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A 7-nordumortenone and other dumortane derivatives from the Argentine liverwort *Dumortiera hirsuta*

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Abstract

Five sesquiterpenes with the novel skeletal type dumortane, four of them new and a known cyclic bisbibenzyl compound were isolated together with other common plant constituents from an Argentine collection of the liverwort *Dumortiera hirsuta*. Their structures were established by spectral analysis.

Keywords: *Dumortiera hirsuta*; Wiesnerellaceae; Hepaticae; Liverwort; Sesquiterpene; Dumortane-type; Nordumortane-type

1. Introduction

Our previous and recent investigations on the thallic liverwort *Dumortiera hirsuta* (Marchantiales) from wet places of the rocky mountains of northwestern Argentina have led to the isolation of two new sesquiterpenoids with a novel skeleton, for which we proposed the name 'dumortane' (Toyota, Bardón, Kamiya, Takaoka, & Asakawa, 1997). These substances only seem to be present in the Argentine collections since, the same species from Japan, furnished sesquiterpene derivatives with other skeletal types (Matsuo, Uto, Nakayama, & Hayashi, 1976), cyclic bisbibenzyls and common plant constituents (Asakawa, 1995; Toyota, Yoshida, Matsunami, & Asakawa, 1997). In the present article, we describe the results of a reinvestigation of the Argentine *D. hirsuta* from which dumortenol (**1**) and two new dumortane derivatives **2** and **3**, a rearranged dumortane-type **4** and a nordumortane-type sesquiterpene **5** were isolated in trace amounts, as well as the cyclic bisbibenzyl marchantin C (**6**), phytol, stigmasterol and the sesquiterpenes β-barbatene and caryophyllene oxide.

2. Results and discussion

The ether extract of *D. hirsuta* furnished a crystalline compound isolated from a previous collection and assigned structure **1** (Toyota et al., 1997) by spectroscopic and X-ray crystallographic analysis. Additionally, two new dumortane-type **2** and **3**, a rearranged dumortane-type **4** and a nordumortane-type sesquiterpene **5** were isolated. Sesquiterpenes possessing a five and an eight membered ring in the molecule, with skeletons named asteriscane and precapnellane, were previously found in the aromatic shrub *Lippia integrifolia* (Griseb.) Hieron (Verbenaceae) (Catalán, De Lampasona, Cerda-García-Rojas, & Joseph-Nathan, 1995), in *Asteriscus aquaticus* L. (Compositae) (San Feliciano et al., 1985) and in soft coral, *Capnella imbricata* (Ayanoglu, Gebreyesus, Beechan, & Djerassi, 1979) but asteriscane- and precapnellane-type skeletons differ from dumortane in the methyl substitution pattern. Compound **2** is a ketone which showed a molecular ion at *m/z* 218. Its HR-mass spectrum indicated a molecular formula $C_{15}H_{22}O$ accounting for five degrees of unsaturation. The FT-IR spectrum showed a band at 1691 cm^{-1} for an α,β-unsaturated ketone and a double bond absorption at 1618 cm^{-1} indicating the

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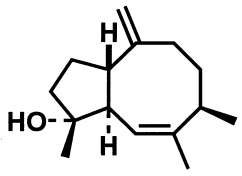

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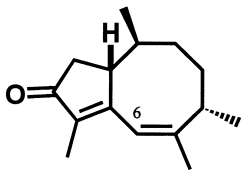

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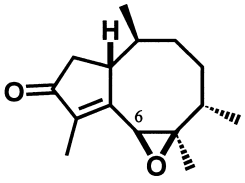

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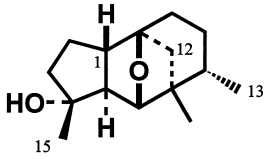

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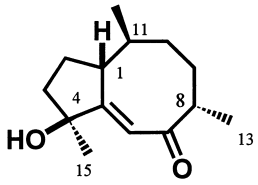

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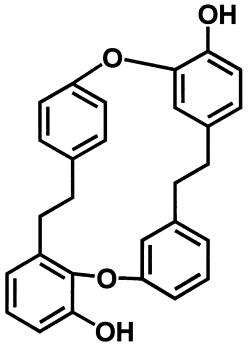

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presence of an additional γ,δ-conjugated double bond. Accordingly, the ^{13}C NMR spectrum disclosed a carbonyl signal at δ 208.6 together with four olefinic carbons at δ 167.2, 149.9, 136.1 and 123.6. Comparison of the ^{1}H NMR and ^{13}C NMR spectra of **2** with those of **1** suggests the same gross structure for both. In the ^{1}H NMR spectrum of **2**, four methyl signals at δ 1.88, 1.77, 1.10 and 1.08 are present and the only vinyl doublet at δ 6.59 assigned to H-6 is 1 ppm down field compared to the corresponding signal in the ^{1}H NMR spectrum of **1**. Therefore, compound **2** has a 4,7,8,11-tetramethylbicyclo[6.3.0]undecane skeleton and, consequently, is also a dumortane-type sesquiterpenoid. Total assignments of all protons and carbons of **2** (Table 1) were supported by COSY, HSQC and HMBC spectra. The proposed stereochemistry at C-1, C-8 and C-11 was established by the NOESY spectrum (Fig. 1). Ring conformations shown in Fig. 1 are also supported by the observed coupling constants $J_{8,9\alpha} = 13$, $J_{8,9\beta} = 6$, $J_{11,1} \sim 8$, $J_{11,10\beta} = J_{11,10\alpha} \sim 3$, $J_{9\alpha,10\alpha} = J_{9\alpha,10\beta} = 3$, $J_{9\beta,10\alpha} = 13.5$, $J_{9\beta,10\beta} = 3$ Hz, $J_{1,2\beta} = 6.5$ Hz and $J_{1,2\alpha} = 0.8$ Hz in the ^{1}H NMR spectrum. Compound **3** is the 6,7 epoxy derivative of **2**. The structure of **3** was easily inferred from its ^{1}H NMR and ^{13}C NMR spectra (Table 2) in comparison with those of **2**. Two signals at δ 65.2 and 60.9 due to oxygenated carbons and the lack of the trisubstituted double bond signals in the ^{13}C NMR spectrum together with the presence of a singlet at δ 3.72 and the absence of vinyl protons in the ^{1}H NMR spectrum led to the proposed structure of **3**. The NOESY spectrum evidenced the β-configuration of the epoxy ring

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

Atom	C-Type	δ_C	δ_H	*J* (Hz)	HMBC correlation
1	CH	47.1	3.17 (β)	*brt* (8)	43.8, 208.6, 136.1, 167.2, 40.5, 19.3
2	CH_2	43.8	2.62 (β)	*dd* (18.4, 6.5)	47.1, 208.6, 167.2, 40.5
			2.00 (α)	*dd* (18.7, 0.8)	47.1, 208.6, 136.1, 40.5
3	C	208.6			
4	C	136.1			
5	C	167.2			
6	CH	123.6	6.59	*d* (1.6)	47.1, 136.1, 167.2, 149.8, 34.7, 22.2
7	C	149.9			
8	CH	34.7	3.28 (β)	*dquint* (13, 6)	123.6, 149.8, 29.2, 22.2
9	CH_2	29.2[a]	1.79 (β)	*tdd* (13.5, 5, 3)	
			1.11 (α)	*tt* (13.2, 3)	
10	CH_2	29.3[a]	1.83 (α)	*tt* (14, 3)	
			1.32 (β)	*dq* (13.5, 3)	40.5
11	CH	40.5	1.42 (α)	*tquint* (3, 7)	
12	CH_3	19.4	1.08 (β)	*d* (7)	
13	CH_3	19.2	1.10 (α)	*d* (7)	
14	CH_3	22.2	1.88	*s*	149.8, 34.7
15	CH_3	8.3	1.77	*d* (1.6)	

[a] Interchangeable.

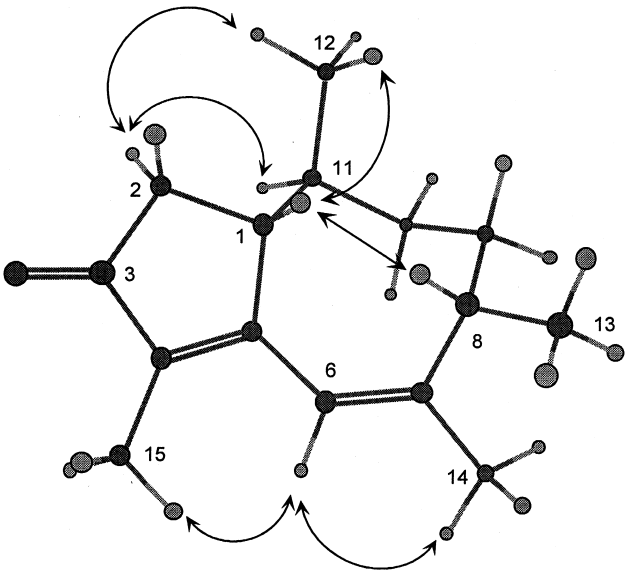

Fig. 1. Partial NOEs observed for compound **2**.

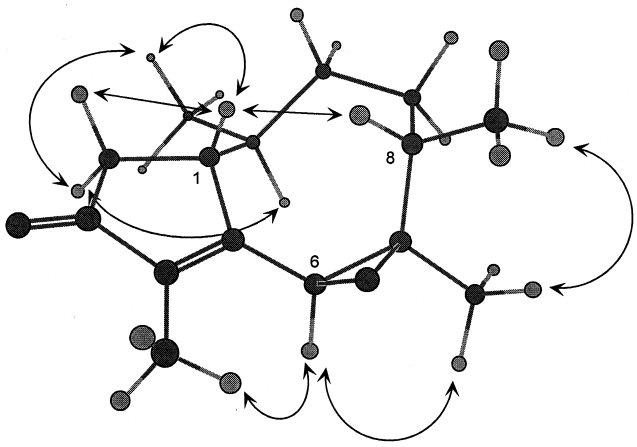

Fig. 2. Partial NOEs observed for compound **3**.

as shown in Fig. 2. Compound **4** showed a molecular ion of m/z 236, ascribable to $C_{15}H_{24}O_2$, which accounts for four degrees of unsaturation and a peak at m/z 218 $[M\text{-}18]^+$ suggesting the presence of a hydroxyl group in the molecule. Since the ^{13}C NMR spectrum showed neither signals for double bonds nor carbonyl carbons, the compound must be tetracyclic. The ^1H NMR spectrum disclosed a doublet and two singlet methyls as well as a doublet methine proton signal at δ 3.50 (d, $J = 11$ Hz). A further HMBC experiment confirmed the position of the tertiary methyl group at C-4 and the oxygenated methine at C-6. Signals at δ_H 1.78 and 1.11 characteristic of an AB system together with the absence of the Me-12 signal indicated that C-12 was involved in a ring leading to a tricarbocyclic skeleton derived from a dumortane precursor. The proposed stereochemistry is supported by NOE difference spectra which showed that H-1 and Me-15 were β-oriented while H-5, H-12α, H-12β and Me-13 were α-oriented (Fig. 3). Total assignments of all protons and carbons of **4** (Table 3) were supported by COSY, HSQC and HMBC spectra.

Compound **5** gave a molecular ion peak at m/z 222 suggesting the molecular formula to be $C_{14}H_{22}O_2$ which accounts for four degrees of unsaturation and a peak at m/z 204 $[M\text{-}18]^+$ which suggests the presence of a hydroxyl group. Its IR spectrum showed a broad band at 1651 cm^{-1} for an α,β-unsaturated ketone as well as a broad band at 3447 cm^{-1} for the hydroxyl group. The absence of a signal for Me-14 agreed with

Table 2
^1H and ^{13}C NMR data of compound **3** ($CDCl_3$)

Atom	C-Type	δ_C	δ_H	J (Hz)	HMBC correlation
1	CH	44.8	2.89 (β)	*brt* (8)	146.4, 166.8, 208.3, 43.7, 41.4, 18.9
2	CH_2	43.7	2.59 (β)	*dd* (18.4, 6)	208.3, 166.8, 44.8, 41.4
			2.06 (α)	*brd* (18.7)	208.3, 166.8, 146.4, 44.8, 41.4
3	C	208.3			
4	C	146.4			
5	C	166.8			
6	CH	60.9	3.72	*s*	166.8, 146.4, 65.1, 44.8, 17.1
7	C	65.2			
8	CH	36.0	2.03 (β)	*dquint* (13, 6)	65.1, 29.5, 28.7, 18.0, 17.1
9	CH_2	28.7	1.79 (β)	*tdd* (14, 5, 3)	65.1, 36.0, 28.7
			1.36 (α)	*tt* (13.5, 3)	36.0
10	CH_2	29.5	1.87 (α)	*tt* (14, 3)	
			1.42 (β)	*dq* (14, 3)	44.8, 41.4, 36.0, 29.5
11	CH	41.4	1.49 (α)	*tquint* (3, 7)	44.8, 29.5, 28.7
12	CH_3	18.9	1.05 (β)	*d* (7)	44.8, 41.4, 28.7
13	CH_3	18.0	1.10 (α)	*d* (6.6)	65.1, 36.0, 29.5
14	CH_3	17.1	1.30 (α)	*s*	65.1, 60.9, 36.0
15	CH_3	8.3	1.90	*d* (1.9)	208.3, 166.8, 146.4

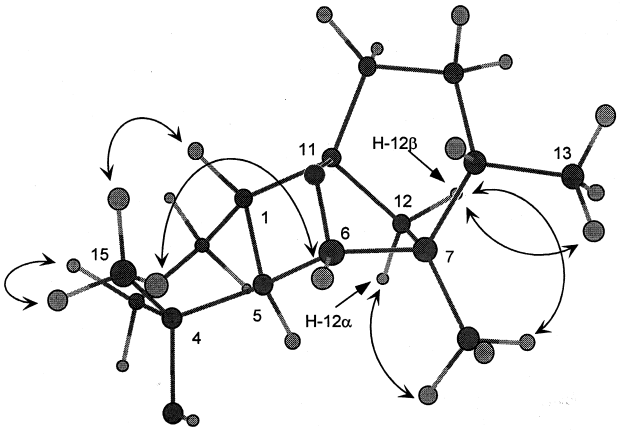

Fig. 3. Partial NOEs observed for compound **4**.

the molecular formula and the ^{13}C NMR spectrum showing only 14 signals suggested that this was a norsesquiterpene. The presence of a carbonyl carbon and a trisubstituted double bond was evident in the signals at δ 205.3 for the carbonyl at C-7, δ 167.5 for C-5 and δ 127.4 for C-6 from the ^1H NMR and ^{13}C NMR spectrum. Since there are two double bonds, the compound should be bicyclic. The HMQC spectrum of **5** allowed us to assign unambiguously the signals of all the remaining carbons. Thus the methine signals at δ 47.2, 42.4 and 36.9 were assigned to C-1, C-8 and C-11, the aliphatic methylene signals at δ 39.5, 29.8, 29.7 and 28.3 were assigned to C-3, C-2, C-10 and C-9, respectively, while the three methyl signals at 26.8, 17.9 and 17.0 were assigned to C-15, C-12 and C-13, respectively. The ^1H NMR spectrum exhibited one vinyl proton signal at δ 6.36 (d, $J = 2.5$ Hz) as well as a singlet and two doublet methyl signals at δ 1.07, 1.12 and 1.33 respectively. The signal for H-8 at δ 3.12 is similar in shape to the signal corresponding to this proton in the ^1H NMR spectra of **2** and **3**. A further HMBC experiment confirmed the correlation between the C-6 vinyl proton whose position was also inferred by the allylic coupling with H-1 and C-4, C-5 and C-8. The gross structure of **5** was related to the known constituent **1** resulting to be a nordumortane-type sesquiterpenoid. Complete assignment of all protons was achieved by COSY, HSQC, HMBC (Table 4) spectra. The NOESY spectrum of **5** allowed us to infer the proposed stereochemistry at C-1, C-4, C-8 and C-11 (Fig. 4). It is important to point out that the NOESY correlation between Me-15 and H-3α and H-11 (α-oriented) of compound **5** supports the proposed stereochemistry at C-4 which differs from that proposed for compounds **1** and **4**. From the biogenetic point of view, the hydroxyl group at C-4 is probably formed from the corresponding 4,5-epoxide derivative which can be α or β-oriented at C-4 according to the proposed biosynthetic scheme shown in Fig. 5.

It is noteworthy that the relative stereochemistry at C-8 of **1** differs from the stereochemistry proposed here for compounds **2**–**5** suggesting that in the biosynthetic step of methyl group migration from C-7 Fig. 5 both methyl groups are able to migrate leading to a different final stereochemistry at C-8.

Table 3
^1H and ^{13}C NMR data of compound **4** ($CDCl_3$)

Atom	C-Type	δ_C	δ_H	J (Hz)	HMBC correlation
1	CH	51.1	1.60–1.70 (β)[a]		
2	CH_2	22.0	1.60–1.70[a]		
			1.70–1.80[a]		
3	CH_2	39.9	1.70–1.80[a]		
			1.95 (α)	*dbr* (12)	
4	C	78.6			
5	C	55.6	2.30 (α)	*dd* (13.5, 11)	22.0, 51.1, 78.6, 79.4
6	CH	79.4	3.50 (α)	*d* (11)	25.8, 28.6, 42.1, 55.6, 78.6
7	C	42.1			
8	CH	28.6	2.06–2.12[a]		
9	CH_2	30.8	2.06–2.12[a]		
			1.42	*dddbr* (11, 7, 4)	
10	CH_2	30.2	1.70–1.80[a]		
			1.92–1.98[a]		
11	CH	70.5			
12	CH_2	46.5	1.11 (H-12α)	*dd* (12.7, 1.7)	28.6, 42.1, 51.1, 70.5
			1.78 (H-12β)	*d* (12.7)	
13	CH_3	18.8	0.98 (α)	*d* (7)	28.6, 30.8, 42.1
14	CH_3	25.8	1.04 (β)	*s*	28.6, 42.1, 46.5, 79.4
15	CH_3	24.4	1.32 (β)	*s*	25.8, 39.9, 55.6, 78.6

[a] Obscured due to overlapping signals.

Table 4
^1H and ^{13}C NMR data of compound **5** (C_6D_6)

Atom	C-Type	δ_C	δ_H	*J* (Hz)	HMBC correlation
1	CH	47.2	3.19 (β)	*brqd* (9, 2.5)	167.5, 36.9, 17.8
2	CH_2	29.8[a]	2.25 (β)	*ddt* (12.4, 4, 8)	80.4
			1.38–1.45[b]		
3	CH_2	39.5	1.9 (α)	*ddd* (12, 7, 4)	
			1.7[b]		
4	C	80.5			
5	C	167.5			
6	CH	127.4	6.36	*d* (2.5)	167.5, 80.4, 47.2, 42.4
7	C	205.3			
8	CH	42.4	3.12 (β)	*dquint* (12.5, 6.5)	205.3, 17.0, 28.3
9	CH_2	28.3	1.85 (β)	*tdd* (13.7, 6.5, 3.5)	
			1.24 (α)	*ddt* (13.7, 12.5, 4)	
10	CH_2	29.7[a]	1.69 (β)	*tt* (14.5, 4)	
			1.38–1.45[b]		
11	CH	36.9	1.51 (α)	*tquint* (3.5, 7)	
12	CH_3	17.9	1.12 (β)	*d* (7) 47.2, 36.9	
13	CH_3	17.0	1.07 (α)	*d* (6.3)	205.3, 28.3, 42.4
Missing					
15	CH_3	26.8	1.33(α)	*s*	167.5, 80.4

[a] Interchangeable.
[b] Obscured due to overlapping signal.

3. Experimental

3.1. General

For TLC detection, Godin reagent (Godin, 1954) was used. For sepn of mixts an HPLC equipped with differential refractometer, a silica gel and a reverse phase C18 columns were employed. The mixt. CH_2Cl_2–MeOH (1:1) was used for sepns with Sephadex LH-20.

3.2. Spectral data

NMR spectra were recorded at 150 MHz for ^{13}C and 600 MHz for ^1H. GC–MS were 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

Dumortiera hirsuta (Sw.) Nees (453 g) was collected in February 1995 in San Javier, Tucumán province, Argentina. A voucher specimen HAB # 3 is on deposit at Fundacion Miguel Lillo, Tucumán.

3.4. Extraction and isolation

The mechanically ground air dried liverwort was successively extracted with Et_2O (30 days) and MeOH (15 days) at room temp. Evapn of the Et_2O extract at red. pres. gave 4.013 g of residue (yield 0.89%) which was chromatographed on silica gel using an *n*-hexane–EtOAc gradient, to give 7 frs (I–VII). Fr. III (290.1 mg) gave a mixture of sesquiterpene hydrocarbons in which β-barbatene and caryophyllene oxide were identified by GC-mass analysis as major constituents. Fr. IV (142.2 mg) was rechromatographed on Sephadex LH-20 and further processed by HPLC on silica gel (*n*-hexane–EtOAc 80–20, 2 ml min^{-1}) to give 3.8 mg of **1**, 4.6 mg of **2** and 13.2 mg of phytol. Fr. V (135.4 mg) was rechromatographed on Sephadex LH-20 and further processed by HPLC on silica gel (*n*-hexane–EtOAc 80–20, 2 ml min^{-1}) to yield 8.2 mg of **6** (marchantin C), 9.1 mg of stigmasterol and 3.0 mg of **3**. Fr. VI was chromatographed by HPLC using a C18

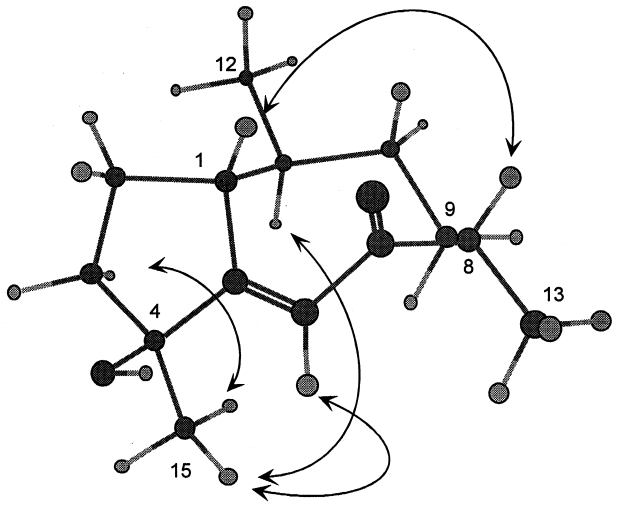

Fig. 4. Partial NOEs observed for compound **5**.

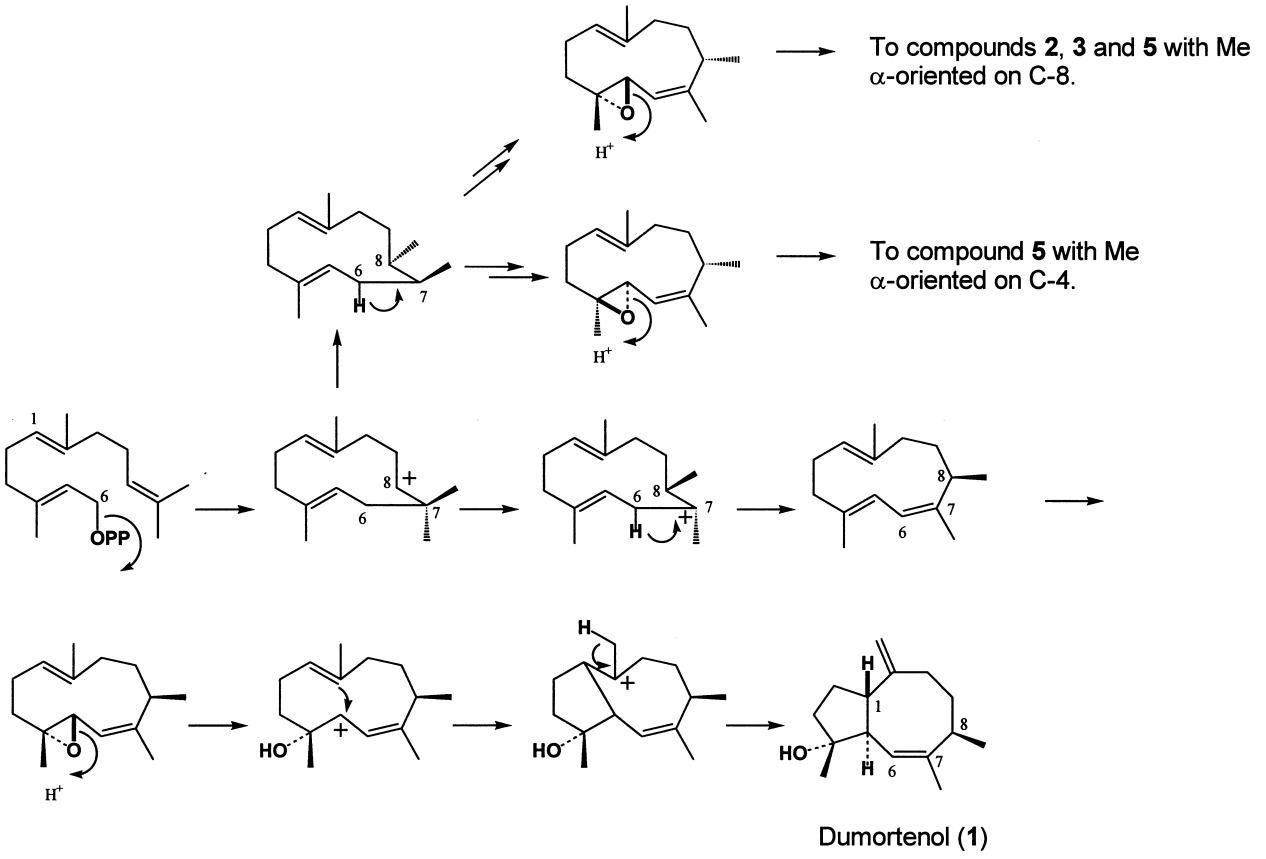

Fig. 5. Proposed biogenesis of dumortane-type compounds from *trans*-farnesylpyrophosphate.

column (MeOH–H_2O 3:2, flow rate 2 ml min^{-1}) to give 8 mg of **5**. Fr. VII contained **4** as major compound purified by HPLC in a C18 column (MeOH–H_2O 1:1, flow rate 1.2 ml min^{-1}) to furnish 3.1 mg of **4**. From the methanolic extract only a new portion of marchantin C (6.8 mg) was obtained.

*3.4.1. Compound **2***

Oil, IR ν_{max} cm^{-1}: 1691, 1618; HRMS. found: $[M]^+$218.1676; $C_{15}H_{18}O$ requires 218.1671; MS *m/z* (rel. int.): 218 (100), 203 (23), 189 (26), 175 (44), 161 (60), 147 (44), 133 (36), 119 (48), 105 (32), 91 (36), 77 (22).

*3.4.2. Compound **3***

Oil, IR ν_{max} cm^{-1}: 1705, 1628, 1260, 1202; MS *m/z* (rel. int.): 234 (10), 219 (2), 216 (2), 205(9), 191 (7), 177 (18), 163 (32), 149 (20), 135 (30), 121 (32), 107 (34), 93 (32), 79 (44), 69 (58), 55 (78), 43 (100).

*3.4.3. Compound **4***

Oil, IR ν_{max} 3451, 1207 cm^{-1}: MS *m/z* (rel. int.): 236 (31), 218 (47), 178 (21), 160 (27), 147 (19), 127 (88), 125 (100), 110 (42), 109 (68), 95 (21), 81 (56), 67 (18), 55 (24), 43 (61).

*3.4.4. Compound **5***

Oil, IR ν_{max} cm^{-1}: 3447, 1651; MS *m/z* (rel. int.): 222 (52), 207 (26), 204 (24) 193 (22), 180 (20), 179 (100), 166 (72), 165 (100), 147 (43), 133 (36), 132 (34), 121 (38), 119 (45), 107 (31), 95 (32), 93 (54).

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