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Sesquiterpenoids, hopanoids and bis(bibenzyls) from the Argentine liverwort *Plagiochasma rupestre*

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Abstract

A new cadinane-type sesquiterpene alcohol named rupestrenol has been isolated from the Argentine liverwort *Plagiochasma rupestre* (Forst.) Steph., belonging to the Aytoniaceae along with the known sesquiterpenoids marsupellone, β-longipinene and caryophyllene oxide, three known hopanoids, and four known bis(bibenzyl) derivatives as well as other common plant constituents. The structure and stereochemistry of rupestrenol was established by a combination of extensive NMR experiments and X-ray crystallographic analysis. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Plagiochasma rupestre; Aytoniaceae; Liverworts; Hepaticae; Rupestrenol; Cadinane; Caryophyllane; Longipinane; Sesquiterpenoids; Hopanoids; Bis(bibenzyls); Chemosystematics

1. Introduction

Liverworts produce not only various pungent and bitter terpenoids but also benzyls and bis(bibenzyl) derivatives which show interesting biological activity, and which are valuable for the chemosystematics of the Hepaticae (Asakawa, 1982, 1990, 1993, 1995, 1997, 1998). The *Plagiochasma* genus (Aytoniaceae) includes *P. appendiculata*, *P. australe*, *P. japonicum*, *P. pterospermum* (= *P. intermedium*) and *P. rupestre*. We have reported the isolation and the structure elucidation of a new bis(bibenzyl), marchantin H (Tori, Toyota, Harrison, Takikawa & Asakawa, 1985) and the presence of elemane-, caryophyllane-, barbatane-, bazzanane-type sesquiterpenoids and the isolation of (+)-gymnomitr-8(12)-en-9α-ol and two bis-bibenzyls, pakyonol and riccardin C (**13**) from *P. pterospermum* (= *P. intermedium*) (Hashimoto, Irita & Asakawa, 1998) and Markham & Porter (1978) reported the presence of three flavonoid glycosides from *P. rupestre*. Harrison, Becker, Connolly & Rycroft (1992) found in *P. rupestre* the longipinane-type sesquiterpenoid, marsupellone (**7**), two elemane-type sesquiterpenoids, and a hopane-type triterpenoid zeorin (**11**) together with a flavone methyl ether.

In order to study the comparative biochemistry of the species belonging to the Aytoniaceae, we reinvestigated the chemical constituents of *P. rupestre* from Argentine and report here the isolation and structure of a new cadinane-type sesquiterpenoid named rupestrenol (**1**), together with known sesqui- and triterpenoids and bis(bibenzyls).

2. Results and discussion

P. rupestre is widespread in humid rocky mountains of northern Argentina. The air-dried and ground *P. rupestre* was extracted with ether and then methanol.

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The ether extract was chromatographed on silica gel and Sephadex LH-20, and each fraction was purified by HPLC to give rupestrenol (**1**), a new cadinane-type sesquiterpene alcohol, the known sesquiterpenoids marsupellone (**7**) (Matsuo, Uto, Uchio, Nakayama & Hayashi, 1979), caryophyllene oxide (**8**) (Asakawa, Toyota, Bischler, Campbell & Hattori, 1984) and *β*-longipinene (**6**) (Andersen et al., 1977), the norhopanoid adiantone (**9**) (Hveding-Bergseth, Bruun & Kjøsen, 1983), the hopanoids diplopterol (**10**) (Ageta, Iwata & Otake, 1963) and zeorin (**11**) (Huneck & Lehn, 1963), the known bis(bibenzyls) marchantin K (**12**) (Asakawa, Tori, Masuya & Frahm, 1990), marchantin B (**13**) (Asakawa, Toyota, Matsuda, Takikawa & Takemoto, 1983), riccardin C (**15**) (Asakawa & Matsuda, 1982) and isoriccardin C (**16**)(Asakawa, Tori, Takikawa, Krishnamurti & Kanti Kar, 1987). The methanol extract was treated in the same manner as described above to afford **1**, **10**, **12** and **16**.

The structure of rupestrenol (**1**) was deduced by spectroscopic data, along with X-ray crystallographic analysis of the 2,4-dinitrophenylhydrazone of the aldehyde (**2**) derived from **1**. The EI-mass spectrum of **1** gave a molecular ion peak at *m*/*z* 220 and its HR-mass spectrum indicated a molecular formula $C_{15}H_{24}O$ that accounted for four degrees of unsaturation. The IR spectrum showed the broad band at 3300 cm^{-1} of a hydroxyl group along with the bands at 1645 and 895 cm^{-1} related to olefinic systems. The broad singlet at δ 4.10 (2H) in the ^{1}H-NMR spectrum suggested that a primary allylic alcohol was present. The signals of 3 vinyl protons and the corresponding 4 olefinic carbons which accounted for two double bonds, led to the assumption of a bicyclic molecule. The ^{1}H–^{1}H COSY, HMQC and HMBC spectra provided evidence for the presence of 4,11(13)-cadinadienol. The antiperiplanar relationships of the four protons at *C*-1, *C*-2, *C*-6 and *C*-10 were indicated by the large coupling constants $J_{1,\,2ax} = 12$ Hz, $J_{1,\,6} = J_{1,\,10} = 10$ Hz. A compound with the structure and stereochemistry we hereby propose for **1** had been previously reported from the Chilean Nolanaceae *Nolana coelestis* (Garbarino, Chami, Montagna & Gambaro, 1993) but the chemical shifts of H-1, C-1, C-2, C-6, C-7, C-8 and C-10 in the corresponding NMR spectra (Table 1 and Table 2) as well as the reported value for the specific optical rotation (Table 1) of the compound assigned formula **1** in the above reference differ from those of our compound **1**. The ^{13}C-NMR spectral data of **1** resembled

Table 1
^{1}H-NMR data for compound **1** ($CDCl_3$-TMS)

	1^a ($[\alpha]_D$ +43.9) (600 MHz)		1^b ($[\alpha]_D$ − 177.3) (360 MHz)	
H-1	0.76	*dddd* (12, 12, 10, 2)[c]	1.20	*ddd* (11, 9, 2.0)
H-2eq	1.92–2.02	*m*		
H-2ax	1.18	*dddd* (12, 12, 4.7, 1.5)		
H-3eq	1.92–2.02	*m*		
H-3ax	1.92–2.02	*m*		
H-5	5.26	*quin* (1.6)	5.39	*br s*
H-6	1.8–1.82	*m*	1.73	*m*[d] (9, 11)
H-7	1.7–1.82	*m*	1.88	*ddd* (9.0,9.0, 2.5)
H-8eq	1.7–1.82	*m*		
H-8ax	1.38	*dddd* (13, 3.6, 3.6, 3.6)		
H-9eq	1.7–1.82	*m*		
H-9ax	1.08	*dddd*(13, 13, 13, 4)		
H-10	1.20	*m*		
H-12	4.10	*br s*	4.00	*br s*
H-13a	4.91	*q* (1.4)	4.93	*d* (1.0)
H-13b	5.15	*q* (1.6)	5.11	*d* (1.0)
H-14	0.92	*d* (6.3)	0.93	*d* (5)
H-15	1.61	*m*	1.61	*s*

[a] Present study.
[b] Garbarino et al. (1993).
[c] Coupling constants (*J* in parentheses) were given in Hz;.
[d] Obscured due to overlapping signals.

Table 2
^{13}C-NMR spectral data for compounds **1** and **5** ($CDCl_3$-TMS)

Carbon	1^a	1^b	5^c
1	46.5	35.3	46.3
2	26.5	17.0	26.2
3	30.9	31.3	26.4
4	134.6	134.1	137.5
5	123.5	125.0	125.5
6	44.1	39.6	51.1
7	47.5	43.5	42.6
8	34.1	29.6	32.5
9	35.9	34.3	35.7
10	36.3	42.0	36.5
11	152.2	152.9	148.4
12	64.9	69.5	19.1
13	109.1	108.2	111.6
14	19.6	19.5	19.7
15	23.5	23.5	67.4

[a] Present study.
[b] Garbarino et al. (1993).
[c] Wei et al. (1995).

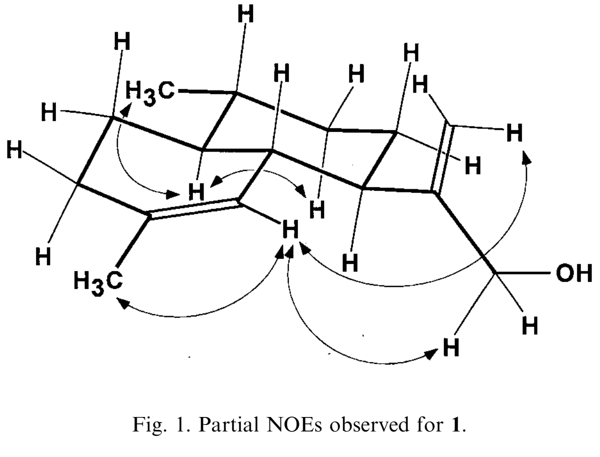


Fig. 1. Partial NOEs observed for **1**.

those of *(1R,6S,7S,10S)*-15-hydroxy-cadina-4,11(13)-diene (**5**) (Table 1), isolated from the Taiwanese liverwort, *Reboulia hemisphaerica* (Wei, Ma & Wu, 1995), indicating that **1** possessed the same relative stereochemistry as **5**. This assumption was further confirmed by the NOE experiment of **1** and the X-ray crystallographic analysis of the 2,4-dinitrophenylhydrazone (**4**) of **2**, which was obtained by oxidation of **1** with pyridinium dichromate (PDC). The partial NOESY spectrum showed NOEs between (i) H-1 and H-9ax, (ii) H-1 and H-14, (iii) H-5 and H-12, (iv) H-5 and H-13, and (v) H-5 and H-12, as shown in Fig. 1. The ORTEP drawing of **4** is shown in Fig. 2. Oxidation and benzoylation of **1** to the corresponding aldehyde (**2**) and *p*-bromobenzoate (3) provided additional chemical evidence. Consequently, the structure of rupestrenol was established to be (+)-($1R^*$, $6S^*$,$7S^*$,$10S^*$)-15-hydroxy-cadina-4,11(13)-diene (**1**). The absolute configuration of **1** remained to be clarified. Thus the structure assigned to compound **1** reported by Garbarino et al. (1993) might need to be revised.

From the methanolic extract, a minor bis-bibenzylic compound, which was assigned as marchantin K (**12**) was isolated. This compound has been isolated from *Marchantia polymorpha* collected in Germany and its strcuture elucidated by the ^{1}H-, ^{13}C-NMR and NOE spectral data of the permethylated product (**14**) of **12** (Asakawa et al., 1990). In Tables 3 and 4, the ^{1}H- and ^{13}C-NMR and HMBC spectral data of the original marchantin K have been shown. These data further confirmed the structure of **12**. Previously, the ^{13}C-NMR data for isoriccardin C (**16**) has been reported (Asakawa et al., 1987). In Table 4, their assignments are newly added.

The structures of the previously known terpenoids and bis(bibenzyls) were established by comparison of spectral and other physical data with those of authentic samples.

In Table 5, the chemical constituents of the *Plagiochasma* have been shown. A previous report on the French *P. rupestre* indicated the presence of compounds **7** and **11** in addition to elemane-type sesquiterpenoids (Harrison et al., 1992). Bis(bibenzyls) were

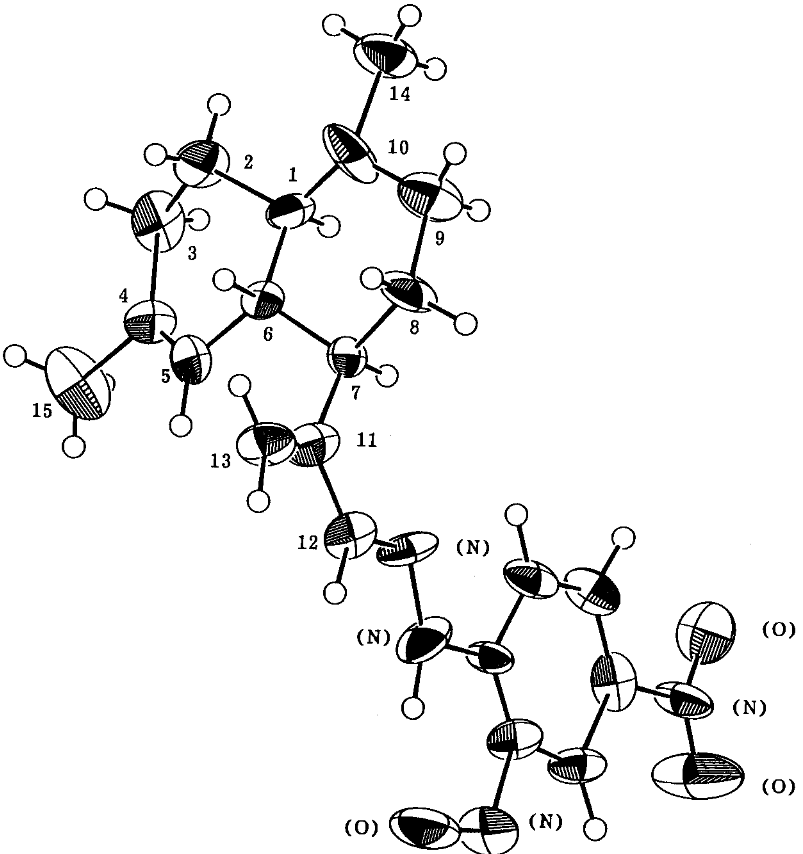


Fig. 2. ORTEP drawing for **4**.

Table 3
^{1}H-NMR (600 MHz, $CDCl_3$-TMS) data for **12**, and ^{13}C-NMR data for **12** and **16** (150 MHz, CD_3OD-TMS)

12				**12**		**16**
H			*J*	C		C
2,6	6.50	*br d*	8	1	155.2	153.2
3,5	6.91	*br d*	8	2	122.7	121.7[a]
7a	3.00–3.05	*m*		3	130.6	130.8
7b,8a	2.90–2.97	*m*		4	140.6	137.2
8b	2.80	*ddd*	15, 10, 2	5	130.6	130.3
10	6.83	*d*	8.5	6	122.7	121.7[a]
11	6.75	*d*	8.5	7	37.1	34.5
3′	4.96	*d*	2	8	30.8	36.1
5′	6.52	*d*	2	9	128.3	143.3
7′	4.06	*dd*	10,4	10	121.3	116.6
8a′	3.03	*dd*	12,4	11	113.5	153.4
8b′	2.56	*dd*	12,10	12	145.8	117.0
10′	6.68	*d*	2	13	139.1	130.5
12′	6.46	*ddd*	8,3,1	14	142.2	122.6
13′	6.85	*t*	8	1′	134.8	143.6
14′	6.02	*br d*	8	2′	148.9	147.8
OMe	3.26	*s*		3′	109.2	114.7
				4′	132.1	133.6
				5′	107.0	121.6[a]
				6′	147.5	114.6
				7′	85.9	38.0
				8′	45.0	36.5
				9′	139.9	121.6
				10′	118.1	120.0
				11′	158.9	154.0
				12′	114.0	113.3
				13′	129.0	130.1
				14′	124.6	142.9

[a] Assignments may be reversed.

absent in this collection. The elemane-type sesquiterpenoids have not been detected in the present species. Hopanoids are triterpenes widely distributed among bacteria and cyanobacteria and have been found in a few genera of liverworts (Toyota & Asakawa, 1993; Grammes, Burkhardt & Becker, 1994), lichens (Huneck & Yoshimura, 1996) and ferns (Murakami & Tanaka, 1988). They have also been isolated from sedimentary organic matter of most varied origin and are ubiquitous molecular fossils derived from cellular constituents of microorganisms (Ourisson, Albrecht & Rohmer, 1979). The Argentine and French *P. rupestre* and the Japanese *P. pterospermum* produce commonly zeorin (**11**). Thus, this triterpene alcohol is one of the chemical markers of the genus *Plagiochasma*. The Argentine *P. rupestre* is chemically similar to *P. pterospermum*, since both species produce cyclic bis(bibenzyl), riccardin C (**15**) and the similar bis(bibenzyls) (**12, 13, 16**) to those of marchantin H and pakyonol (Hashimoto et al., 1998). There is chemical affinity between the *Plagiochasma* species and *Reboulia hemisphaerica* which belongs to the Aytoniaceae, because both genera biosynthesize barbatane-, and cadinane-type sesquiterpenoids and marchantin- and riccardin-type bis(bibenzyls) (Wei et al., 1995).

It is also noteworthy that adiantone (**9**) and diplopterol (**10**) and their related hopanoids have been isolated from in vitro cultured liverwort *Fossombronia alaskana* and *F. pusilla* belonging to the order Metzgeriales (Grammes et al., 1994), although these species are morphologically different from the *Plagiochasma* classified into the order Marchantiales.

Table 4
C–H correlation for **12** observed by HMBC spectrum

C	H	C	H
1	2,6,3,5	1′	3′, 5′
2,6	2,6,3,5	2′	3′
3,5	3, 5, 7a,7b	3′	5′
4	2, 6, 7a, 7b, 8a, 8b,	4′	3′, 5′, 8a′, 8b′,
7	3, 5, 8a, 8b	5′	3′
8	7a, 7b, 10	6′	5′
9	8a, 8b, 10	7′	3′, 8b′, OMe
10	8a, 8b	8′	10′, 14′
12	10, 11	9′	8a′, 8b′, 13′
13	11	10′	12′, 14′
14	8a, 8b, 10	11′	10′, 12′. 13′
		12′	10′, 13′, 14′
		13′	14′
		14′	8a′, 8b′, 10′, 12′

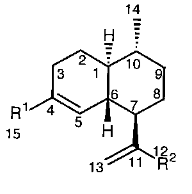
Table 5
Distribution of terpenoids and phenolic compounds in *Plagiochasma* species

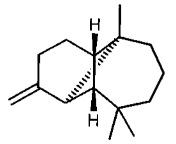
Compounds	*P. rupestre*[a]	*P. rupestre*[b]	*P. pterospermum*[c]
Sesquiterpenoids			
Barbatanes			+
Bazzananes			+
Cadinanes	+		
Caryophyllanes	+		+
Elemanes		+	
Longipinanes	+	+	
Hopanoids			
Adianton	+		
Zeorin	+	+	+
Diplopterol	+		
Bis(bibenzyls)			
Marchantin B	+		
Marchantin H			+
Marchantin K	+		
Pakyonol			+
Riccardin C	+		+
Isoriccardin C	+		

[a] Present study.

[b] Harrison et al. (1992).

[c] Tori, Toyota, Harrison, Takikawa & Asakawa (1985) and Hashimoto et al. (1998).





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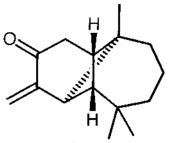

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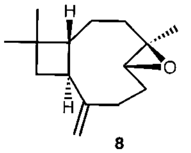
1: R^1=Me, R^2= CH_2OH
2: R^1=Me, R^2= CHO
3: R^1=Me, R^2= $CH2OCOC_6H_4Br$
4: R^1=Me, R^2= CH=N-NH-2,4-dinitrophenyl
5: R^1=CH_2OH, R^2=Me



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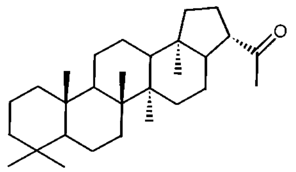

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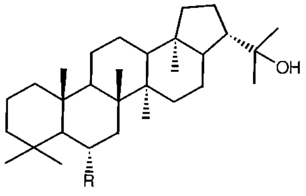
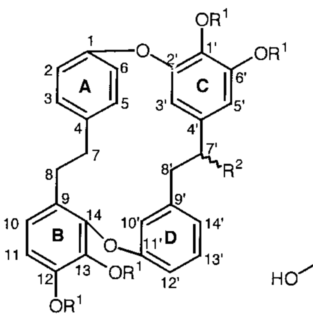
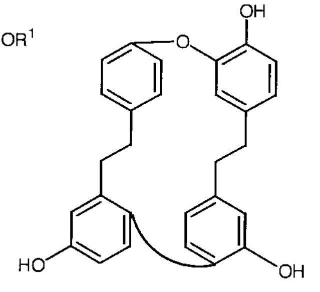


10: R= H
11: R= OH



12: R^1=H, R^2=OMe
13: R^1=R^2=H
14: R^1=Me, R^2=OMe



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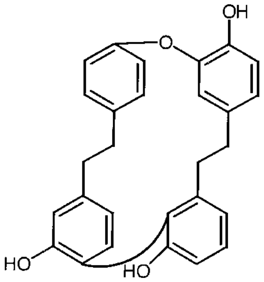

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3. Experimental

3.1. General

For separation of a mixture of the chemical constituents, a silica gel column and HPLC with a differential refractometer were used. Flow rate was 2 ml min^{-1}. Retention times were measured from the solvent peak. A mixture of CH_2Cl_2–MeOH (1:1) was used for separation with Sephadex LH-20.

3.2. Spectral data

NMR spectra were recorded at 150 or 50 MHz for ^{13}C and 600 or 200 MHz for ^{1}H. GC–MS was performed at 70 eV using a fused silica column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm).

3.3. Plant material

Plagiochasma rupestre (Forst.) Steph. was collected in February 1996 in La Heladera, Tucumán province, Argentina. A voucher specimen is on deposit at Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

3.4. Extraction and isolation

The mechanically ground air dried liverwort was successively extracted with Et_2O (15 days) and MeOH (20 days) at room temp. Evaporation of the Et_2O extract in vacuo gave a residue (9.30 g) which was chromatographed on silica gel using *n*-hexane with increasing amounts of EtOAc (0–100%) to give 4 fractions (I–V). Fr. I (1.13 g) was chromatographed on Sephadex LH-20 to give a mixture further rechromatographed on silica gel using *n*-hexane with increasing amounts of EtOAc (0–20%) to give fractions 1 to 3. HPLC of Fr. 1 with *n*-hexane–EtOAc (95:5) yielded marsupellone (**7**) (332 mg, R_t 4 min) and adiantone (**9**) (12.4 mg, R_t 8 min), Fr. 2 gave a mixture containing caryophyllene oxide (**8**) the retention time and mass spectrum (measured by GC–MS) of which were identical to those of an authentic sample. Fr. 3 gave diplopterol (**10**) (130 mg). Fr. II (1.40 g) was subjected to CC on Sephadex LH-20, followed by CC on silica gel using *n*-hexane and increasing amounts of EtOAc (10–50%) to give a new portion of **10** (84 mg), stigmasterol (71 mg) and rupestrenol (**1**) (239 mg). Fr. III (202 mg) was chromatographed on Sephadex LH-20 to give a mixture further processed by HPLC using *n*-hexane–EtOAc (80:20), to give zeorin (**11**) (46 mg, R_t 3 min). Fr IV (1.00 g) was subjected to CC on Sephadex LH-20 and the mixture further chromatographed on silica gel to give 2 fractions. Fr. 1 gave marchantin B (**13**) (630 mg) and Fr. 2 was processed by HPLC (*n*-hexane-EtOAc 70:30) to give riccardin C (**15**) (12 mg, R_t 14 min). Evaporation of MeOH extract in vacuo gave a residue (2.05 g) which was chromatographed on silica gel using *n*-hexane and increasing amounts of EtOAc (20–100%) to give 3 fractions (I–III). All fractions were separately subjected to CC on Sephadex LH-20. Fr. I was then rechromatographed on silica gel to give **10** (26 mg) and **1** (13 mg). HPLC of Fr. II (*n*-hexane-EtOAc 80:20) gave **10** (2 mg, R_t 16 min), **1** (22 mg, R_t 32 min) and phytol (7 mg, R_t 38 min). Fr. III was subjected to HPLC (*n*-hexane-EtOAc 1:1) to give isoriccardin C (**16**) (8 mg, R_t 10 min) and a mixture further subjected to HPLC (*n*-hexane-EtOAc 70:30) to give marchantin K (**12**) (5 mg, R_t 19 min).

3.5. Rupestrenol [(+)-(1R,6S*,7S*,10S*)-12-hydroxy-4,11(13)-cadinadiene] (**1**)*

Colourless solid; mp 50–52°; $[\alpha]_D$ +43.9 ($CHCl_3$; *c* 0.85). FT–IR (neat) ν_{max}: 3300, 1645, 1378, 1050 and 895 cm^{-1}. HR–MS: found 220.1820 $C_{15}H_{24}O$, requires 220.1829. EI–MS *m/z* (rel.int.): 220 $[M]^+$ (52), 202 (65), 189 (40), 187 (41), 132 (55), 121(100), 119 (72), 105 (37), 93 (48), 81 (35). ^1H- (600 MHz) and ^{13}C-NMR spectral data (150 MHz, $CDCl_3$) (see Table 1 and 2).

*3.6. Oxidation of **1** with pyridinium dichromate (PDC)*

To compound (**1**) (18 mg) in CH_2Cl_2 (5 ml) PDC (60 mg) was added and the mixture stirred for 6 h at room temperature. After purification by HPLC (*n*-hexane-EtOAc 95:5) 4,11(13)-cadinadien-12-al (**2**) (7.2 mg) was obtained. Colourless oil, FT–IR (neat) ν_{max}: 2699, 1694 and 1620 cm^{-1}, UV λ_{max} (EtOH) nm (log ε): 221 (3.80). ^1H-NMR spectrum (600 MHz, C_6D_6): δ 9.33 (*s*, H-12), 5.71 (*s*, H-13), 5.41 (*s*, H-13′), 5.17 (*t*, *J* = 1 Hz, H-5), 1.80–1.92 (5H, *m*,[1], *H*-6, *H*-8eq, H-2eq, H-3ax, H-3eq), 1.69 (*ddd*, *J* = 13, 13, 3.3 Hz, H-7), 1.56 (3*H*, *dd*, *J* = 2, 1 Hz, *H*-15), 1.56 (*dddd*, *J* = 12, 3, 3, 3Hz, *H*-9eq), 0.96–1.11 (*m*[1], *H*-8ax, *H*-9ax, *H*-10), 1.08 (*dddd*, *J* = 13, 13, 8, 1.5 *H*-2ax), 0.84 (3H, *d*, *J* = 6.5 Hz, *H*-14), 0.74 (*dddd*, *J* = 12, 12, 10, 2 Hz, H-1). ^{13}C-NMR spectrum (150 MHz, $CDCl_3$): δ 193.7 (C-12) 154.1 (C-11), 134.4 (C-4), 133.4 (C-13), 123.7 (C-5), 46.5 (C-1), 44.2 (C-6), 36.5 (C-10), 35.9 (C-9), 34.1 (C-7), 31.2 (C-3), 26.8 (C-8, C-2), 23.6 (C-15), 19.8 (C-14).

*3.7. Benzoylation of **1***

To compound **1** (25 mg) in C_5H_5N (2 ml) *p*-bromobenzoyl chloride (500 mg) was added and the mixture was stirred for 72 h at room temperature. The resulting product was purified by CC on silica gel and by HPLC (*n*-hexane–EtOAc 95:5) to give *p*-bromobenzoate (**3**) (8 mg), colourless gum, FT–IR (neat) ν_{max}: 1725, 1591, 1269, 1013, 897 and 756 cm^{-1}. ^1H-NMR spectrum (600 MHz, $CDCl_3$): δ 7.93 (2*H*, *d*, *J* = 8.5 Hz, *H*-2′, *H*-6′), 7.58 (2*H*, *d*, *J* = 8.5 Hz, *H*-3′, *H*-5′), 5.34 (*brs*, *H*-5), 5.23 (*q*, *J* = 1.5 Hz, *H*-13), 5.04 (*d*, *J* = 1 Hz, *H*-13′), 4.74 (*ddd*, *J* = 13.5, 1, 0.5 Hz, *H*-12), 4.77 (*ddd*, *J* = 13.5, 1, 0.5 Hz, *H*-12′), 1.92–2.24 (3H, *m*[1], *H*-2eq, *H*-3eq, *H*-3ax), 1.82–1.88 (2*H*, *m*[1], *H*-6, *H*-7), 1.81 (*ddd*, *J* = 13, 3.5, 3.5 Hz, *H*-8eq), 1.75 (*ddd*, *J* = 13, 3.5, 3.5 Hz, *H*-9eq), 1.62 (3 *H*, *s*, *H*-15), 1.44 (*dddd*, *J* = 13, 13, 13, 4, $H\text{-}8_{ax}$), 1.21 (*dddd*, *J* = 12, 10, 6.5, 3.5 Hz, *H*-10), 1.15 (*dddd*, *J* = 13, 13, 8, 1.5 Hz, *H*-2ax), 1.09 (*dddd*, *J* = 13, 13, 13, 3.5 Hz, *H*-9ax), 0.92 (3*H*, *d*, *J* = 6.5 Hz, *H*-14), 0.78 (*dddd*, *J* = 12, 12, 10, 2.5 Hz, *H*-1). ^{13}C-NMR spectrum (150 MHz, $CDCl_3$): δ 165.5 (C′=O), 146.8 (C-11), 134.6 (C-4), 131.7 (C-3′, C-5′), 131.2 (C-2′, C-6′) 129.2[2] (C-1′), 128.0[2] (C-4′), 123.6 (C-5), 113.2 (C-13), 66.8 (C-12), 47.9 (C-7), 46.5 (C-1), 44.1 (C-6), 36.3 (C-10), 35.9 (C-9), 33.9 (C-8), 30.9 (C-3), 26.5 (C-2), 23.5 (C-15), 19.6 (C-14).

*3.8. 2,4-Dinitrophenylhydrazone of **2***

To **2** (6 mg) in EtOH, was added dropwise 1 ml of a solution that contains 2,4-dinitrophenylhydrazine (150 mg) dissolved in H_2SO_4 (1 ml), EtOH (3.3 ml) and H_2O (7.6 ml). An orange solid precipitated immediately. After filtration and purification by CC on silica gel using C_6H_6, the hydrazone (**4**) was recrystallized from C_6H_6.

*3.9. X-ray crystal data of **4***

Data collection: MXC (MAC Science). Cell refinement: MXC (MAC Science). Data reduction: CRYSTAN. Program used to solve structure: CRYSTAN SIR 92. Refinement: Full matrix least square. Diffractometer: Mac Science MX C18. $C_{21}H_{26}O_4N_4$, MW = 398.00, Triclinic, *P*1, a = 6.082 (7) Å, b = 7.495 (3) Å, *c* = 23.03 (1) Å, V = 1033.93 (1) $Å^3$, Z = 2, *D*x = 1.278 Mg m^{-3}, *D*m = 1.300 Mg m^{-3}, Cu–Kα *radiation*, $\lambda = 1.54178$ Å, $\theta = 20 - 25°$, $\mu = 7.003$ mm^{-1}, Refractions: 1570, Parameters: 520, *R*int = 0.0061, *R* = 0.101, $\omega R = 0.127$, $S = 2.092$.

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[1] Obscured due to overlapping signals.

[2] Interchangeable carbons.

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