resulted from the NOESY spectrum (Fig. 5). Direct rigorous hydrogenation of **14** yielded products identical to those obtained from (−)-**2** as well as from **7**, **9**, **10** and **11**. Thus, **14** was assigned to show a (1*R*,2*S*,6*R*,8*S*,10*S*)-configuration. The α-orientation of the ring was deduced by the spatial interactions of H-2 and H-1. Treatment of **14** with an acidic ion exchange resin (Amberlyst) for 2 h at room temperature gave cadalene and two sesquiterpenes, the major one being identical to α-calacorene and the minor one could not be identified.

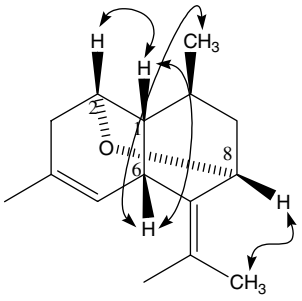


Figure 5. NOE correlations of **14**.

2.10. Structure elucidation of (+)-(1*S*,5*S*,6*R*,9*R*,10*R*)-5,9-epoxyamorpha-3,7(11)-diene 15

Compound **15** was obtained as a trace component (0.05%) and showed a molecular ion signal at *m*/*z* at 218 corresponding to $C_{15}H_{22}O$ as determined by HR-EIMS (*m*/*z* 218.1679 [M^+]). It also exhibited a similar ^1H NMR spectrum (C_6D_6) as **14**, except that the position of the two methine protons at the oxygenated carbons shifted to δ 3.70 (1H, s, H-9) and 3.93 (1H, s, H-5). In addition, the endocyclic double bond was situated at C_3 compared to C_4 in **14**. Additional structural information was obtained from HMBC and HMQC which confirmed the presence of two carbon–oxygen-linkages with signals at δ 72.9 (d, C-9) and 73.9 (d, C-5). In addition, the methine carbon at δ 120.0 (d, C-3) and three quaternary carbons at δ 121.9 (s, C-11), 129.8 (s, C-7) and 137.8 (s, C-4) were confirmed from the HMBC spectrum. The relative configuration of **15** was deduced from the NOESY spectrum, which indicated the interactions of protons H-1 with H-14 and H-6. In addition, proton H-5 also interacts with H-6 (Fig. 6). The hydrogenation of **15** yielded the same products as **14**, establishing the compound to show (1*S*,5*S*,6*R*,9*R*,10*R*)-configuration. Subjected to acidic ion exchange resin (Amberlyst) treatment, 10% of compound **15** formed similar compounds as **14**. Regarding the yield of degradation, product **15** was relatively stable when compared to **14**.

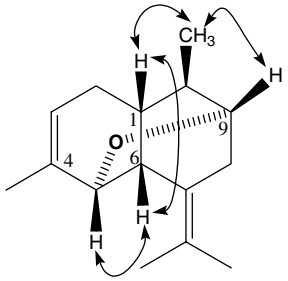


Figure 6. NOE correlations of **15**.

2.11. Structure elucidation of (−)-(1*R*,2*S*,6*R*,10*S*)-2α-hydroxyamorpha-4,7(11)-diene 16

Compound **16**, $C_{15}H_{24}O$ (*m*/*z* 220.1809 [M^+]), which is the deacetylated form of (−)-2α-acetoxyamorpha-4,7(11)-diene **3**[6] also occurs in the essential oil. The 2D ^1H–^1H-COSY, HMQC and HMBC spectra in addition to the ^{13}C NMR (Table 1) confirmed the structure. The relative configuration of **16** was determined by NOE spectroscopy (Fig. 7). Its absolute configuration was deduced from the direct hydrogenation of **16** and comparison with the hydrogenation products of **3** using enantioselective GC. Thus, a (1*R*,2*S*,6*R*,10*S*)-configuration was assigned to **16**.

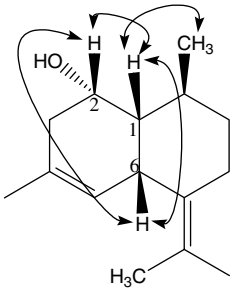


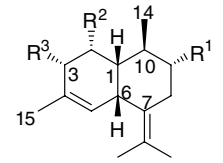
Figure 7. NOE correlations of **16**.

2.12. Structure elucidation of (−)-(1*R*,2*R*,6*R*,10*S*)-2β-acetoxyamorpha-4,7(11)-diene 17

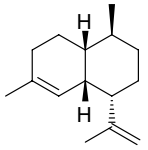
(−)-2β-Acetoxyamorpha-4,7(11)-diene **17** (1.2%) has the molecular formula $C_{17}H_{26}O_2$. Although this could not be confirmed by mass spectrometry (the largest ion detected under EI conditions corresponds to $C_{15}H_{22}$ due to immediate loss of acetic acid). The ^{13}C NMR spectrum (Table 1) revealed the presence of 17 carbon resonances. ^1H NMR and HMBC demonstrated that compound **17** had a total of 26 protons 23 protons of which are directly attached to the carbon skeleton. The ^1H NMR and ^{13}C NMR were identical to those of (−)-2α-acetoxyamorpha-4,7(11)-diene **3** except for slight chemical shift changes. The 2D ^1H–^1H-COSY, HMQC and HMBC spectra confirmed the structure of **17**. The spatial interactions of protons were derived from the NOESY spectrum, which is similar to that of **3** (Fig. 7), except for the lack of spatial interactions between protons H-2 and H-6. The absolute configuration of **17** was confirmed by deacetylation, followed by pyridiniumdichromate (PDC) oxidation. The oxidised product (+)-amorpha-4,7(11)-dien-2-one **18**, gave identical ^1H NMR data, the same GC–MS characteristics and the same retention times on achiral polysiloxane and chiral cyclodextrin derived GC phases as the deacetylated and PDC oxidised product of (−)-**3**. Compound **18** could also be identified as a trace constituent in the essential oil. As a result, the stereochemistry at the stereogenic centres C-1, C-2, C-6 and C-10 was confirmed as (1*R*,2*R*,6*R*,10*S*) for **17**.

3. Conclusion

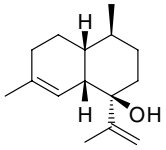
In conclusion, the co-existence of compounds **1**, **2**, **3**, **7**, **9**, **10**, **12**, **13**, **14**, **15**, **16** and **17** with (−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxy-11-methoxyamorpha-4,7-diene **19** and (−)-(1*R*,2*S*,6*R*,10*S*)-2α-acetoxyamorpha-4,7(11)-dien-8-one **20**[5] which were identified on the basis of their mass spectral fragmentation patterns and retention indices, suggested that the essential oil of the Austrian *M. aquatica* consists



2 $R^1 = R^2 = R^3 = H$
3 $R^1 = R^3 = H$; $R^2 = OAc$
9 $R^1 = OH$; $R^2 = R^3 = H$
10 $R^1 = R^2 = H$; $R^3 = OH$
12 $R^1 = R^2 = H$; $R^3 = OAc$
13 $R^1 = R^2 = H$; $R^3 = =O$
16 $R^1 = R^3 = H$; $R^2 = OH$
17 $R^1 = R^3 = H$; $R^2 = \beta$-OAc
18 $R^1 = R^3 = H$; $R^2 = = O$



1



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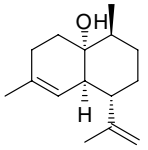
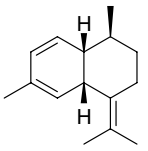


8



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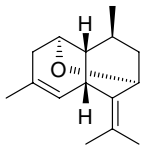
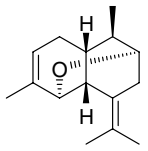


14



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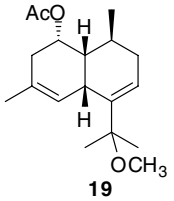

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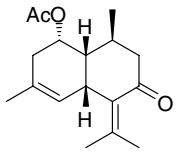

20

of mainly amorphanes, traces of cadinane/muurolane, for example, (+)-*epi*-cubenol, few gymnomitrane based compounds (α- and β-barbatene) with traces of longipinane (α-longipinene).

4. Experimental

4.1. General experimental procedures

4.1.1. Gas chromatography. Carlo Erba HRGC 5300 Mega series instrument fitted with 25 m fused silica capillaries coated with polysiloxane CPSil-5 and polysiloxane CPSil-19 (Chrompack); Carlo Erba Fractovap 2150 or 4160 gas chromatographs with 25 m fused silica capillaries coated with octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin,[14] heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin[14] or heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin[15] in OV 1701 (50%, w/w), split injection; split ratio approx. 1:30; FID; carrier gas 0.5 bar H_2; injector and detector temperatures were 200 and 250 °C, respectively.

4.1.2. Preparative GC. Modified Varian 1400 and 2800 instruments, equipped with stainless steel columns (1.85 m × 4.3 mm) with 10% polydimethylsiloxane SE-30 on Chromosorb W-HP or with 2.5% octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin in OV-1701 (50%, w/w) on Chromosorb G-HP or with 6% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in SE-52 (50%, w/w) on Chromosorb W-HP; FID; helium as carrier gas at a flow rate of 120 ml/min; injector and detector temperatures were 200 and 250 °C, respectively.[16]

4.1.3. GC–MS. Electron impact (70 eV) and chemical ionisation (NH_3) GC–MS was carried out on a Hewlett Packard HP 5890 gas chromatograph coupled with a VG Analytical 70-250S magnetic field mass spectrometer. All compounds were identified by comparison of their mass spectra and gas chromatographic retention indices with a spectral library established under identical experimental conditions.[7,8]

4.1.4. NMR spectroscopy. NMR measurements were carried out with a Bruker WM 500 (500 MHz) instrument in C_6D_6 using TMS as internal standard. NMR-experiment acquisition for ^1H–^1H COSY was with 4096 (F2) and 256 (F1) data points. Delay was 1.1 s. F2 and F1 were each processed as Qsine with a sinus shift of 0. HSQC was recorded as a phase sensitive experiment with TD = 4096 (F2) and TD = 128 (F1) data points and with a delay of 1/4 * 145 Hz. Processing was done as an exponential multiplication in F2 with line broadening of 4.00 Hz and with QSINE in F1 with a sinus shift of 2. HMBC was recorded as a *J* filtered experiment with TD = 4096 (F2) and TD = 256 (F1). Delay was 0.065 s. F2 and F1 were each processed as Qsine with a sinus shift of 4. NOESY was recorded as phase sensitive with TD = 4096 (F2) and TD = 400 (F1) data points, respectively. Mixing time was 600 ms. F2 and F1 were each processed as Qsine with a sinus shift of 2.

4.1.5. Polarimetry. Measurements were performed with a polarimeter 341 (Perkin–Elmer) at 589 nm at 20 °C. Due to the very small amounts of isolated compounds, only the direction of the optical rotation is given in order to avoid inaccuracies.

4.1.6. Thin-layer chromatography. Thin-layer chromatography was carried out using glass and aluminium plates coated with silica 60 F_{254} (Merck). An ethanolic solution of sulfuric acid (10%) and anisaldehyde was used as the spray reagent. The solvent system used was *n*-hexane/ethyl

acetate (3:1, v/v). The R_f values were also determined in this solvent system.

4.1.7. Reactions. *Hydrogenation* reactions were performed by bubbling hydrogen gas through a stirred solution of ca. 1 mg of sample in 1 ml *n*-hexane and 0.5 mg Pd/C at room temp. for 1 h. The reaction mixture was filtered, and the reaction products analysed by GC–MS and by GC on several capillary columns with modified cyclodextrin derivatives.

Dehydration was carried out with ca. 1 mg of sample in 0.5 ml of pyridine and 1 drop of phosphoryl chloride under ice cooling. After 1 h of stirring at room temperature the reaction was quenched by adding a few drops of water, and the mixture was extracted three times with *n*-hexane. The organic phase was washed several times with water and dried over Na_2SO_4.

Oxidation reactions were carried out using ca. 1 mg of sample in dry CH_2Cl_2 and ca. 2 mg of PDC. After 3 h of stirring at room temperature Et_2O (5 ml) was added, and the resulting mixture was filtered through a short column packed with florisil.

Deacetylation reactions were carried out by treating ca. 1 mg of samples in methanol (1 ml) with ca. 2 mg of K_2CO_3. The reaction mixture was stirred at room temperature for 12 h. After work-up, the product was analysed by GC and GC–MS.

Acidic transformations were carried out by treatment of ca. 0.3 mg of samples in hexane with a few granules of Amberlyst® 15 at room temperature for about 2 h. The reaction mixture was monitored by GC.

4.2. Origin of *M. aquatica*

M. aquatica was collected near Gaschurn/Montafon, Austria, in July 2001. A plant voucher is deposited at the Botanik und Ökologie department Universität Ulm, D-89091 Ulm, Germany.

4.3. Isolation of compounds

The hydrodistillate of *M. aquatica* was submitted to flash column chromatography (column packed dry with silica gel; elution with *n*-hexane to yield the hydrocarbon fraction and gradient elution with ethyl acetate in hexane to yield the oxygenated fractions). Oxygenated fractions were further subjected to prep-TLC and final isolations were carried out using GC Varian 1400 and 2800 on a packed column with SE-30- and/or SE-52-columns combined with at least one cyclodextrin phase column.

4.4. (−)-Myltayl-4-ene 6

Colourless oil, $RI_{CPSIL\,5} = 1380$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.88 (3H, br s, H-13), 0.94 (3H, s, H-14), 0.99 (3H, s, H-15), 0.98–1.04 (2H, m, H-1a, H-2a), 1.21–1.26 (1H, m, H-10a), 1.39–1.48 (2H, m, H-9a, H-10b), 1.52–1.61 (2H, m, H-8a, H-9b), 1.62 (3H, s, H-12), 1.71–1.76 (1H, m, H-1b), 1.77–1.82 (1H, m, H-2b), 1.88–1.94 (2H, m, H-3, H-8b), 5.47 (1H, s, H-5); ^{13}C NMR (125.7 MHz, C_6D_6): δ 16.1 (q, C-12), 20.0 (t, C-9), 21.5 (q, C-13), 23.0 (q, C-14), 25.2 (t, C-1), 25.4 (t, C-2), 29.4 (q, C-15), 30.3 (t, C-8), 33.2 (s, C-11), 39.3 (t, C-10), 56.1 (s, C-7), 58.5 (d, C-3), 60.8 (s, C-6), 131.0 (d, C-5), 142.4 (s, C-4); MS (EI, 70 eV), *m*/*z* (rel. int.): 204 [M^+] (16), 176 (44), 161 (100), 147 (8), 133 (12), 119 (21), 105 (18), 91 (17), 77 (8), 55 (8), 41 (15).

4.5. (+)-(1*R*,6*S*,7*S*,10*S*)-7β-Hydroxyamorpha-4,11-diene 7

Colourless oil, $RI_{CPSIL\,5} = 1614$; $R_f = 0.75$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): 0.92 (3H, d, H-14, $J = 6.3$ Hz), 1.34–1.46 (4H, m, H-2a, H-8a, H-9a, H-10), 1.47–1.52 (1H, m, H-9b), 1.54–1.62 (1H, m, H-8b), 1.57 (3H, br s, H-15), 1.62–1.68 (1H, m, H-3a), 1.75–1.82 (1H, m, H-3b), 1.81 (3H, s, H-12), 1.84–1.93 (2H, m, H-1, H-2b), 2.49 (1H, s, H-6), 4.85 (1H, s, H-13a), 4.90 (1H, s, H-13b), 5.15 (1H, s, H-5); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1. MS (EI, 70 eV), *m*/*z* (rel. int.): 220 [M^+] (15), 202 (85), 187 (100), 173 (17), 159 (80), 146 (33), 145 (94), 134 (60), 132 (75), 133 (62), 121 (39), 119 (65), 105 (52), 91 (53), 81 (38), 79 (47), 77 (46), 69 (30), 55 (40), 41 (75); EIHRMS calcd for $C_{15}H_{24}O_1$ [M^+] *m*/*z* 220.1827, found [M^+] *m*/*z* 220.1832.

4.6. (−)-(1*S*,6*S*,9*R*,10*R*)-9α-Hydroxyamorpha-4,7(11)-diene 9

White, viscous oil; $RI_{CPSIL\,5} = 1680$; $R_f = 0.56$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 1.03 (3H, d, H-14, $J = 6.6$ Hz), 1.24–1.29 (1H, m, H-1), 1.44–1.55 (3H, m, H-2, H-10), 1.58 (3H, s, H-15), 1.61–1.68 (1H, m, H-3a), 1.66 (6H, br s, H-12, H-13), 1.78–1.86 (2H, m, H-3b, H-8a), 2.71 (1H, dd, H-8b, $J = 4.1$, 12.6 Hz), 2.98 (1H, dt, H-9, $J = 4.1$, 11.4 Hz), 3.36 (1H, br s, H-6), 5.09 (1H, s, H-5); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1. MS (EI, 70 eV), *m*/*z* (rel. int.) 220 [M^+] (63), 202 (60), 187 (100), 173 (30), 159 (52), 147 (60), 145 (65), 131 (42), 119 (48), 105 (59), 91 (61), 77 (43), 55 (42), 41 (80); EIHRMS calcd for $C_{15}H_{24}O_1$ [M^+] *m*/*z* 220.1827, found [M^+] *m*/*z* 220.1816.

4.7. (−)-(1*R*,3*R*,6*S*,10*S*)-3α-Hydroxyamorpha-4,7(11)-diene 10

Colourless oil; $RI_{CPSIL\,5} = 1666$; $R_f = 0.68$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.88–0.97 (1H, m, H-9a), 1.01 (3H, d, H-14, $J = 6.6$ Hz), 1.24–1.28 (1H, m, H-1), 1.48–1.53 (1H, m, H-2a), 1.60–1.65 (1H, m, H-9b), 1.67 (3H, d, H-12, $J = 0.9$ Hz), 1.68 (3H, d, H-13, $J = 2.2$ Hz), 1.73 (3H, br s, H-15), 1.80–1.88 (1H, m, H-8a), 1.99–2.05 (1H, m, H-10), 2.09–2.15 (1H, m, H-2b), 2.52–2.57 (1H, m, H-8b), 3.30 (1H, br s, H-6), 3.68 (1H, s, H-3), 5.16 (1H, s, H-5); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1. MS (EI, 70 eV), *m*/*z* (rel. int.): 220 [M^+] (8), 218 (12), 202 (43), 187 (27), 177 (48), 159 (77), 145 (60), 131 (39), 119 (47), 105 (61), 91 (75), 77 (49), 67 (31), 53 (41), 41 (100). HRMS calcd for $C_{15}H_{24}O_1$ [M^+] *m*/*z* 220.1827, found [M^+] 220.1826.

4.8. (+)-(1*S*,6*S*,10*S*)-Amorpha-2,4,7(11)-triene 11

Colourless oil, $RI_{CPSIL\,5} = 1449$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.76–0.85 (1H, m), 0.87 (3H, d, $J = 6.3$ Hz), 1.49–1.57 (2H, m), 1.62 (3H, d, $J = 1.9$ Hz), 1.66 (3H, s), 1.69 (3H, br s), 1.70–1.76 (1H, m), 1.85–1.92 (1H, m), 2.59—2.64 (1H, m), 3.77 (1H, s), 5.20 (1H, s), 5.85 (1H, d, $J = 9.5$ Hz), 6.08 (1H, dd, $J = 6.0$, 9.4 Hz); MS (EI, 70 eV), *m*/*z* (rel. int.): 202 [M^+] (82), 187 (25), 173 (8), 160 (32), 159 (100), 145 (82), 131 (42), 119 (39), 105 (51), 91 (38), 77 (22), 67 (20), 53 (18), 41 (39).

4.9. (−)-(1*R*,3*R*,6*S*,10*S*)-3α-Acetoxyamorpha-4,7(11)-diene 12

Colourless oil; $RI_{CPSIL\,5} = 1780$; $R_f = 0.94$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.86 (3H, d, H-14, $J = 6.3$ Hz), 0.91 (1H, dt, H-9a, $J = 3.5$, 13.6 Hz), 1.14–1.20 (1H, m, H-1), 1.52–1.61 (2H, m, H-2a, H-9b), 1.65 (9H, br s, H-12, H-13, H-15), 1.74 (3H, s, H-17), 1.75–1.84 (1H, m, H-8a), 2.02–2.11 (1H, m, H-10), 2.29 (1H, dd, H-2b, $J = 1.9$, 15.5 Hz), 2.54 (1H, br dd, H-8b, $J = 1.6$, 13.9 Hz), 3.25 (1H, s, H-6), 5.24 (1H, br s, H-5), 5.33 (1H, br d, H-3, $J = 5.0$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m*/*z* (rel. int.): 262 [M^+] (20), 220 (4), 202 (98), 187 (50), 177 (21), 160 (55), 159 (100), 145 (70), 131 (33), 119 (42), 105 (44), 91 (38), 77 (23), 67 (18), 55 (23), 43 (63).

4.10. (−)-(1*R*,6*S*,10*S*)-Amorpha-4,7(11)-dien-3-one 13

Colourless oil; $RI_{CPSIL\,5} = 1676$; $R_f = 0.86$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.72 (3H, d, H-14, $J = 6.3$ Hz), 0.73–0.81 (1H, m, H-9a), 1.33–1.40 (1H, m, H-1), 1.41–1.58 (3H, m, H-8a, H-9b, H-10), 1.59 (6H, s, H-12, H-13), 1.82 (3H, s, H-15), 2.12 (1H, dd, H-2a, $J = 4.7$, 16.1 Hz), 2.41 (1H, br d, H-8b, $J = 13.2$ Hz), 2.72 (1H, dd, H-2b, $J = 2.5$, 16.1 Hz), 3.52 (1H, br s, H-6), 5.91 (1H, s, H-5); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m*/*z* (rel. int.): 218 [M^+] (100), 203 (12), 189 (4), 176 (25), 175 (89), 161 (49), 147 (28), 133 (30), 119 (41), 107 (35), 105 (47), 91 (64), 77 (48), 67 (26), 55 (40), 41 (68); EIHRMS calcd for $C_{15}H_{22}O_1$ [M^+] *m*/*z* 218.1671, found [M^+] *m*/*z* 218.1668.

4.11. (+)-(1*R*,2*S*,6*R*,8*S*,10*S*)-2,8-Epoxyamorpha-4,7(11)-diene 14

Colourless oil; $RI_{CPSIL\,5} = 1597$; $R_f = 0.75$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.87 (3H, d, H-14, $J = 7.3$ Hz), 1.02–1.06 (1H, m, H-9a), 1.37–1.40 (1H, m, H-1), 1.39 (3H, s, H-13), 1.58 (3H, s, H-12), 1.61 (3H, br s, H-15), 1.75–1.81 (1H, m, H-10), 1.95 (1H, br d, H-3a, $J = 18.0$ Hz), 2.18 (1H, d, H-3b, $J = 18.6$ Hz), 2.21–2.26 (1H, m, H-9b), 3.07 (1H, s, H-6), 4.04 (1H, s, H-2), 4.49 (1H, s, H-8), 5.72 (1H, d, H-5, $J = 6.0$ Hz); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m*/*z* (rel. int.): 218 [M^+] (49), 200 (9), 185 (12), 175 (7), 157 (12), 143 (10), 138 (11), 133 (22), 121 (100), 119 (66), 105 (28), 93 (36), 91 (32), 77 (26), 55 (21), 41 (48); EIHRMS calcd for $C_{15}H_{24}O_1$ [M^+] *m*/*z* 218.1671, found [M^+] *m*/*z* 218.1680.

4.12. (+)-(1*R*,5*S*,6*R*,9*R*,10*R*)-5,9-Epoxyamorpha-3,7(11)-diene 15

Colourless oil; $RI_{CPSIL\,5} = 1595$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.71 (3H, d, H-14, $J = 6.9$ Hz), 1.21–1.32 (1H, m, H-1), 1.57 (3H, s, H-12), 1.59 (3H, s, H-13), 1.75 (1H, d, H-2a, $J = 17.0$), 1.88 (3H, s, H-15), 1.87–1.95 (1H, m, H-10), 2.05–2.13 (1H, d, H-2b, $J = 18.0$ Hz), 2.15–2.23 (1H, d, H-8a, $J = 16.4$ Hz), 2.42 (1H, s, H-6), 2.45 (1H, d, H-8b, $J = 13.6$ Hz), 3.70 (1H, s, H-9), 3.93 (1H, s, H-5), 5.32 (1H, s, H-3); MS (EI, 70 eV), *m*/*z* (rel. int.): 218 [M^+] (98), 203 (5), 185 (12), 175 (11), 157 (13), 145 (16), 135 (100), 119 (69), 105 (30), 93 (32), 91 (35), 77 (26), 55 (21), 41 (49); EIHRMS calcd for $C_{15}H_{22}O_1$ [M^+] *m*/*z* 218.1671, found [M^+] *m*/*z* 218.1679.

4.13. (−)-(1*R*,2*S*,6*R*,10*S*)-2α-Hydroxyamorpha-4,7(11)-diene 16

Colourless oil; $RI_{CPSIL\,5} = 1684$; $R_f = 0.56$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.98–1.05 (1H, m, H-9a), 1.21 (3H, d, H-14, $J = 6.6$ Hz), 1.31–1.35 (1H, m, H-1), 1.56 (3H, br s, H-15), 1.56–1.60 (1H, m, H-9b), 1.67 (3H, s, H-12), 1.70 (3H, d, H-13, $J = 1.9$ Hz), 1.70–1.82 (2H, m, H-8a, H-10), 1.94–2.09 (2H, m, H-3), 2.49 (1H, br d, H-8b, $J = 13.9$ Hz), 3.40 (1H, s, H-6), 3.78-3.82 (1H, m, H-2), 4.97 (1H, s, H-5); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI, 70 eV), *m*/*z* (rel. int.): 220 [M^+] (100), 205 (22), 202 (24), 187 (78), 177 (33), 159 (85), 145 (53), 131 (29), 121 (30), 119 (31), 105 (38), 91 (42), 55 (39), 41 (76); EIHRMS calcd for $C_{15}H_{24}O_1$ [M^+] *m*/*z* 220.1827, found [M^+] *m*/*z* 220.1809.

4.14. (−)-(1*R*,2*R*,6*R*,10*S*)-2β-Acetoxyamorpha-4,7(11)-diene 17

Colourless oil; $RI_{CPSIL\,5} = 1721$; $R_f = 0.94$; sense of optical rotation (benzene): (−); ^{1}H NMR (500 MHz, C_6D_6): δ 0.86 (3H, d, H-14, $J = 6.6$ Hz), 0.87–0.94 (1H, m, H-9a), 1.21–1.32 (1H, m, H-10), 1.46–1.51 (1H, m, H-9b), 1.54 (3H, br s, H-15), 1.56–1.62 (1H, m, H-1), 1.67 (3H, s, H-12), 1.71 (3H, s, H-17), 1.75 (3H, s, H-13), 1.75–1.83 (1H, m, H-8a), 1.95–2.08 (2H, m, H-3), 2.52 (1H, br d, H-8b, $J = 13.6$ Hz), 3.83 (1H, s, H-6), 5.18 (1H, s, H-5), 5.49 (1H, s, H-2); ^{13}C NMR (125.7 MHz, C_6D_6): see Table 1; MS (EI 70 eV), *m*/*z* (rel. int.): 262 [M^+], 202 (98), 187 (89), 174 (11), 159 (98), 145 (97), 131 (41), 119 (32), 105 (55), 91 (50), 77 (30), 67 (21), 55 (29), 43 (100).

4.15. (+)-(1*R*,6*R*,10*S*)-Amorpha-4,7(11)-dien-2-one 18

Colourless oil; $RI_{CPSIL\,5} = 1645$; sense of optical rotation (benzene): (+); ^{1}H NMR (500 MHz, C_6D_6): δ 0.79 (3H, d, $J = 6.6$ Hz), 1.16 (3H, dd, $J = 10.4$, 17.7 Hz), 1.41 (3H, d, $J = 0.9$ Hz), 1.49 (3H, d, $J = 1.9$ Hz), 1.58 (3H, d, $J = 0.9$ Hz), 2.12 (1H, dd, $J = 5.0$, 11.4 Hz), 2.41–2.62 (4H, m), 3.69 (1H, br s), 5.02 (1H, s); MS (EI 70 eV),

m/*z* (rel. int.): 218 [M^+] (100), 203 (21), 190 (8), 185 (8), 175 (82), 161 (51), 147 (48), 133 (38), 119 (42), 105 (43), 91 (41), 77 (28), 55 (27), 41 (67).

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