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New Diterpenoids from the Alga *Dictyota dichotoma*

Rosario Durán, Eva Zubía, María J. Ortega, and Javier Salvá*

Departamento de Química Orgánica, Facultad de Ciencias del Mar, Apdo. 40, 11510 Puerto Real, Cádiz, SPAIN.

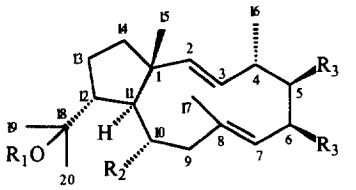
Abstract: The marine alga *Dictyota dichotoma* from Cortadura (Cádiz, Spain) contains, in addition to nineteen known diterpenes (**8-26**), the new dolabellanes, (1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-5,6,18-triacetoxy-10-hydroxy-2,7-dolabelladiene (**1**), (1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-18-acetoxy-10-hydroxy-2,7-dolabelladiene (**2**), (1*R*,2*E*,4*S*,5*R*,7*E*,10*S*,11*S*,12*R*)-5-acetoxy-10,18-dihydroxy-2,7-dolabelladiene (**3**), (1*R**,3*E*,7*S**,8*S**,11*S**,12*R**)-7,8-epoxy-3,18-dolabelladiene (**4**), and (1*R**,2*E*,4*R**,7*E*,11*S**,12*R**)-18-acetoxy-2,7-dolabelladiene (**5**) together with two new hydroazulene diterpenes, isopachydictyol A (**6**) and dictyotatriol A (**7**). The structures of the new compounds were elucidated by interpretation of spectroscopic data and the absolute configurations of compounds **3**, **7** and **10** were established using the Mosher's method and of compounds **1**, **2**, and **11** by chemical interconversions. The new diterpenes isolated from *D. dichotoma* showed mild cytotoxicity against four tumor cell lines excepting compounds **1** and **7** that were inactive. Dolabellane **2** exhibited the greatest activity.

Marine algae have been the group of organisms that has received more attention of marine natural products chemists over the last 25 years. However, since many of the algal metabolites were described before the current pharmacological bioassays were available the potencial of most of these metabolites remains unexplored.[1]

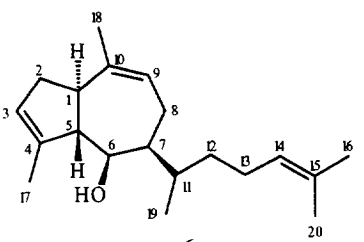
Brown algae of the Dictyotaceae Family have given rise to a great number of diterpenes generally grouped in three types: xenicanes, extended sesquiterpenes, and dolabellanes. *Dictyota dichotoma* is a member of this Family which has been extensively studied affording diterpenes of these three groups although these studies have noted a wide range of variations among its constituents depending upon time and location of collection.[2]

In the development of our research project directed towards the search for biologically active compounds from marine organisms of the southern coast of Spain we collected specimens of the brown alga *Dictyota dichotoma* (Huds.) Lamouroux in Cortadura (Cádiz, Spain). Our specimens contained five new dolabellane diterpenes (**1-5**), two new hydroazulenoid diterpenes (**6**, **7**) together with nineteen known diterpenes (**8-26**).

Specimens of *D. dichotoma* (62 g dry wt) were collected by hand and immediately frozen. The less polar material of an acetone extract was chromatographed on silica gel. Final purification of selected fractions using normal phase HPLC allowed isolation of the following compounds: (1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-



1 R_1 = Ac; R_2 = OH; R_3 = OAc
2 R_1 = Ac; R_2 = OH; R_3 = H
5 R_1 = Ac; R_2 = R_3 = H
8 R_1 = H; R_2 = OH; R_3 = OAc
9 R_1 = H; R_2 = R_3 = OAc
10 R_1 = H; R_2 = OH; R_3 = H
10a R_1 = H; R_2 = (*R*)-OMTPA; R_3 = H
10b R_1 = H; R_2 = (*S*)-OMTPA; R_3 = H
11 R_1 = H; R_2 = OAc; R_3 = H
12 R_1 = R_2 = R_3 = H
27 R_1 = H; R_2 = R_3 = OH



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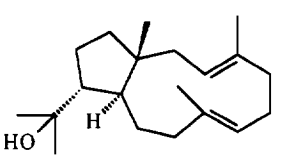

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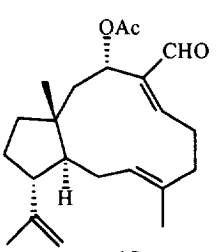

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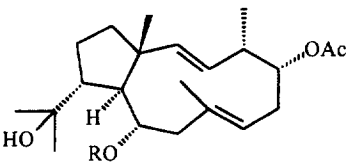

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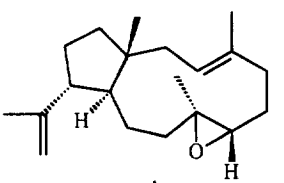
3a R = (*R*)-MTPA
3b R = (*S*)-MTPA



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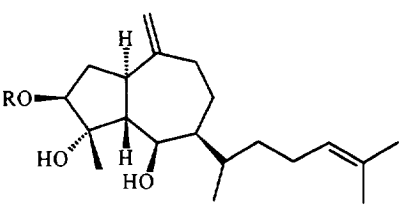
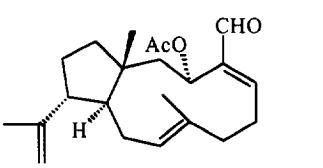


7 R = H
7a R = (*R*)-MTPA
7b R = (*S*)-MTPA



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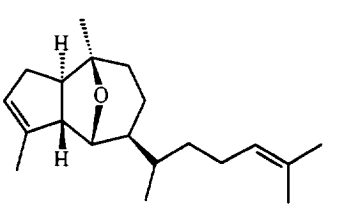

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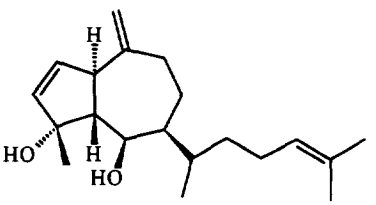

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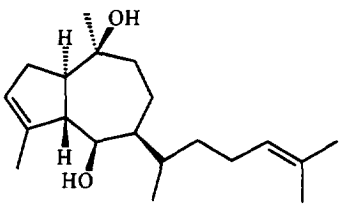

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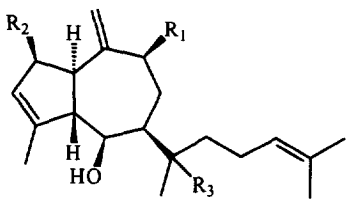
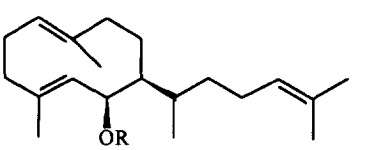
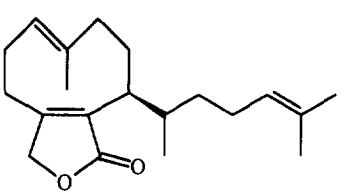


19 $R_1 = R_2 = R_3 = H$
20 $R_1 = OAc$; $R_2 = R_3 = H$
21 $R_1 = R_3 = H$; $R_2 = OAc$
22 $R_1 = R_2 = H$; $R_3 = OH$



23 R = H
24 R = Ac



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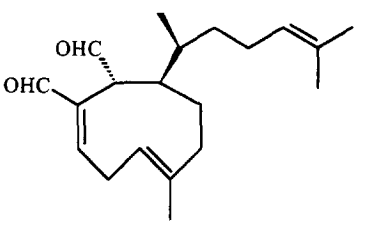

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5,6,18-triacetoxy-10-hydroxy-2,7-dolabelladiene (**1**, 0.140% dry wt), (1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-18-acetoxy-10-hydroxy-2,7-dolabelladiene (**2**, 0.018% dry wt), (1*R*,2*E*,4*S*,5*R*,7*E*,10*S*,11*S*,12*R*)-5-acetoxy-10,18-dihydroxy-2,7-dolabelladiene (**3**, 0.021% dry wt), (1*R**,3*E*,7*S**,8*S**,11*S**,12*R**)-7,8-epoxy-3,18-dolabelladiene (**4**, 0.009% dry wt), (1*R**,2*E*,4*R**,7*E*,11*S**,12*R**)-18-acetoxy-2,7-dolabelladiene (**5**, 0.016% dry wt), isopachydictyol A (**6**, 0.010% dry wt), and dictyotatriol A (**7**, 0.031% dry wt), together with the known compounds **8-26** listed in Table 1.

Compound **1** was isolated as a colorless oil. The molecular formula, $C_{26}H_{40}O_7$, was obtained from the high resolution mass measurement. The absorption at 1738 cm^{-1} together with the ^1H NMR signals at δ 2.14 (s, 3H), 2.00 (s, 3H), and 1.99 (s, 3H) indicated the presence of three acetoxyl groups in the structure of **1** and the IR absorption at 3550 cm^{-1} indicated that compound **1** was an alcohol. These functionalities together with the presence of two double bonds which gave rise to the ^{13}C NMR signals at δ 137.5 (d), 136.1 (s), 127.4 (d), and 124.2 (d) suggested that **1** was a bicyclic diterpene. A careful analysis of ^1H and ^{13}C NMR, COSY,

Table 1. Known Compounds Isolated from *Dictyota dichotoma*.

#	Compound	% dry wt	Ref.
8	(1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-5,6-diacetoxy-10,18-dihydroxy-2,7-dolabelladiene	0.056	3
9	(1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-5,6,10-triacetoxy-18-hydroxy-2,7-dolabelladiene	0.031	3
10	(1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-10,18-dihydroxy-2,7-dolabelladiene	0.011	4,5
11	(1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-10-acetoxy-18-hydroxy-2,7-dolabelladiene	0.071	4,6
12	(1*R**,2*E*,4*R**,7*E*,11*S**,12*R**)-18-hydroxy-2,7-dolabelladiene	0.009	4,7
13	(1*R**,3*E*,7*E*,11*S**,12*R**)-18-hydroxy-3,7-dolabelladiene	0.006	8
14	(1*R*,3*S*,4*E*,8*E*,11*S*,12*R*)-3-acetoxy-4,8,18-dolabellatrien-16-al	0.015	9
15	(1*R*,3*S*,4*Z*,8*E*,11*S*,12*R*)-3-acetoxy-4,8,18-dolabellatrien-16-al	0.043	9,10
16	dictyoxide	0.006	11
17	dictyotadiol	0.295	12,13
18	dictyol C	0.026	14
19	pachydictyol A	0.048	15
20	dictyol B acetate	0.003	12,16
21	dictyol D acetate	0.015	17
22	dictyol E	0.077	14
23	dilophol	0.113	18,19
24	dilophol acetate	0.004	20
25	neodictyolactone	0.006	21
26	dictyodial	0.403	22,23

HETCOR, and ^{1}H-^{13}C long range experiments demonstrated that **1** possessed a dolabellane carbon skeleton.

The singlets at δ 1.57 (s, 3H) and 1.52 (s, 3H) showed long range couplings with the ^{13}C NMR singlet at δ 86.7 and with the doublet at δ 47.7 indicating the presence of an isopropyl acetate moiety attached to C-12. The singlet at δ 0.99 (s, 3H) was assigned to the angular methyl group Me-15 of the dolabellane system.

The ^{13}C NMR singlet at δ 136.1 and a doublet at δ 124.2 that was correlated in the HETCOR experiment with the ^{1}H NMR signal at δ 5.38 (br d, J = 10.0 Hz, 1H) together with the vinylic methyl signals at δ 1.74 (br s, 3H) in the ^{1}H NMR spectrum and at δ 21.3 (s) in the ^{13}C NMR spectrum were assigned to an *E*-trisubstituted double bond bearing a methyl group. The olefinic proton signal at δ 5.38 showed a cross peak in the COSY spectrum with the signal at δ 5.66 (dd, J = 10.0 and 1.8 Hz, 1H) which, in addition, showed coupling with the signal at δ 5.15 (dd, J = 6.0 and 1.8 Hz, 1H) indicating the presence of two secondary acetoxyl groups in α,β to the double bond. The second double bond present in the structure of **1** gave rise to the ^{1}H NMR signals at δ 5.49 (dd, J = 16.5 and 5.0 Hz, 1H) and 5.04 (dd, J = 16.5 and 1.3 Hz, 1H) consistent with an *E*-disubstituted double bond. The olefinic proton signal at δ 5.49 showed a cross peak in the COSY spectrum with the signal at δ 2.63 (m, 1H) which was, in addition, coupled with the methyl signal at δ 1.09

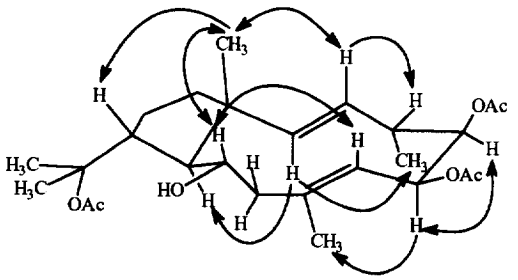
(d, J = 7.2 Hz, 3H) and with the signal at δ 5.15 mentioned above. The long range couplings observed between the olefinic carbon signal at δ 137.5 and the Me-15 signal at δ 0.99 in the ^{1}H-^{13}C long range experiment indicated that the disubstituted double bond was located in α,β to the C-1 bridgehead carbon atom. Finally, the hydroxyl group was located at C-10 on the eleven membered ring because the geminal proton signal at δ 3.50 (br dd, J = 12.1 and 12.1 Hz, 1H) showed cross peaks in the COSY spectrum both with the vinylic methylene signals at δ 2.52 (br d, J = 11.7 Hz, 1H) and 1.90 (dd, J = 12.1 and 11.7 Hz, 1H) and with the bridgehead methine proton at δ 1.47 (d, J = 10.5 Hz, 1H).

A series of NOE difference spectroscopy experiments provided confirmation of the proposed structural assignments and defined the relative stereochemistry of dolabellane **1** (Figure 1). Irradiation of the Me-15 signal caused enhancements of the H-10 and H-12 signals indicating a *cis* orientation of H-11 with respect tothe adjacents acetoxy-isopropyl and hydroxyl groups and the *trans* fusion of the two rings of the dolabellane. Irradiation of the H-6 signal enhanced the Me-17 and H-5 signals and, irradiation of the H-3 signal produced enhancement of Me-15 and H-4 signals defining the relative stereochemistry on the eleven membered ring of compound **1**. Treatment of **1** with lithium aliminium hydride afforded a tetrol **27** identical in all respects to the reduction product of the known compound **8** whose absolute stereochemistry had been determined using the Horeau's method.[3] It was concluded that compound **1** had an absolute stereochemistry as that depicted in formula **1** and that, therefore, belongs to the same enantiomeric series as the known dolabellane **8**.

Compound **2** was isolated as a colorless oil. The molecular formula, $C_{22}H_{36}O_3$, was obtained from the high resolution mass measurement. The IR absorptions at 3450 cm^{-1} and 1735 cm^{-1} together with the ^{1}H NMR signal at δ 2.01 (s, 3H) indicated that **2** contained an acetoxyl and a hydroxyl group. These data together with a general examination of the spectroscopic data indicated that **2** was a dolabellane diterpene.

The methyl proton signals at δ 1.58 (s, 3H) and 1.56 (s, 3H) together with the ^{13}C NMR singlet at δ 87.0 indicated the presence of an isopropyl acetate on C-12 similar to compound **1** above discussed. Two double bonds with identical location and stereochemistry as those of **1** were proposed upon observation of the ^{1}H NMR signals at δ 5.19 (dd, J = 16.0 and 7.6 Hz, 1H) and 5.05 (d, J = 16.0 Hz, 1H) assigned to the C-2, C-3 double bond and at δ 1.61 (br s, 3H) and 4.99 (br dd, J = 7.8 and 7.5 Hz, 1H) assigned to the methyl substituted C-7,C-8 double bond. The signal at δ 4.99 showed a cross peak in the COSY spectrum with the methylene proton signal at δ 2.15 (m, 2H) which was in addition coupled with the methylene proton signals at δ 1.60 (m, 1H) and 1.48 (m, 1H) indicating that **2** lacked the two acetoxyl groups on C-5 and C-6. The doublet on the ^{13}C NMR spectrum at δ 68.9 was due to the presence of a secondary hydroxyl group whose geminal proton appeared at δ 3.43 (ddd, J = 12.1, 11.1 and 2.0 Hz, 1H) and which was coupled with the allylic methylene protons at δ 2.24 (br d, J = 11.8 Hz, 1H) and 2.00 (m, 1H) and with the methine bridgehead proton at δ 1.70 (dd, J = 10.8 and 2.0 Hz, 1H) indicating that the hydroxyl group was located at C-10.

The relative stereochemistry of **2** was defined by a series of NOE difference spectroscopy experiments. Irradiation of the Me-15 proton signal caused enhancement of the H-12, H-10 and H-3 proton signals whereas irradiation of the H-7 signal produced enhancement of H-10 and H-4 signals defining the relative



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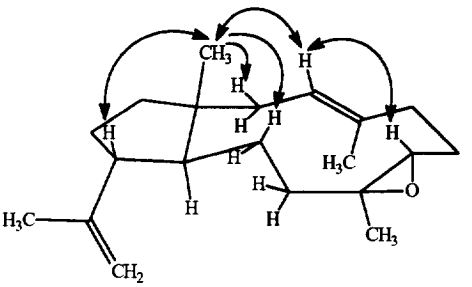

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Figure 1. Selected NOE (indicated by arrows) for dolabellanes 1 and 4.

stereochemistry of compound **2**.

The reduction of **2** with lithium aluminium hydride afforded the known diol **10**.[4,5] The absolute stereochemistry of diol **10** was assigned using the Mosher's method.[24,25] The (*R*)- and (*S*)-MTPA esters **10a** and **10b** were prepared by treatment of **10** with (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, respectively. The Δδ (δ_S- δ_R) values found for H-2, H-15, H-14β, H-14α, H-13β, H-13α, H-12, H-11, H-19, H-20, H-9β, H-9α and H-7 were + 0.02, +0.11, +0.08, +0.08, +0.14, +0.15, +0.50, +0.05, +0.03, +0.16, -0.08, -0.08, -0.01 and -0.02 ppm, respectively. From the MTPA rules, the positive and negative Δδ observed for the signals of the protons on the left and on the right side of the MTPA plane respectively, indicated an *S* configuration for C-10 in compound **10**, and therefore an absolute stereochemistry as depicted in formula **10**. Since the reduction of **2** afforded a compound identical in all respects to the diol **10** it was assumed that **2** belongs to the same enantiomeric series and that its absolute stereochemistry is that depicted in formula **2**. Furthermore, the known compound **11**, whose relative stereochemistry had been determined by single-crystal X-ray analysis,[6] had also been converted into the diol **10** allowing us to assign compound **11** to the same enantiomeric series as compound **10**.

Compound **3** was isolated as a colorless oil of molecular formula, $C_{22}H_{36}O_4$, as indicated by the high resolution mass measurement. The IR spectrum showed a strong hydroxyl band at 3380 cm^{-1} and a carbonyl band at 1738 cm^{-1}. The ^1H NMR singlet at δ 2.09 (s, 3H) indicated the presence of an acetoxyl group. The similarities in the spectroscopic data with the compounds above described suggested that **3** was a dolabellane diterpene. However, the methyl proton signals at δ 1.28 (s, 3H) and 1.23 (s, 3H) together with the ^{13}C NMR signal at δ 73.6 (s) indicated that **3** contained an isopropyl alcohol at C-12 rather than the isopropyl acetate present in compounds **1** and **2**. Analyses of ^1H NMR, ^{13}C NMR, and COSY spectra indicated that compound **3** contained two *E* double bonds located at C-2,C-3 and C-7,C-8 and a hydroxyl group at C-10 as dolabellanes **1** and **2**. The acetoxyl group was located at C-5 upon observation of the COSY cross peak between the geminal proton signal at δ 4.82 (ddd, *J* = 11.8, 11.8 and 4.0 Hz, 1H) and the H-6 allylic methylene proton signals at δ 2.35 (m, 1H) and 2.22 (m, 1H).

Table 2. NMR Data for Dolabellanes **1-5**[a,b]

#C	**1**[c]		**2**		**3**		**4**		**5**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	47.9		47.0		47.1		45.8		46.4	
2	137.5	5.04 dd (16.5,1.3)	134.7	5.05 d (16.0)	137.5	5.13 d (16.1)	43.3	2.26 m; 1.75 m	135.5	5.16 d (16.0)
3	127.4	5.49 dd (16.5,5.0)	135.0	5.19 dd (16.0,7.6)	129.2	5.32 dd (16.1,6.7)	125.0	5.40 br dd (12.0,3.8)	133.8	5.11 dd (16.0,6.2)
4	35.7	2.63 m	37.6	2.12 m	40.8	2.35 m	134.8		38.8	2.05 m
5	77.7	5.15 dd (6.0,1.8)	36.0[d]	1.60 m; 1.48 m	76.5	4.82 ddd (11.8,11.8,4.0)	38.0	2.26 m	35.6	1.54 m; 1.32 m
6	68.8	5.66 dd (10.0,1.8)	27.1	2.15 m	32.3	2.35 m; 2.22 m	23.8	1.88 m; 1.60 m	28.1	2.16 m; 2.11 m
7	124.2	5.38 br d (10.0)	129.6	4.99 br dd (7.8,7.5)	123.7	5.15 br t (7.5)	65.3	2.74 m	127.4	4.99 br dd (9.9,5.4)
8	136.1		128.0		130.5		61.8		133.0	
9	46.8	2.52 br d (11.7) 1.90 dd (12.1,11.7)	49.8	2.24 br d (11.8) 2.00 m	49.0	2.21 m	36.9	1.93 m; 1.27 m	40.2	2.01 m; 1.79 m
10	71.9	3.50 br t (12.1)	68.9	3.43 br ddd (12.1,11.1,2.0)	69.0	3.45 ddd (10.1,3.2,3.0)	23.6	1.33 m	27.4	1.48 m; 1.45 m
11	54.0	1.47 d (10.5)	57.2	1.70 dd (10.8,2.0)	56.9	1.57 m	43.1	1.77 m	53.4	1.45 m
12	47.7	2.68 ddd (11.1,10.5,5.2)	47.8	2.67 ddd (11.1,10.8,5.0)	47.6	2.57 ddd (11.0,11.0,5.9)	50.8	2.74 m	53.9	2.32 ddd (11.0,10.1,4.7)
13	25.8	1.97 m; 1.38 m	25.7	1.93 m; 1.37 m	26.3	1.88 m; 1.31 m	28.7	1.69 m; 1.65 m	25.7	1.79 m; 1.37 m
14	40.0	1.61 m; 1.38 m	38.8[d]	1.60 m; 1.48 m	39.6	1.57 m; 1.50 m	42.0	1.60 m; 1.45 m	39.8	1.60 m; 1.37 m
15	16.9	0.99 s	19.3	0.98 s	19.2	0.99 s	23.7	1.06 s	20.6	0.86 s
16	14.7	1.09 d (7.2)	21.4	0.94 d (6.8)	17.5	0.95 d (6.7)	15.8	1.66 s	22.7	0.92 d (6.8)
17	21.3	1.74 br s	18.4	1.61 br s	19.0	1.61 br s	19.0	1.25 s	17.1	1.50 s
18	86.7		87.0		73.6		146.3		86.1	
19	19.7	1.52 s	25.9[e]	1.58 s	23.6[d]	1.23 s	23.7	1.75 s	22.3[d]	1.48 s
20	25.9	1.57 s	19.5[e]	1.56 s	31.9[d]	1.28 s	112.2	4.88 br s; 4.69 br s	25.4[d]	1.50 s
$\underline{C}H_3CO$	21.2	2.14 s	22.9	2.01 s	21.3	2.09 s			22.6	1.93 s
$CH_3\underline{C}O$	171.4		169.1		170.5				170.5	
$\underline{C}H_3CO$	21.2	1.99 s								
$CH_3\underline{C}O$	170.1									
$\underline{C}H_3CO$	22.9	2.00 s								
$CH_3\underline{C}O$	169.0									
O<u>H</u>		2.92 d (12.1)		2.88 d (12.1)						

a: Assignments were aided by COSY and HETCOR experiments. b: Coupling constants are presented in Hertz units. c: Assignments were aided by ^{1}H-^{13}C long range experiment d-e: Values with the same superscript in the same column may be interchanged.

The proposed structural assignments were confirmed by a series of NOE difference spectroscopy experiments which, in addition, provided the relative stereochemistry of compound **3**. Irradiation of the H-12 signal produced enhancement on the Me-15 signal, irradiation of H-10 signal caused enhancements of the Me-15 and H-7 signals, irradiation of H-5 signal enhanced the H-7 and H-3 signals, and, finally, irradiation of Me-16 signal produced enhancement on the H-2 signal.

The absolute stereochemistry of **3** was elucidated using the Mosher's method. The (*R*)- and (*S*)-MTPA esters **3a** and **3b** were obtained by treatment of **3** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, respectively. Positive $\Delta\delta$ (δ_S- δ_R) values were found for H-3, H-2, H-15, H-14β, H-14α, H-13β, H-13α, H-12, H-11, H-19 and H-20 protons (+0.02, +0.02, +0.10, +0.09, +0.12, +0.15, +0.17, +0.48, +0.05, +0.03 and +0.15, respectively) while negative $\Delta\delta$ values were found for H-9β, H-9α, H-17 and H-7 protons (-0.08, -0.08, -0.01 and -0.02, respectively). Following the MTPA rules, these data indicated an *S* configuration for C-10, and therefore an absolute stereochemistry as depicted in formula **3**.

A less polar component isolated from *D. dichotoma* had the molecular formula $C_{20}H_{32}O$, as indicated by the high resolution mass measurement. The IR spectrum did not contain any hydroxyl band but the ^{13}C NMR signals at δ 65.2 (d) and 61.7 (s) were assigned to carbons bearing oxygen. The signal at δ 65.2 (d) was correlated in a HETCOR experiment with the methine proton signal at 2.74 (m, 1H) which showed a long range coupling in the COSY spectrum with the methyl proton signal at δ 1.25 (s, 3H). These data together with a general analysis of the ^1H and ^{13}C NMR, COSY and HETCOR spectra indicated that **4** was a dolabellane diterpene containing, on the eleven membered ring, a trisubstituted epoxide bearing a methyl group.

The ^1H NMR spectrum contained two signals at δ 4.88 (br s, 1H) and 4.69 (br s, 1H) coupled with a methyl proton signal at δ 1.75 (br s, 3H). The olefinic proton signal at δ 4.69 showed, in addition, a cross peak in the COSY spectrum with the signal at δ 2.74 (m, 1H) assigned to H-12, which was also coupled with the signal at δ 1.77 (m, 1H) assigned to H-11. These data indicated the presence of a C-18,C-20 double bond. A broad double doublet at 5.40 (br dd, J = 12.0 and 3.8 Hz, 1H) showed cross peaks in the COSY spectrum with the methylene proton signals at δ 2.26 (m, 1H) and 1.75 (m, 1H) and, in addition, allylic coupling with the methyl proton signal at δ 1.66 (br s, 3H). The allylic methylene signals at δ 2.26 and 1.75 were correlated in the HETCOR experiment with the ^{13}C NMR signal at δ 43.3 (t) attributable to an allylic methylene linked to a quaternary carbon and, therefore, indicating a C-3,C-4 double bond. Finally, the epoxide was located at C-7, C-8 upon observation of the coupling between the signal at δ 2.74 with the methylene proton signals at δ 1.88 (m, 1H) and 1.60 (m, 1H) and of these two signals with the allylic methylene signal at δ 2.26 (m, 2H).

A series of NOE difference spectroscopy experiments provided confirmation of the proposed structural assignments and defined the relative stereochemistry of compound **4** (Figure 1). Irradiation of the Me-15 signal caused enhancements of the H-12 and H-3 proton signals and irradiation of H-3 signal produced enhancement on the H-7 signal indicating that Me-15, H-12, H-3 and H-7 were located on the β face of the molecule. In the absence of an independent determination for the absolute stereochemistry of compound **4** it is depicted as belonging to the same enantiomeric series as the other components of *D. dichotoma* whose absolute

configuration is described in the present paper (**1-3**, **10**, **11**) or had already been determined (**8**, **9**).[3]

Compound **5** was isolated as a colorless oil. The molecular formula, $C_{22}H_{36}O_2$, was deduced from the high resolution mass measurement. The IR absorption at 1737 cm^{-1} indicated the presence of a carbonyl group and the ^1H and ^{13}C NMR data of **5** closely resembled those of the known dolabellane **12**.[4,7] The most significant difference in the ^1H NMR spectrum was the presence of an acetate signal at δ 1.94 (s, 3H) and the downfield shift of H-12 signal at δ 2.32 (ddd, J = 11.0, 10.1 and 4.7 Hz, 1H). This acetate group gave rise in the ^{13}C NMR spectrum to the signals at δ 170.5 (s) and 22.5 (q). These data clearly indicated that **5** was the acetate of the known dolabellane alcohol **12**.

The proposed structural assignments and relative stereochemistry were confirmed by a series of NOE difference spectroscopy experiments. Irradiation of the Me-15 signal enhanced the H-12 and H-3 proton signals and irradiation of the Me-16 produced enhancement on the H-2 signal.

The acetate **5** was converted into the known alcohol **12** by treatment with lithium aluminium hydride, and in the absence of an independent determination for the absolute configuration it is depicted as belonging to the same enantiomeric series as the other components of *D. dichotoma*.

Compound **6** was isolated as a colorless oil of molecular formula $C_{20}H_{32}O$. This data together with IR absorption at 3450 cm^{-1} suggested that **6** was a diterpenic alcohol. The ^1H and ^{13}C NMR data of compound **6** closely resembled to those reported for pachydictyol A (**19**)[15] and therefore suggested that compound **6** possessed an hydroazulene skeleton. Similarly to pachydictyol A (**19**) compound **6** contained the 1,5-dimethyl-4-hexenyl side chain which gave rise to the ^{13}C NMR signals at δ 135.5 (s), 124.7 (d), 34.7 (t), 33.6 (d), 25.7 (q), 25.4 (t), 17.7 (q), and 17.5 (q), the C-3,C-4 double bond as ascertained by the ^{13}C NMR signals at δ 142.8 (s) and 124.3 (d) and by the ^1H NMR signal at δ 5.36 (br s, 1H), and a 6β-hydroxy substituent as indicated by the ^{13}C NMR doublet at 74.5 and the H-6α signal at δ 3.92 (m, 1H). However, the ^1H NMR of **6** contained a broad doublet at δ 5.50 (br d, J = 8.6 Hz, 1H) and a vinylic methyl signal at δ 1.71 (d, J = 1.5 Hz, 3H) and lacked the exomethylene proton signals present in the ^1H NMR of pachydictyol A (**19**) indicating that **6** was its endo C-9,C-10 isomer. A series of NOE difference spectroscopy experiments provided confirmation of the proposed assigments, in particular, the enhancements produced on H-6 and H-7 signals upon irradiation of H-1. In the absence of an independent determination isopachydictyol A has been depicted in formula **6** as belonging to the same enantiomeric series as pachydictyol A (**19**) whose absolute configuration had been determined by single-crystal X-ray analysis.[15]

The most polar component of *D. dichotoma* was isolated as an amorphous powder of molecular formula $C_{20}H_{34}O_3$, as indicated by the high resolution mass measurement. This data together with the strong IR absorption at 3400 cm^{-1} and the ^{13}C NMR signals at δ 80.5 (s), 79.6 (d) and 72.7 (d) suggested that **7** was a diterpenic triol. The ^{13}C NMR signal at δ 131.6 (s), 124.7 (d), 35.2 (t), 34.5 (d), 25.7 (q), 25.7 (t), 17.7 (q) and 17.5 (q) indicated the presence of a 1,5-dimethyl-4-hexenyl side chain. The ^{13}C NMR signals at δ 152.1 (s) and 106.7 (t) together with the ^1H NMR signals at δ 4.69 (br s, 1H) and 4.68 (br s, 1H) were attributable to an exocyclic double bond, and the ^{13}C NMR doublet at δ 72.7 that was correlated in a HETCOR

experiment with the signal at δ 4.21 (br d, J = 8.9 Hz, 1H) indicated that compound **7** contained an hydroazulene skeleton with a seven membered ring identical to that of pachydictyol A (**19**). The substitution on the five membered ring was elucidated as follows: the ^{13}C NMR signals at δ 80.5 (s) and 25.7 (q) indicated the presence of a hydroxyl group at C-4, and the remaining hydroxyl group was located at C-3 upon observation of the COSY cross peaks between the geminal H-3 proton at δ 3.97 (dd, J = 9.4 and 5.9 Hz, 1H) and the H-2 methylene proton signals at δ 2.00 (m, 1H) and 1.68 (m, 1H) which were in addition coupled to the allylic heteroanular proton H-1 at δ 2.33 (m, 1H).

The relative stereochemistry of compound 7 was defined by a series of NOE difference spectroscopy experiments. Irradiation of the H-6 signal produced enhancements of H-1 and H-7 signals consistent with a relative stereochemistry of the seven membered ring identical to that of pachydictyol A (**19**). Irradiation of the H-3 signal caused enhancement on the H-1 signal and irradiation of the Me-17 signal enhanced the H-5 signal defining the relative stereochemistry both about the five membered ring and of the ring junction. It was therefore proposed structure 7 for dictyotatriol A.

The absolute stereochemistry of 7 was determined using the Mosher's method. Treatment of 7 with (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride yielded the monoesters **7a** and **7b**, respectively. The values of $\Delta\delta$ (δ_S- δ_R) found for H-18a, H-18b, H-1, H-2β, H-2α, H-17 and H-6 were -0.05, -0.08, -0.02, -0.06, -0.02, +0.09 and +0.01, respectively. Following the MTPA rules these data indicated an absolute configuration *S* for C-3 in compound 7, and therefore an absolute stereochemistry as showed in formula 7.

In general, the new diterpenes showed a mild activity in bioassays directed to detect in vitro cytoxicity aganist P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma tumor cell lines. However, dolabellane **1** and dictyotatriol A (**7**) were inactive in these tests. Compounds **3-6** were mildly active with ED_{50} values of 5 μg/mL in all cases. Dolabellane **2** exhibited the greatest activity with ED_{50}= 1.2 μg/mL against P-388 and A-549 tumor cell lines, and ED_{50} = 2.5 μg/mL against HT-29 and MEL-28 tumor cell lines.

EXPERIMENTAL SECTION

General: Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer and UV spectra were recorded on a Philips PU 8710 spectrophotometer. ^1H NMR and ^{13}C NMR were made on a Varian Unity 400 at 400 MHz and 100 MHz respectively, using $CDCl_3$ as solvent. The resonance of residual chloroform at δ_H 7.26 and of $CDCl_3$ at δ_C 77.0 were used as internal reference for ^1H and ^{13}C spectra, respectively. Assignments with identical supercripts may be interchanged. Mass spectra were recorded on a VG 12250 or on Kratos MS 80RFA spectrometer. In High Performance Liquid Chromatography separations LiChrosorb Si 60 were used in normal phase mode using a differential refractometer. All solvents were spectral grade or were distilled from glass prior to use.

Collection, Extraction and Purification: The alga *Dictyota dichotoma* (62 g dry weight) was collected by hand in the intertidal zone of Cortadura (Cádiz, Spain) in July 1994 and was immediately frozen. The frozen tissue

was extracted exhaustively with acetone at room temperature. The filtered Me_2CO solution was evaporated under reduced pressure and the aqueous residue was extracted with Et_2O. The solvent was evaporated to give an oil residue (5 g) which was chromatographed on a SiO_2 column using solvents of increasing polarity from hexane to diethyl-ether and, subsequently, EtOAc and chloroform-methanol (8:2). Fractions of the general cromatography eluted with 10% ether in hexane were further separated by normal phase HPLC eluting with hexane/EtOAc (99:1) to afford (1*R**,2*E*,4*R**,7*E*,11*S**,12*R**)-18-acetoxy-2,7-dolabelladiene (**5**, 10 mg, 0.016% dry wt), dilophol acetate (**24**, 2.5 mg, 0.004% dry wt), and dictyoxide (**16**, 3.5 mg, 0.006% dry wt). Fractions eluted with 20% ether in hexane were subjected to normal phase HPLC separation eluting with hexane/EtOAc (98:2) yielding isopachydictyol A (**6**, 6 mg, 0.010% dry wt), pachydictyol A (**19**, 30 mg, 0.048% dry wt), and (1*R**,3*E*,7*S**,8*S**,11*S**,12*R**)-7,8-epoxy-3,18-dolabelladiene (**4**, 5.7 mg, 0.009% dry wt). Fractions eluted with 30% ether in hexane were further separated by normal phase HPLC eluting with hexane/EtOAc (97:3) to obtain (1*R**,3*E*,7*E*,11*S**,12*R**)-18-hydroxy-3,7-dolabelladiene (**13**, 3.5 mg, 0.006% dry wt), and dilophol (**23**, 70 mg, 0.113% dry wt). Fractions of the general chromatography eluted with 40% ether in hexane were further separated by normal phase HPLC eluting with 5% EtOAc in hexane to yield neodictyolactone (**25**, 4 mg, 0.006% dry wt), (1*R**,2*E*,4*R**,7*E*,11*S**,12*R**)-18-hydroxy-2,7-dolabelladiene (**12**, 5.3 mg, 0.009% dry wt), and dictyol B acetate (**20**, 2 mg, 0.003% dry wt). Fractions eluted with 50% ether in hexane were further separated by normal phase HPLC eluting with hexane/EtOAc (9:1) to afford dictyodial (**26**, 250 mg, 0.403% dry wt), (1*R*,3*S*,4*Z*,8*E*,11*S*,12*R*)-3-acetoxy-4,8,18-dolabellatrien-16-al (**15**, 26.6 mg, 0.043% dry wt), (1*R*,3*S*,4*E*,8*E*, 11*S*,12*R*)-3-acetoxy-4,8,18-dolabellatrien-16-al (**14**, 9.1 mg, 0.015% dry wt), dictyol D acetate (**21**, 9 mg, 0.015% dry wt), (1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-18-acetoxy-10-hydroxy-2,7-dolabelladiene (**2**, 11.3 mg, 0.018% dry wt), (1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-10-acetoxy-18-hydroxy-2,7-dolabelladiene (**11**, 44 mg, 0.071% dry wt), and dictyol E (**22**, 48 mg, 0.077% dry wt). Fractions eluted with 60% ether in hexane were further subjected to normal phase HPLC separation eluting with hexane/EtOAc (8:2) to obtain dictyotadiol (**17**, 183 mg, 0.295% dry wt), (1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-10,18-Dihydroxy-2,7-dolabelladiene (**10**, 7 mg, 0.011% dry wt), and dictyol C (**18**, 16 mg, 0.026% dry wt). Fractions eluted with 70% ether in hexane were subjected to normal phase HPLC separation eluting with hexane/EtOAc (7:3) to afford (1*R*,2*E*,4*S*,5*R*,7*E*,10*S*,11*S*,12*R*)-5-acetoxy-10,18-dyhydroxy-2,7-dolabelladiene (**3**, 13 mg, 0.021% dry wt), (1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-5,6,18-triacetoxy-10-hydroxy-2,7-dolabelladiene (**1**, 87 mg, 0.140% dry wt), and (1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-5,6,10-triacetoxy-18-hydroxy-2,7-dolabelladiene (**9**, 19 mg, 0.031% dry wt). (1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-5,6-diacetoxy-10,18-dihydroxy-2,7-dolabelladiene (**8**, 35 mg, 0.056% dry wt) was crystallized from the fractions eluted with Et_2O. Fractions eluted with EtOAc were purified by HPLC on normal phase mode using 45% EtOAc in hexane to obtain dictyotatriol A (**7**, 25 mg, 0.031% dry wt).

(1*R*,2*E*,4*S*,5*R*,6*S*,7*E*,10*S*,11*S*,12*R*)-5,6,18-Triacetoxy-10-hydroxy-2,7-dolabelladiene (1): Colorless oil; $[\alpha]_D^{25}$ +10° (*c* = 0.11, $CHCl_3$); IR (film) 3550, 1738, 1249 cm^{-1}; EIMS (70 eV) *m/z* (rel. int.) 404 (M^+-AcOH, 3), 344 (M^+-2AcOH, 6), 302 (19), 284 (M^+-3AcOH, 12), 226 (3), 205 (29), 159 (29), 135 (100), 97 (85), 78 (31); HREIMS Obsd. *m/z* = 464.2769 (M^+), $C_{26}H_{40}O_7$ requires *m/z* = 464.2774; see Table 2 for NMR data.

(1*R*,2*E*,4*R*,7*E*,10*S*,11*S*,12*R*)-18-Acetoxy-10-hydroxy-2,7-dolabelladiene (2): Colorless oil; $[\alpha]_D^{25}$ -36.2° (*c* = 0.47, $CHCl_3$); IR (film) 3550, 1735, 1248 cm^{-1}; EIMS (70 eV) *m/z* (rel. int.) 288 (M^+-AcOH, 13), 270 (M^+-AcOH-H_2O, 6), 227 (7), 177 (18), 151 (32), 122 (100), 107 (67), 80 (66), 69 (57); HREIMS Obsd. *m/z* = 348.2644 (M^+), $C_{22}H_{36}O_3$ requires *m/z* = 348.2664; see Table 2 for NMR data.

(1*R*,2*E*,4*R*,5*R*,7*E*,10*S*,11*S*,12*R*)-5-Acetoxy-10,18-dihydroxy-2,7-dolabelladiene (3): Colorless oil; $[\alpha]_D^{25}$ -6.7° (*c* = 0.15, $CHCl_3$); IR (film) 3379, 1738, 1248 cm^{-1}; EIMS (70 eV) *m/z* (rel. int.) 346 (M^+-H_2O, 7), 286 (M^+-

H_2O-AcOH, 36), 271 (13), 231 (8), 205 (11), 189 (19), 175 (42), 135 (88), 122 (100), 107 (86), 80 (73), 69 (91), 55 (58); HREIMS Obsd. *m/z* = 364.2597 (M^+), $C_{22}H_{36}O_4$ requires *m/z* = 364.2614; see Table 2 for NMR data.

(1*R,3*E*,7*S**,8*S**,11*S**,12*R**)-7,8-Epoxy-3,18-dolabelladiene (4):** Colorless oil; $[\alpha]_D^{25}$ +27.4° (*c* = 0.46, $CHCl_3$); IR (film) 1648, 889 cm^{-1}; EIMS (70 eV) *m/z* (rel. int.) 288 (M^+, 3), 270 (M^+-H_2O, 2), 255 (M^+-H_2O-CH_3, 3), 219 (3), 187 (10), 175 (12), 151 (35), 133 (79), 121 (100), 107 (85), 93 (88), 81 (77), 67 (74), 55 (38); HREIMS Obsd. *m/z* = 288.2445 (M^+), $C_{20}H_{32}O$ requires *m/z* = 288.2453; see Table 2 for NMR data.

(1*R,2*E*,4*R**,7*E*,11*S**,12*R**)-18-Acetoxy-2,7-dolabelladiene (5):** Colorless oil; $[\alpha]_D^{25}$ -53.2° (*c* = 0.41, $CHCl_3$); IR (film) 1737, 1254 cm^{-1}; EIMS (70 eV) *m/z* (rel. int.) 272 (M^+-AcOH, 8), 257 (M^+-AcOH-CH_3, 5), 229 (25), 201 (5), 189 (10), 175 (55), 161 (20), 147 (36), 135 (20), 121 (33), 93 (46), 81 (49), 55 (23), 43 (100); HREIMS Obsd. *m/z* = 332.2713 (M^+), $C_{22}H_{36}O_2$ requires *m/z* = 332.2715; see Table 2 for NMR data.

Isopachydictyol A (6): Colorless oil; $[\alpha]_D^{25}$ -25.7° (*c* = 0.28, $CHCl_3$); IR (film) 3450, 1639 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 5.50 (br d, 1H, *J* = 8.6 Hz, H-9), 5.36 (br s, 1H, H-3), 5.12 (br t, 1H, *J* = 7.1 Hz, H-14), 3.92 (m, 1H, H-6), 2.68 (m, 1H, H-1), 2.55 (m, 1H, H-5), 2.35 (m, 1H, H-2), 2.27 (m, 1H, H-8), 2.16 (m, 1H, H-2), 2.04 (m, 1H, H-13), 1.94 (m, 1H, H-13), 1.85 (td, 3H, *J* = 2.8, 1.5 Hz, H-17), 1.79 (m, 1H, H-8), 1.71 (d, 3H, *J* = 1.5 Hz, H-18), 1.69 (d, 3H, J = 0.9 Hz, H-16), 1.64 (m, 1H, H-7), 1.61 (s, 3H, H-20), 1.54 (m, 1H, H-12), 1.49 (m, 1H, H-11), 1.21 (m, 1H, H-12), 0.97 (d, 3H, *J* = 6.6 Hz, H-19); ^{13}C NMR (100 Mz, $CDCl_3$) δ 142.8 (s, C-4), 138.7 (s, C-10), 131.5 (s, C-15), 126.0 (d, C-9), 124.7 (d, C-14), 124.3 (d, C-3), 74.5 (d, C-6), 57.4 (d, C-5), 46.9 (d, C-7), 46.0 (d, C-1), 35.4 (t, C-2), 34.7 (t, C-12), 33.6 (d, C-11), 25.7 (q, C-16), 25.4 (t, C-13), 24.5 (t, C-8), 23.1 (q, C-18), 17.7 (q, C-20), 17.5 (q, C-19), 16.2 (q, C-17); EIMS (70 eV) *m/z* (rel. int.) 288 (M^+, 17), 270 (M^+-H_2O, 10), 255 (M^+-H_2O-CH_3, 9), 227 (6), 175 (19), 159 (71), 157 (60), 145 (36), 131 (41), 109 (52), 107 (100), 105 (66), 95 (63), 81 (66), 69 (75), 55 (75); HREIMS Obsd. *m/z* = 288.2444 (M^+), $C_{20}H_{32}O$ requires *m/z* = 288.2453.

Dictyotatriol A (7): Amorphous solid; $[\alpha]_D^{25}$ +27.8 (*c* = 0.09, $CHCl_3$); IR (film) 3400, 1646 cm^{-1}; ^1H NMR (400 Mz, $CDCl_3$) δ 5.12 (br dd, 1H, *J* = 7.3, 7.0 Hz, H-14), 4.69 (br s, 1H, H-18), 4.68 (br s, 1H, H-18), 4.21 (br d, 1H, *J* = 8.9 Hz, H-6), 3.97 (dd, 1H, *J* = 9.4, 5.9 Hz, H-3), 2.57 (m, 1H, H-9), 2.33 (m, 1H, H-1), 2.06 (m, 1H, H-9), 2.00 (m, 1H, H-2α), 1.97 (m, 2H, H-13), 1.71 (m, 1H, H-5), 1.68 (m, 1H, H-2β), 1.68 (br s, 3H, H-16), 1.60 (s, 3H, H-20), 1.58 (m, 1H, H-11), 1.54 (m, 3H, H-8, H-12), 1.51 (m, 1H, H-7), 1.30 (br s, 3H, H-17), 1.25 (m, 1H, H-12), 0.97 (d, 3H, *J* = 6.2 Hz, H-19); ^{13}C NMR (100 Mz, $CDCl_3$) δ 152.1 (s, C-10), 131.6 (s, C-15), 124.7 (d, C-14), 106.7 (t, C-18), 80.5 (s, C-4), 79.6 (s, C-3), 72.7 (d, C-6), 57.8 (d, C-5), 47.1 (d, C-7), 39.3 (t, C-9), 38.9 (d, C-1), 35.2 (t, C-12), 34.5 (d, C-11), 34.4 (t, C-2), 25.7 (q, C-16), 25.7 (q, C-17), 25.7 (t, C-13), 23.4 (t, C-8), 17.7 (q, C-20), 17.5 (q, C-19); EIMS (70 eV) *m/z* (rel. int.) 322 (M^+, 2), 304 (M^+-H_2O, 10), 268 (M^+-3H_2O, 2) 237 (3), 204 (12), 161 (10), 133 (12), 109 (41), 95 (19), 81 (100), 69 (38), 55 (28); HREIMS Obsd. *m/z* = 322.2511 (M^+), $C_{20}H_{34}O_3$ requires *m/z* = 322.2508.

Treatment of compounds 1, 2, 5, 8, 9 and 11 with $LiAlH_4$: To a solution of **1** (5.3 mg) in dry Et_2O, 0.1 mL of $LiAlH_4$ 1M were added and the resulting suspension was stirred overnight at room temperature. Excess reagent was destroyed by careful addition of Et_2O and the mixture was filtered through a small silica gel column using EtOAc. The residue was purified by HPLC using hexane/EtOAc (95:5) to obtain **27**[3] (3.2 mg). Treatment of compounds **8** (6.2 mg) and **9** (3.6 mg) with $LiAlH_4$ following the same procedure yielded tetrol **27** (1.1 and 1.7 mg, respectively). Compounds **2** (2.5 mg) and **11** (9.0 mg) upon treatment with $LiAlH_4$ yielded

the diol **10**[4,5] (1.2 and 3.5 mg, respectively).

Synthesis of the (*R*)-MTPA ester (3a): Treatment of **3** (2.5 mg) with CH_2Cl_2 solutions of dicyclohexylcarbodiimide (13 mg in 0.6 mL), *N,N*-dimethylaminopyridine (1.5 mg in 0.25 mL) and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (12 mg in 0.5 mL) and the mixture was stirred at room temperature for 24 h. After evaporation of the solvent under reduced pressure the residue was purified on a Si gel TLC plate to obtain the (*R*)-MTPA ester **3a** (1.5 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) (selected data) δ 5.36 (dd, 1H, *J* = 16.3, 7.2 Hz, H-3), 5.28 (br t, 1H, *J* = 7.7 Hz, H-7), 5.12 (dd, 1H, *J* = 16.3, 0.8 Hz, H-2), 4.92 (br d, 1H, *J* = 10.7 Hz, H-10), 4.84 (ddd, 1H, *J* = 8.3, 8.1, 3.2 Hz, H-5), 2.50 (dd, 1H, *J* = 12.1, 11.3 Hz, H-9α), 2.23 (br d, 1H, *J* = 11.3, H-9β), 2.11 (s, 3H, CH_3CO-), 1.71 (m, H-12), 1.68 (br s, 3H, H-17), 1.67 (m, 1H, H-11), 1.65 (m, 1H, H-13β), 1.40 (m, 1H, H-14β), 1.33 (m, 1H, H-14α), 1.16 (m, 1H, H-13α), 1.04 (s, 3H, H-19)[a], 0.92 (s, 3H, H-20)[a], 0.81 (s, 3H, H-15).

Synthesis of the (*S*)-MTPA ester (3b): Treatment of **3** (4.5 mg) with CH_2Cl_2 solutions of dicyclohexylcarbodiimide (23 mg in 1 mL), *N,N*-dimethylaminopyridine (3 mg in 0.5 mL) and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (12 mg in 0.5 mL) as described above yielded the (*S*)-MTPA ester **3b** (1.8 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) (selected data) δ 5.38 (dd, 1H, *J* = 16.1, 7.2 Hz, H-3), 5.26 (br t, 1H, *J* = 7.7 Hz, H-7), 5.14 (dd, 1H, *J* = 16.1, 0.9 Hz, H-2), 4.89 (ddd, 1H, *J* = 11.0, 2.5, 2.5 Hz, H-10), 4.84 (ddd, 1H, *J* = 8.3, 8.1, 3.3 Hz, H-5), 2.42 (dd, 1H, *J* = 12.1, 11.2 Hz, H-9α), 2.19 (m, 1H, H-12), 2.15 (m, 1H, H-9β), 2.11 (s, 3H, CH_3CO-), 1.80 (m, 1H, H-13β), 1.72 (dd, 1H, *J* = 10.0, 2.4 Hz, H-11), 1.66 (br s, 3H, H-17), 1.49 (m, 1H, H-14β), 1.45 (m, 1H, H-14α), 1.33 (m, 1H, H-13α), 1.08 (s, 3H, H-19)[a], 1.07 (s, 3H, H-20)[a], 0.91 (s, 3H, H-15).

Synthesis of the (*R*)-MTPA ester (7a): To a solution of **7** (3.0 mg) in CH_2Cl_2 (1,5 mL) at 0 °C containing *N,N*-dimethylaminopyridine (4.0 mg) and Et_3N (0.5 mL), (*S*)-MTPA chloride (25 μL) was added, and the mixture was stirred under N_2 at room temperature for 24 h. After evaporation of the the solvent under reduced pressure the residue was purified on a Si gel TLC plate to obtain the (*R*)-MTPA ester **7a** (1.1 mg). ^{1}H NMR ($CDCl_3$, 400 MHz) (selected data) δ 5.12 (dd, 1H, *J* = 8.5, 6.5 Hz, H-3), 4.73 (br s, 1H, H-18a), 4.63 (br s, 1H, H-18b), 4.26 (m, 1H, H-6), 2.55 (m, 1H, H-9), 2.50 (m, 1H, H-1), 2.25 (m, 1H, H-2α), 2.07 (m, 1H, H-9), 1.80 (m, 1H, H-2β), 1.20 (s, 3H, H-17).

Synthesis of the (*S*)-MTPA ester (7b): To a solution of **7** (3.0 mg) in CH_2Cl_2 (1.5 mL) containing *N,N*-dimethylaminopyridine (4.0 mg) and Et_3N (0.5 mL), (*R*)-MTPA chloride (30 μL) was added as described above yielding the (*S*)-MTPA ester **7b** (1.1 mg). ^{1}H NMR ($CDCl_3$, 400 MHz) (selected data) δ 5.07 (dd, 1H, *J* = 8.4, 6.3 Hz, H-3), 4.68 (br s, 1H, H-18a), 4.55 (br s, 1H, H-18b), 4.27 (m, 1H, H-6), 2.51 (m, 1H, H-9), 2.48 (m, 1H, H-1), 2.23 (m, 1H, H-2α), 2.06 (m, 1H, H-9), 1.74 (m, 1H, H-2β), 1.29 (s, 3H, H-17).

Synthesis of the (*R*)-MTPA ester (10a): To a solution of **10** (2.5 mg) in dry pyridine (1 mL), (*S*)-MTPA chloride (12 μL) was added and the mixture was stirred at room temperature for 24 h. After evaporation of the solvent under reduced pressure the residue was purified on a Si gel TLC plate to obtain the (*R*)-MTPA ester **10a** (1.0 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) (selected data) δ 5.25 (dd, 1H, *J* = 16.1, 7.4 Hz, H-3), 5.15 (br dd, 1H, *J* = 8.2, 7.2 Hz, H-7), 5.04 (d, 1H, *J* = 16.1 Hz, H-2), 4.92 (ddd, 1H, *J* = 11.0, 2.4, 2.3, H-10), 2.50 (dd, 1H, *J* = 11.7, 11.5 Hz, H-9α), 2.18 (m, 1H, H-9β), 1.72 (dd, 1H, *J* = 10.1, 2.4 Hz, H-11), 1.71 (m, 1H, H-12), 1.67 (m, 1H, H-13β), 1.67 (br s, 3H, H-17), 1.47 (m, 1H, H-14β), 1.36 (m, 1H, H-14α), 1.16 (m, 1H, H-13α), 1.06 (s, 3H, H-19)[a], 0.93 (s, 3H, H-20)[a], 0.79 (s, 3H, H-15).

Synthesis of the (*S*)-MTPA ester (10b): Treatment of **10** (2.7 mg) with (*R*)-MTPA chloride (15 μL) in pyridine as described above yielded the (*S*)-MTPA ester **10b** (1.7 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (dd, 1H, *J* = 16.1, 7.3 Hz, H-3), 5.13 (br dd, 1H, *J* = 8.3, 7.8 Hz, H-7), 5.06 (d, 1H, *J* = 16.1 Hz, H-2), 4.89 (ddd, *J* = 12.0, 2.3, 2.3, H-10), 2.42 (dd, 1H, *J* = 12.0, 11.2 Hz, H-9α), 2.21 (ddd, *J* = 11.3, 10.3, 4.2 Hz, 1H, H-12), 2.10 (br d, 1H, *J* = 11.2 Hz, H-9β), 1.81 (m, 1H, H-13β), 1.77 (dd, 1H, *J* = 10.3, 2.3 Hz, H-11), 1.66 (br s, 3H, H-17), 1.55 (m, 1H, H-14β), 1.44 (m, 1H, H-14α), 1.31 (m, 1H, H-13α), 1.09 (br s, 6H, H-19, H-20), 0.90 (s, 3H, H-15).

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