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Secondary metabolites from the liverwort *Tylimanthus renifolius*

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Abstract

Two new herbertane sesquiterpenoids, (−)-herbertene-1,12-diol and (−)-γ-herbertenol, a new aromadendrane, 9-acetoxy-10-hydroxyaromadendrane, a new rosane diterpenoid, 5,15-rosadiene-3,11-dione and a new phytane, 2*Z*-phytene-1,15,20-triol, along with the known (−)-α-herbertenol have been isolated from the liverwort *Tylimanthus renifolius*. Several flavones and flavanones with an unsubstituted ring B were also identified. This is the first report of this class of flavonoids from the Hepaticae. All structures were elucidated by NMR spectroscopy.

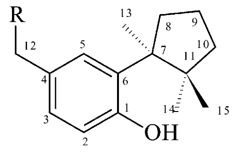
Keywords: Tylimanthus renifolius; Acrobolbaceae; Hepaticae; Herbertane; Aromadendrane; Rosane; Phytane; Flavone; Flavanone

1. Introduction

Liverworts are known to be a rich source of terpenoids and phenolic compounds (Zinsmeister et al., 1991; Asakawa, 1995 and 2001). In the course of our investigation of these compounds (Scher et al., 2003; Hertewich et al., 2003) we have analysed the constituents of the species *Tylimanthus renifolius*. This paper describes the isolation and characterization of the lipophilic compounds.

2. Results and discussion

A combination of size exclusion chromatography, vacuum liquid chromatography and HPLC of the dichloromethane extract of the plant led to the isolation of six terpenoids (**1**–**6**), among them the known (−)-α-herbertenol (**1**) (Matsuo et al., 1986) and ten flavonoids (**7**–**16**), all with an unsubstituted ring B.



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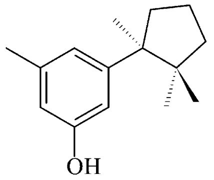

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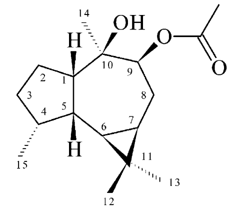

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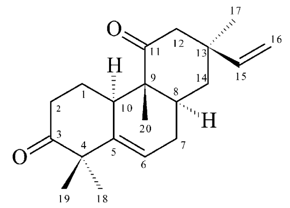

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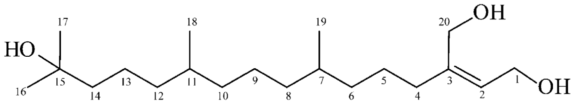

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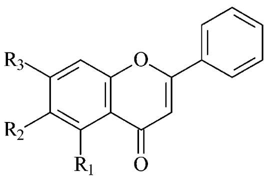
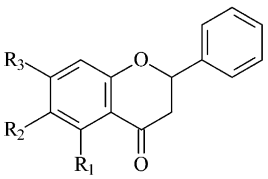


7 -12

	R1	R2	R3
7	OH	H	OMe
8	OMe	H	OMe
9	OH	OMe	OMe
10	OMe	OH	OMe
11	OH	OMe	OH
12	OMe	OMe	OMe



13 -16

	R1	R2	R3
13	OH	H	OMe
14	OMe	H	OMe
15	OH	OMe	OMe
16	OMe	OMe	OMe

Compound **2** was obtained as a colourless oil with a molecular formula of $C_{15}H_{22}O_2$, as indicated by the EI mass spectrum (m/z 234 $[M]^+$). The ^{13}C NMR spectrum showed the signals of six aromatic carbons (δ_C 154.3, *s*; 133.5, *s*; 132.4, *s*; 128.9, *d*; 126.1, *d*; 117.0, *d*), one of them phenolic and of a primary alcohol (65.6, *t*). The ^1H NMR spectrum displayed the signals of 3 aromatic protons, their couplings indicating a 1,2,4-substituted aromatic system (δ_H 7.28, *d*, $J=2.0$ Hz; δ_H 7.05, *dd*, $J=2.0$, 8.0 Hz; δ_H 6.64, *d*, $J=8.0$ Hz), a hydroxylic methylene (δ_H 4.57, 2H, *s*) and the singlets of three methyl groups (δ_H 1.40, 1.17, 0.73). The spectroscopic data were quite similar to those of (−)-α-herbertenol (**1**) except for the absence of the aromatic methyl and the appearance of a primary alcohol. Considering this difference the structure of **2** could be identified as (−)-4-hydroxymethyl-2-(1′,2′,2′-trimethylcyclopentyl)phenol or (−)-herbertene-1,12-diol. This is the first report of **2** as a natural product, but recently it was mentioned as an intermediate in the synthesis of formylherbertenol (Abad et al., 2000).

Compound **3**, a colourless oil, had the molecular ion peak at m/z 218 in the EIMS and thus a molecular formula of $C_{15}H_{22}O$. Its ^{13}C NMR spectrum showed that it was a phenol similar to α-herbertenol. However, in the ^1H NMR spectrum the differences became apparent. Instead of the 1,2,4-substitution pattern of α-herbertenol compound **3** showed the poorly resolved signals of three *meta*-coupled protons (δ_H 6.72, *brs*; δ_H 6.62, *t*, $J=2.0$ Hz; δ_H 6.46, *brs*) indicating a 1,3,5-substituted benzene ring. On the basis of the above spectral data and correlations in the HSQC and HMBC spectra the structure of **3** was established as (−)-5-methyl-3-(1′,2′,2′-trimethylcyclopentyl)phenol. We suggest the trivial name (−)-γ-herbertenol for compound **3**.

The EI mass spectrum of compound **4** ($[M]^+=m/z$ 280) was in agreement with a molecular formula of $C_{17}H_{28}O_3$ and hence four double bond equivalents. The ^{13}C NMR spectrum had resonances for five methyls, three methylenes, six methines and three quaternary carbons. Two of these resonances indicated alcohol functions (δ_C 76.9, *d*; 75.7, *s*), one of which was acetylated (δ_C 170.1, *s*; 21.2, *q*). The ^1H NMR spectrum showed one doublet and four singlet methyls. One of them belonged to the acetate group (δ_H 2.05, *s*), another one to a methyl group attached to an oxygen bearing carbon (δ_H 1.15, *s*). The remaining two methyls (δ_H 1.02 and 0.98, both *s*) were geminal to each other. The appearance of two signals in the high field of the spectrum (δ_H 0.14, *dd*, $J=9.5$, 9.5 Hz; δ_H 0.72, *ddd*, $J=6.0$, 9.5, 12.0) suggested the presence of a cyclopropane moiety. The four double bond equivalents along with the data mentioned above suggested an acetylated aromadendranediol. The positions of the acetoxy group at C-9 and the hydroxyl group at C-10 were deduced from the $^1H,^1H$ COSY, HSQC, and HMBC spectra (Fig. 1). The stereochemistry was deduced by comparison of the chemical shifts of **4** with those of known aromadendranes. An NMR study of the ^1H and ^{13}C chemical shifts of the 10-aromadendranols globulol and viridiflorol (Faure, 1991) showed significant differences in the shifts of C-6 ($\delta_{C\,viridiflorol}$ 22.5, $\delta_{C\,globulol}$ 28.6), H-5 ($\delta_{H\,viridiflorol}$ 1.84, $\delta_{H\,globulol}$ 1.23) and H-6 ($\delta_{H\,viridiflorol}$ δ_H 0.11, $\delta_{H\,globulol}$ 0.51).With C-6 at δ_C 22.3, H-5 at δ_H 1.78 and H-6 at δ_H 0.14 compound **4** should be of the viridiflorol type. These assumptions were confirmed by a NOESY experiment which established the configuration of the acetate as 9β. Thus **4** is the (1β,4α,5β,6α,7α,9β,10β)-form of 9-acetoxy-10-hydroxyaromadendrane.

The optical rotation of **5** was positive and the CD spectrum showed a positive cotton effect. Its EIMS gave a parent ion at m/z 300 $[M]^+$ which corresponds to a molecular formula of $C_{20}H_{28}O_2$ and hence seven double bond equivalents. The ^{13}C NMR spectrum displayed the signals of two ketones (δ_C 214.6 and 214.4), a vinyl group (δ_C 147.7, *d*; 110.3, *t*) and a trisubstituted double bond (δ_C 144.2, *s*; 118.2, *d*). The molecule is therefore tricyclic. The ^1H NMR spectrum revealed four tertiary methyl groups (δ_H 1.26, 1.22, 1.01, 0.98) and four olefinic protons which confirmed the presence of the vinyl group (δ_H 5.82, 4.96, 4.94) and the trisubstituted double bond (δ_H 5.59). The DEPT spectra showed four methyls, six methylenes, four methines and six quaternary carbons. Pimarane, isopimarane and rosane

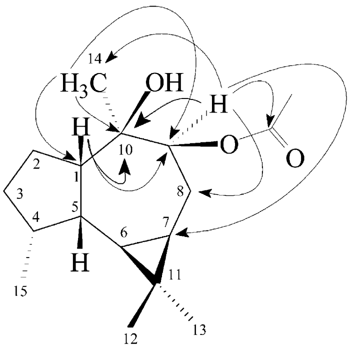


Fig. 1. Significant HMBC correlations of compound **4**.

skeletons, differing in the position of the angular methyl group C-20, seemed likely candidates. The placement of this methyl group at C-9 (rosane-type) as well as the positions of the ketones at C-3 and C-11 and the 5,15-diene double bonds could be readily established by 2D NMR $^1H,^1H$-COSY and HMBC measurements (see Fig. 2). A NOESY experiment determined the relative stereochemistry of the molecule. Key correlations included: H-10 to H-8 and H-18; H-8 to H-17. Thus H-8, H-10, Me-17 and Me-18 are α- whereas Me-19 and Me-20 are β-orientated. Rosanes are unusual diterpenoids for the Hepaticae. The only previous example is from the liverwort *Gackstroemia decipiens* (Geis and Becker, 2000). Rosanes arise by migration of the C-10 methyl group of pimaranes to C-9 and occur in both enantiomeric series predominantly in higher plants (Connolly and Hill, 1992).

Compound **6** has a molecular formula $C_{20}H_{40}O_3$ ($m/z = 329$ $[M+H]^+$) and thus only one double bond equivalent. The ^{13}C NMR spectrum showed signals for one tertiary alcohol (δ_C 71.2), two primary alcohols (δ_C 60.9, 58.6) and a trisubstituted double bond (δ_C 144.1, *s*; 126.3, *d*) indicating an acyclic diterpenoid triol. Comparison of the ^1H and ^{13}C NMR data with those of known phytanes led to the conclusion that compound **6** is a derivative of phytol (Brown, 1994). In contrast to phytol, the signals for the vinyl methyl C-20 were missing. Therefore C-20 is hydroxylated. The resulting hydroxy methylene group was found as a singlet at δ_H 4.15 (2H) in the ^1H NMR spectrum. Its correlations with both double bond carbons (C-2 and C-3) in the HMBC confirmed this assumption. The second hydroxyl was located at C-15 establishing an isopropyl alcohol, whose methyl groups could be identified by the signal at δ_H 1.19 (6H). Its correlation to one of the oxygen bearing carbons (C-15, δ_C 71.2) provided additional proof of structure. The stereochemistry of the double bond was deduced to be *Z* from the chemical shifts of C-4 and C-20 (Guerreiro, 1986). Compound **6** is thus 2*Z*-phytene-1,15,20-triol.

The above terpenoids were only the minor components of *Tylimanthus renifolius*. Flavone and flavanone derivatives with an unsubstituted ring B were isolated as the main constituents. According to Chemical Abstracts such compounds have not thus far been reported as constituents of bryophytes. The following flavonoids were identified: 5-hydroxy-7-methoxyflavone (**7**) (Thusoo et al., 1981), 5,7-dimethoxyflavone (**8**) (Häberlein and Tschiersch, 1994), 5-hydroxy-6,7-dimethoxyflavone (**9**) (Biekofsky et al., 1991), 6-hydroxy-5,7-dimethoxyflavone (**10**) (Osorio-Olivares et al., 1999), 5,7-dihydroxy-6-methoxyflavone (**11**) (Buschi et al., 1981), 5,6,7-trimethoxyflavone (Biekofsky et al., 1991) (**12**), 5-hydroxy-7-methoxyflavanone (**13**) (González et al., 1989), 5,7-dimethoxyflavanone (**14**) (Mayer, 1990), 5-hydroxy-6,7-dimethoxyflavanone (**15**) (Kamperdick et al., 2002), 5,6,7-trimethoxyflavanone (**16**) (Achenbach and Hemrich, 1991). They were identified by their ^1H and ^{13}C NMR data which were consistent with the cited literature.

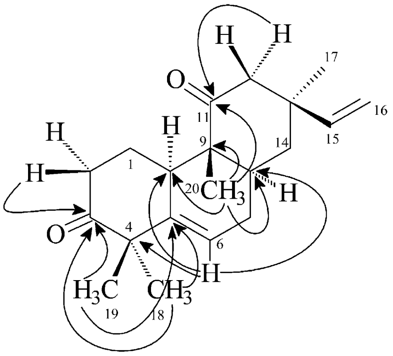


Fig. 2. Significant HMBC correlations of compound **5**.

Tylimanthus species do not grow in isolation but are usually associated with other liverworts. This makes it difficult to collect large amounts of these plants and may explain why these species have been neglected phytochemically. Apart from *T. renifolius*, only *T. urvilleanus* has been investigated chemically (Asakawa and Inoue, 1984a) and it contains a completely different set of metabolites. In contrast to *T. renifolius*, the lipophilic extract of *T. urvilleanus* contained neither flavonoids nor terpenoids, apart from *trans* β-farnesene, but the three bibenzyl dihydrobenzofuran derivatives tylimanthins A–C. Therefore, a phytochemical evaluation of the genus *Tylimanthus* is not possible unless more species are analysed.

The occurrence of herbertane sesquiterpenoids in *T. renifolius* is remarkable. For years, herbertanes seemed to be restricted to *Herbertus* (Asakawa, 1995; Buchanan et al., 1996; Matsuo et al., 1981, 1986) and *Mastigophora* species (Asakawa, 1995; Fukuyama et al., 1988, 1991) and their potential as a chemical marker of these genera has been discussed (Hashimoto et al., 2000). Now their occurrence in species like *T. renifolius* as well as in South American *Plagiochila bispinosa* and *P. subdura* (Asakawa and Inoue, 1984b), in *Marchantia polymorpha* (Asakawa et al., 1984; Matsuo et al., 1985) and in Panamanian *Bazzania hookeri* (Saar, S. and Becker, H., unpublished results), which are, according to Schuster (1984) or Crandall-Stotler and Stotler (2000), neither related to one another nor to *Herbertus* or *Mastigophora,* casts serious doubt on the use of herbertanes as chemosystematic markers.

3. Experimental

3.1. Spectroscopy and spectrometry

Optical rotations were measured in $CHCl_3$. NMR Spectra were recorded in $CDCl_3$ (^1H NMR: 500 MHz,

^{13}C NMR: 125 MHz) relative to $CDCl_3$ at δ_H 7.24, δ_C 77.0. ^{13}C multiplicities were determined using the DEPT pulse sequence. 2D Spectra were recorded as H,H-COSY, HSQC and HMBC experiments.

3.2. Plant material

T. renifolius Hässel et Solari was collected 30 km east from Ushuaia in a *Nothofagus pumilio* forest, Tierra del Fuego, Argentina, in March 1997 and identified by Prof. Dr. R. Mues and Prof. Dr. U. Drehwald. A voucher specimen is deposited at the Herbarium Drehwald (No. 3088).

3.3. Extraction and isolation

The extraction scheme followed the standard procedure of our group (Adam et al., 1998). For VLC either silica gel 15 μm or diol silica gel 40–63 μm was used, for HPLC either a silica gel column (Knauer LiChrospher 100 Si, 5 μm) or a diol silica gel column (Knauer LiChrospher 100 DIOL 5 μm) was used. The plant material (305 g) was powdered and extracted with CH_2Cl_2. After removal of the solvent the crude extract, which contained high amounts of chlorophyll and other by-products, was chromatographed by CC on Sephadex LH-20 (150×2.5 cm i.d.) with MeOH–CH_2Cl_2 (1:1) as eluent, to give three fractions. Fraction 2 (9.0 g) was separated by VLC (silica gel, *n*-hexane–EtOAc gradient) and gave the fractions 2.1 (0–0.5% EtOAc, 60 mg), 2.2 (0.5–1.5% EtOAc, 59 mg), 2.3 (1.5–3.5% EtOAc, 190 mg), 2.4 (3.5–7% EtOAc, 372 mg), 2.5 (7–10% EtOAc, 391 mg), 2.6 (12–35% EtOAc, 867 mg), 2.7 (35–70% EtOAc, 1657 mg), 2.8 (80–90% EtOAc, 1467 mg), 2.9 (90–100% EtOAc, 1118 mg). The following compounds crystallized overnight in the test tubes of the VLC separation: fr. 2.5: 30 mg of 5-hydroxy-7-methoxyflavone (**7**) as yellow crystals; fr. 2.6: 208 mg of 5,6,7-trimethoxyflavanone (**16**) as colourless crystals; fr. 2.7: 109 mg of 5-hydroxy-6,7-dimethoxyflavone (**9**) as yellow crystals, fr. 2.8: 1333 mg of 5,7-dimethoxyflavone (**8**) as yellow crystals. Fr. 2.9 was pure 5,6,7-trimethoxyflavone (**12**). After separation of fraction 2.4 with HPLC (silica gel, *n*-hexane–EtOAc 95:5) 7 fractions, 2.41 to 2.47, were obtained. 2.41 was pure (−)-α-herbertenol (**1**, 27 mg). 2.43 was purified by HPLC (silica gel, *n*-hexane–EtOAc 96:4) and yielded 12 mg of 5-hydroxy-7-methoxyflavanone (**13**) and 1 mg of (−)-γ-herbertenol (**3**). HPLC separation of 2.45 (silica gel, *n*-hexane-TBME 93:7) yielded 6 mg of 5,15-rosadiene-3,11-dione (**5**). HPLC of fr. 2.5 (silica gel, *n*-hexane–EtOAc 90:10) yielded 8 fractions (2.51–2.58). HPLC of 2.56 (silica gel, *n*-hexane–EtOAc 90:10) gave 26 mg of 5-hydroxy-6,7-dimethoxyflavanone (**15**), HPLC of fr. 2.57 (diol silica gel, *n*-hexane–EtOAc 90:10) gave 1 mg of 9-acetoxy-10-hydroxyaromadendrane (**4**). Fr. 2.6 was separated by VLC (silica gel, *n*-hexane–EtOAc gradient) and gave the fractions 2.61 to 2.65. Fr. 2.64 (18–30% EtOAc) was separated with HPLC (diol silica gel, *n*-hexane–EtOAc 78:22) and yielded 3 mg of (−)-herbertene-1,12-diol (**2**). Fr. 2.7 was separated by VLC (diol silica gel, 25–35% EtOAc in *n*-hexane) and yielded 24 mg of 5,7-dimethoxyflavanone (**14**). Frs. 2-8 was separated by VLC (diol silica gel, *n*-hexane–EtOAc gradient) and gave the fractions 2.81–2.85. Fr. 2-85 (60% EtOAc) was separated by HPLC (diol silica gel, *n*-hexane–EtOAc 20:80) and yielded 2*Z*-phytene-1,15,20-triol (**6**, 2 mg), 5,7-dihydroxy-6-methoxyflavone (**11**, 0.5 mg) and 6-hydroxy-5,7-dimethoxyflavone (**10**, 0.5 mg).

3.4. (−)-4-Hydroxymethyl-2-(1′,2′,2′-trimethylcyclopentyl)phenol or (−)-herbertene-1,12-diol (2)

Colourless oil; EIMS: *m*/*z* (rel. int.) = 234 (39) $[M]^+$, 201 (23), 174 (36), 164 (100), 151 (96), 147 (81), 133 (32), 121 (33), 107 (19), 91 (21), 77 (16), 55 (15); ^{1}H NMR ($CDCl_3$): 7.28 (*d*, *J* = 2.0 Hz, H-5), 7.05 (*dd*, *J* = 8.0, 2.0 Hz, H-3), 6.64 (*d*, *J* = 8.0 Hz, H-2), 4.57 (*s*, 2×H-12), 2.59 (*m*, H-8a), 1.76 (*m*, H-8b), 1.75 (*m*, 2×H-9), 1.66 (*m*, H-10a), 1.53 (*m*, H-10b), 1.40 (*s*, 3×H-13), 1.17 (*s*, 3×H-14), 0.73 (*s*, 3×H-15); ^{13}C NMR ($CDCl_3$): 154.3 (*s*, C-1), 133.5 (*s*, C-6), 132.4 (*s*, C-4), 128.9 (*d*, C-5), 126.1 (*d*, C-3), 117.0 (*d*, C-2), 65.6 (*t*, C-12), 51.1 (*s*, C-7), 44.7 (*s*, C-11), 41.3 (*t*, C-10), 39.5 (*t*, C-8), 27.0 (*q*, C-15), 25.6 (*q*, C-14), 22.8 (*q*, C-13), 20.3 (*t*, C-9).

3.5. (−)-5-Methyl-3-(1′,2′,2′-trimethylcyclopentyl)phenol or (−)-γ-herbertenol (3)

Colourless oil, EIMS: *m*/*z* (rel. int.) = 218 (18) $[M]^+$, 161 (13), 148 (40), 136 (100), 121 (22), 108 (9), 91 (12), 77 (11), 55 (10); IR ν_{max}^{KBr}: cm^{-1}: 3380, 2920, 2860, 1690, 1630, 1590, 1440, 1280, 1150, 830; ^{1}H NMR ($CDCl_3$): 6.72 (*brs*, H-5), 6.62 (*t*, *J* = 2.0 Hz, H-1), 6.46 (*brs*, H-3), 4.53 (*s*, 2-OH), 2.43 (*ddd*, *J* = 12.5, 9.0, 9.0 Hz, H-8a), 2.27 (*s*, 3×H-12), 1.74 (*m*, H-9a), 1.67 (*m*, H-10a), 1.65 (*m*, H-8b), 1.55 (*m*, H-10b), 1.21 (*s*, 3×H-13), 1.05 (*s*, 3×H-14), 0.82 (*m*, H-9b), 0.56 (3H, *s*, 3×H-15); ^{13}C NMR ($CDCl_3$): 154.7 (*s*, C-2), 149.6 (*s*, C-6), 138.3 (*s*, C-4), 120.6 (*d*, C-5), 112.9 (*d*, C-3), 111.3 (*d*, C-1), 50.4 (*s*, C-7), 44.2 (s, C-11), 39.7 (*t*, C-10), 36.8 (*t*, C-8), 26.5 (*q*, C-15), 24.4 (*q*, C-13), 24.3 (*q*, C-14), 21.6 (*q*, C-12), 19.6 (*t*, C-9).

3.6. 9-Acetoxy-10-hydroxyaromadendrane (4)

Colourless oil, $[\alpha]_D^{20}$ + 34.4° ($CHCl_3$; *c* 0.1); EIMS: *m*/*z* (rel. int.) = 280 (21) $[M]^+$, 262 (6), 238 (1), 220 (62), 202 (100), 187 (45), 177 (97), 159 (57), 149 (26), 139 (31), 125 (42), 107 (37), 81 (24), 54 (26); IR ν_{max}^{KBr}: cm^{-1}: 3500, 3440, 2920, 2870, 1720, 1450, 1370, 1240, 1190, 1020, 890; ^{1}H NMR ($CDCl_3$): 4.79 (*dd*, *J* = 1.5, 10.5 Hz, H-9α), 2.05 (*s*, CH_3COOR), 1.96 (*m*, H-4β), 1.82 (*m*, H-1β), 1.78 (*m*, H-8β), 1.78 (*m*, H-5β), 1.74 (*m*, H-3β), 1.63 (*m*, H-2β), 1.62 (*m*, H-8α), 1.53 (*m*, H-2α), 1.20 (*m*, H-3α), 1.15 (*s*, 3×H-14α), 1.02 (*s*, 3×H-13α), 0.98 (*s*, 3×H-12β), 0.91 (*d*, *J* = 7.0 Hz, 3×H-15α), 0.72 (*ddd*, *J* = 6.0, 9.5, 12.0 Hz, H-7α), 0.14 (*dd*, *J* = 9.5, 9.5 Hz, H-6α); ^{13}C NMR ($CDCl_3$): 170.1 (*s*, $\underline{C}OOMe$), 76.9 (*d*, C-9), 75.7 (*s*, C-10), 56.0 (*d*, C-1), 39.3 (*d*, C-5), 38.1 (*d*, C-4), 28.7 (*t*, C-3), 28.5 (*q*, C-13), 27.7 (*q*, C-14), 26.5 (*t*, C-8), 25.2 (*t*, C-2), 23.5 (*d*, C-7), 22.3 (*d*, C-6), 21.2 (*q*, $COO\underline{C}H_3$), 19.1 (*s*, C-11), 16.1 (*q*, C-15), 15.9 (*q*, C-12).

*3.7. 5,15-Rosadiene-3,11-dione (**5**)*

Colourless oil, $[\alpha]^{20}_D$ + 40.3° ($CHCl_3$; *c* 0.4); CD (*n*-hexane) λ (Δε) 329 (0), 298 (+2.6), 240 (0), 210 (−6.2), 204 (−5.6), 192 (−15.8); GC-MS (EI): *m*/*z* (rel. int.) = 300 (28) $[M]^+$, 257 (41), 203 (40), 189 (32), 171 (56), 145 (50), 133 (41), 119 (57), 105 (100), 91 (94), 77 (58), 69 (45), 55 (66); IR ν^{KBr}_{max}: cm^{-1}: 2880, 2820, 1690, 1670, 1440, 1415, 1360, 1210, 890; ^1H NMR ($CDCl_3$): 5.82 (*dd*, *J* = 10.5, 17.5 Hz, H-15β), 5.59 (*ddd*, *J* = 5.0, 2.5, 2.5 Hz, H-6), 4.96 (*dd*, *J* = 17.5, 1.0 Hz, H-16a), 4.94 (*dd*, *J* = 10.5, 1.0 Hz, H-16b), 3.02 (*m*, H-10α), 2.74 (*d*, *J* = 12.5 Hz, H-12β), 2.69 (*ddd*, *J* = 5.0, 13.0, 15.0 Hz, H-2α), 2.31 (*ddd*, *J* = 4.5, 9.0, 15.0 Hz, H-2β), 2.20 (*dddd*, *J* = 4.5, 5.0, 5.0, 13.0 Hz, H-1α), 1.98 (*m*, H-12α), 1.96 (*m*, 2× H-7), 1.88 (*m*, H-8α), 1.74 (*dd*, *J* = 13.0, 13.0 Hz, H-14β), 1.41 (*m*, H-1β), 1.37 (*m*, H-14α), 1.26 (*s*, 3×H-18α), 1.22 (*s*, 3×H-19β), 1.01 (*s*, 3×H-20β), 0.98 (*s*, 3×H-17α) ^{13}C NMR ($CDCl_3$): 214.6 (*s*, C-3), 214.4 (*s*, C-11), 147.7 (*d*, C-15), 144.2 (*s*, C-5), 118.2 (*d*, C-6), 110.3 (*t*, C-16), 51.0 (*s*, C-4), 49.4 (*s*, C-9), 48.5 (*t*, C-12), 41.6 (*s*, C-13), 38.6 (*d*, C-10), 38.4 (*t*, C-14), 38.2 (*d*, C-8), 37.8 (*t*, C-2), 29.4 (*q*, C-18), 29.0 (*t*, C-7), 25.2 (*t*, C-1), 23.5 (*q*, C-17), 22.8 (*q*, C-19), 12.0 (*q*, C-20).

*3.8. 2Z-Phytene-1,15,20-triol (**6**)*

Colourless oil, $[\alpha]^{20}_D$ −7.1° ($CHCl_3$; *c* 0.2); CIMS: *m*/*z* (rel. int.) = 329 (21) $[M+H]^+$, 311 (7), 293 (100), 275 (92), 149 (13), 137 (8), 123 (10), 111 (11), 95 (8), 69 (21); IR ν^{KBr}_{max}: cm^{-1}:3400, 2920, 2850, 1720, 1450, 1370, 1150, 1010, 930, 900; ^1H NMR ($CDCl_3$): 5.61 (*t*, *J* = 7.0 Hz, H-2), 4.19 (*d*, *J* = 7.0 Hz, 2×H-1), 4.15 (*s*, 2×H-20), 2.09 (*m*, 2×H-4), 1.42 (*m*, 2×H-5), 1.41 (*m*, 2×H-14), 1.38 (*m*, H-7), 1.38 (*m*, H-11), 1.30 (*m*, 2×H-13), 1.29 (*m*, H-9a), 1.28 (*m*, H-6a), 1.27 (*m*, H-12a), 1.24 (*m*, H-10a), 1.24 (*m*, H-8a), 1.19 (6H, *s*, H-16 and H-17), 1.17 (*m*, H-9b), 1.11 (*m*, H-6b), 1.09 (*m*, H-12b), 1.06 (*m*, H-10b), 1.06 (*m*, H-8b), 0.84* (3H, *d*, *J* = 6.5 Hz, H-18), 0.83* (3H, *d*, *J* = 6.5 Hz, H-19). ^{13}C NMR ($CDCl_3$): 144.1 (*s*, C-3), 126.3 (*d*, C-2), 71.2 (*s*, C-15), 60.9 (*t*, C-20), 58.6 (*t*, C-1), 44.2 (*t*, C-14), 37.4# (*t*, C-12), 37.2# (*t*, C-10), 37.1# (*t*, C-8), 36.6# (*t*, C-6), 36.1 (*t*, C-4), 32.7 (*d*, C-11), 32.6 (*d*, C-7), 29.2 (*q*, C-16 and C-17), 25.5 (*t*, C-5), 24.3 (*t*, C-9), 21.7 (*t*, C-13), 19.8 (*q*, C-18 and C-19). *, # Assignments interchangeable.

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