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TETRAHEDRON

Metabolites From the Sponge *Plakortis simplex*. II.[1] Isolation of Four Bioactive Lactone Compounds and of A Novel Related Amino Acid

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Abstract: Seven lactone metabolites, namely the known plakortones B-D (**1-3**), the novel plakortones E (**4**) and F (**5**), and two novel δ-lactones, named simplactones A (**6**) and B (**7**), have been isolated from the Caribbean sponge *Plakortis simplex*, and their structures characterized by spectroscopic and chemical means. In addition, the unprecedented amino acid **8** has been isolated from the same source, and a hypothesis of its biogenetic relationship with simplactones is herein reported. Plakortones B-F and compound **8** exhibited in vitro cytotoxic activity on WEHI 164, murine fibrosarcoma cell line.

Keywords: Marine metabolites; Amino acids; δ-Lactones; Biosynthesis

INTRODUCTION

Since isolation of plakortin in 1978,[2] a series of bioactive cyclic peroxides have been found as secondary metabolites in sponges of the genus *Plakortis*, differing from the parent molecule for length, branching, and functionalization of the alkyl chains. In addition, some structurally related compounds have been isolated, and they essentially belong to two classes: furano-derivatives (for which a biogenetic origin from the corresponding cycloperoxides has been hypothesized)[1,3] endowed with cytotoxic and anti-protozoan activities; and plakortones, bicyclic lactones recently reported from *Plakortis halicondrioides*.[4] Their activity as activators of cardiac sarcoplasmic reticulum Ca^{2+} ATPase may be valuable in correcting relaxation abnormalities related to some forms of human heart failure, and makes plakortones metabolites of pharmacological interest. A synthetic approach to their 2,6-dioxabicyclo[3.3.0]octan-3-one nucleus has been very recently described.[5]

Our research group has been interested for a long time in the chemistry of pharmacologically active marine secondary metabolites, and, having found that the methanolic extract of *Plakortis simplex* displayed in vitro cytotoxic activity, we recently undertook a survey of this Caribbean sponge. As a result of these investigations, we have already described the isolation of two novel cytotoxic furano compounds, found as components of the apolar layer of the crude extract together with plakortin and its novel 9,10-dihydroderivative. Absolute configurations of all these molecules have been determined after chemical derivatization.[1]

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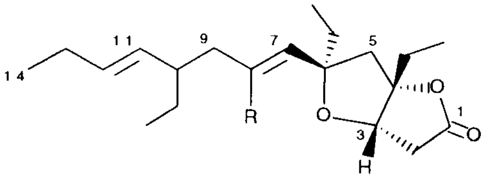
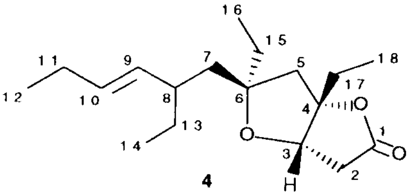
Continuing the analysis of *P. simplex* guided by the search of new bioactive compounds, we have now isolated seven lactone metabolites: five of them belong to the class of plakortones, namely the known plakortones B-D (**1-3**) and the novel plakortones E (**4**) and F (**5**), while the others are two novel δ-lactones, which we named simplactones A (**6**) and B (**7**). In addition, the unprecedented amino acid (**8**) has been isolated, and its structural determination, together with an hypothesis of its biogenetic relationship with simplactones, have been described within the present paper.



1 R = CH_3 **2** 7,8-dihydro, R = CH_3
3 7,8-dihydro, R = H



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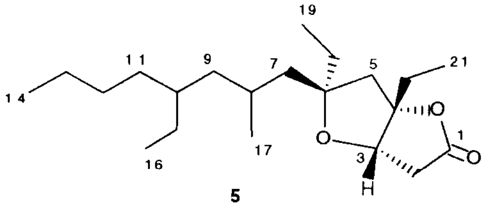

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RESULTS AND DISCUSSION

A specimen of *P. simplex* (order Homosclerophorida, family Plakinidae) was collected by hand during an expedition to the Caribbean Sea, and immediately frozen. After homogenization, the organism (57 g dry wt.) was exhaustively extracted first with methanol and then with chloroform. The methanol extract was partitioned between *n*-BuOH and water, and then the organic phase, combined with the $CHCl_3$ extract, was subjected to chromatography over a column packed with reversed phase silica gel (RP18) and eluted with a system of solvents from H_2O/MeOH 9:1 to H_2O/MeOH 1:9. Five fractions of decreasing polarity were so obtained, named A_1-A_5. The less polar fractions, A_4 and A_5, were combined and purified by a medium pressure liquid chromatography (MPLC) over silica gel, and fractions obtained eluting with *n*-hexane/EtOAc 7:3 and 8:2 were further purified by HPLC (eluent *n*-hexane/EtOAc 94:6) allowing us to isolate pure plakortones B-F (**1-5**). Compounds **1-3** were identified as plakortones B, C and D, respectively, considering that their $[\alpha]_D$ values, ^1H and ^{13}C NMR spectra were identical with those reported in the literature.[4]

Plakortone E (**4**) was isolated as a colorless oil, $[\alpha]_D = -10$. Its EI (45 eV) mass spectrum showed a weak molecular ion at *m/z* 294, and intense fragmentation peaks at *m/z* 265 (M - CH_2CH_3) and *m/z* 183 (base peak, corresponding to the loss of the branched side chain). The molecular formula $C_{18}H_{30}O_3$, requiring four unsaturation equivalents, was deduced from a high resolution mass measurement and supported by ^{13}C NMR data. The presence of a five-membered lactone carbonyl functionality was suggested by the absorption band in the FT-IR (KBr) spectrum at ν_{max} 1786 cm^{-1}.

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR Data of Plakortone E (**4**) and F (**5**) in $CDCl_3$

Pos.	**4**		**5**	
	δC, mult.	δH, mult., J in Hz	δC, mult.	δH, mult., J in Hz
1	175.5, C		176.0, C	
2	37.5, CH_2	2.70, dd, 18.0, 4.8 2.63, bd, 18.0	37.2, CH_2	2.71, dd, 18.3, 5.1 2.64, dd, 18.3, 0.8
3	80.2, CH	4.31, bd, 4.8	80.5, CH	4.35, bd, 5.1
4	98.0, C		97.9, C	
5	46.0, CH_2	2.18, d, 14.6 1.97, d, 14.6	46.2, CH_2	2.32, d, 14.7 1.83, d, 14.7
6	88.2, C		88.0, C	
7	43.5, CH_2	1.62[a] 1.45, dd, 13.2, 9.0	45.3, CH_2	1.41, dd, 14.0, 8.0 1.34, dd, 14.0, 3.7
8	45.3, CH	1.92, m	31.0, CH	1.60, m
9	133.1, CH	5.09, dd, 15.3, 9.0	43.1, CH_2	1.12, m 1.06, m
10	132.0, CH	5.38, dt, 15.3, 6.2	29.7, CH	1.28[a]
11	26.0, CH_2	2.00, m	32.3, CH_2	1.24[a]
12	14.5, CH_3	0.97, t, 7.6	28.5, CH_2	1.26[a]
13	29.5, CH_2	1.40, dq, 13.9, 6.9 1.21, dq, 13.9, 6.9	22.8, CH_2	1.29[a]
14	12.0, CH_3	0.82, t, 6.9	13.4, CH_3	0.88, t, 7.3
15	32.0, CH_2	1.63[a] 1.57, m	26.6, CH_2	1.29[a] 1.22[a]
16	8.1, CH_3	0.85, t, 7.6	10.7, CH_3	0.83, t, 6.6
17	30.3, CH_2	1.76, dq, 15.3, 6.9 1.70, dq, 15.3, 6.9	21.8, CH_3	0.93, d, 6.6
18	9.2, CH_3	1.00, t, 6.9	26.8, CH_2	1.62[a] 1.58[a]
19			8.7, CH_3	0.87, t, 7.3
20			30.3, CH_2	1.77, m 1.74, m
21			8.3, CH_3	1.02, t, 7.3

a. Overlapped with other signals.

^1H and ^{13}C NMR spectra of **4** (Table 1) revealed some analogies with those reported for plakortones[4] and, therefore, their analysis was aided by comparison with the data of known molecules. A 2D HOHAHA spectrum allowed us to divide the ^1H NMR signals of **4** into five spin systems: an alkyl chain containing one double bond, two ethyl groups linked to quaternary carbons, an AB system attributed to an isolated methylene group (H_2-5) and, finally, an O-CH-CH_2 moiety. The proton sequence within each spin system was assigned by inspection of the ^1H-^1H COSY spectrum. Moreover, correlation peaks in HMQC and HMBC (Fig. 1) spectra of **4** allowed to assign all the carbon resonances and also to interconnect the identified substructures of the new molecule. The obtained information, in accordance with the above MS data, led to the conclusion that compound **4** is a new plakortone with a shorter alkyl chain (precisely, it possesses the same alkyl chain of plakortin).

The relative stereochemistry around the bicyclic system of plakortone E was deduced by some key dipolar couplings, evidenced by a 2D NMR ROESY spectrum (Fig. 1). In particular, H-3 (δ 4.31) was coupled with H_2-17 (δ 1.76 and 1.70) and H-7a (δ 1.62), and this suggested that these protons must be located on the same face of the bicyclic nucleus, thus requiring a *cis* junction between the two five-membered rings of **4**. The obtained relative configuration is the same of that reported for known plakortones B-D (**1-3**).[4] Finally, the *E* geometry of the $\Delta^{9,10}$ double bond was inferred by H-9/H-10 coupling constant (J = 15.3 Hz) and confirmed by the dipolar couplings H-9/H_2-11 and H-10/H-8.

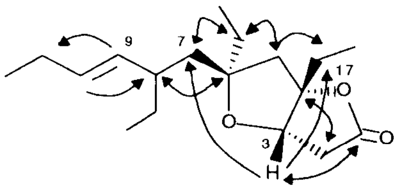


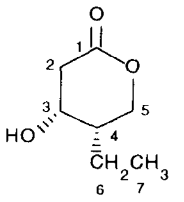
Fig. 1 Diagnostic ROESY (→) and HMBC (↔) correlation peaks detected for plakortone E

Plakortone F (**5**; $[\alpha]_D$ -11), has been easily characterized as the 11,12-dihydro derivative of plakortone C (**2**), taking into account its HR-EIMS (45 eV) spectrum, indicating the molecular formula $C_{21}H_{38}O_3$, and, above all, its NMR data. Both ^{1}H and ^{13}C NMR spectra of **5** appeared similar to those of **2**, lacking in the two olefin signals, substituted by two overlapping methylene multiplets at δ 1.26 and 1.24 in the ^{1}H NMR spectrum of **5**, and by two signals at δ 28.5 and 32.3, respectively, in the ^{13}C NMR spectrum of **5**. In addition, an upfield shift of H-10/C-10, H_2-13/C-13, and H_3-14/C-14 signals was observed. Furthermore, the structure of plakortone F (**5**) was confirmed by 2D NMR spectroscopy and, in particular, combined analysis of COSY, HMQC, and HMBC spectra was used to assign all its ^{1}H and ^{13}C NMR resonances as reported in Table 1. Finally, also the relative stereochemistry around the bicyclic system of plakortone F, deduced by cross peaks in a ROESY spectrum, follows that of plakortone C (**2**).

Isolation of plakortones E and F from *P. simplex* appears noteworthy because it enriches a class of pharmacologically significant metabolites. As a part of our ongoing screening for antitumor compounds from marine sponges, and considering that data about in vitro cytotoxic activity of plakortones were not available, we evaluated all the isolated molecules of this class against WEHI 164, murine fibrosarcoma cell line, at 72 h. The known plakortones B, C and D, and the novel plakortones E and F exhibited cytotoxicity with IC_{50} of 10.0, 9.0, 9.0, 8.0, and 11.0 µg/mL, respectively; the most active of the series being the novel plakortone E. In addition, as already noted for cytotoxic activity of plakortin and of its 9,10-dihydro derivative,[1] the completely saturated analogues are usually less active.

Fraction A_2 was subjected to MPLC over silica gel (230-400 mesh) eluting with a gradient system from EtOAc to MeOH. Fractions eluted with EtOAc were chromatographed by HPLC (eluent EtOAc/*n*-hexane 8:2) and afforded simplactones A (**6**) and B (**7**). A more polar fraction, eluted with EtOAc/MeOH 6:4, was purified by HPLC on reverse phase (C_{18}, eluent H_2O/MeOH 7:3) and furnished the novel amino acid **8** in the pure state.

Simplactone A (**6**), was isolated as a colorless amorphous solid (mg 4.5), $[\alpha]_D$ -3, and its molecular formula, determined from HR-EIMS, is $C_7H_{12}O_3$, in accordance with two degrees of unsaturation. The presence of an hydroxyl group was initially inferred by IR (KBr) absorption band centered at ν_{max} 3420 cm^{-1} and by the diagnostic fragmentation peak at *m/z* 126 (M – H_2O) in the EIMS spectrum. The two remaining oxygen atoms of the molecular formula were attributed to a lactone group, as suggested by IR (KBr) peaks at ν_{max} 1718 and 1233 cm^{-1}, and confirmed by the quaternary carbon atom resonating at δ 170.2 in the ^{13}C NMR spectrum. This contained also the resonances of an oxymethine (δ 69.0), a methine, a methyl, and three methylene groups, one of them bearing an oxygen atom (δ 68.1) (multiplicities distinguished by a DEPT experiment). The above data, together with cross peaks in the ^1H-^1H COSY spectrum and key correlations in the HMBC spectrum (e.g. H_2-5/C-1; H-3/C-1; H_3-7/C-4), allowed us to determine the plane structure of **6**.



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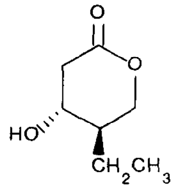

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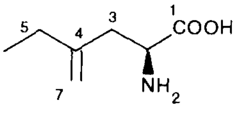

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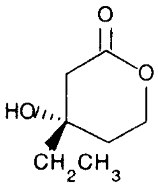
Coupling constants [$J_{2ax\text{-}3}$ = 5.3 Hz; $J_{2eq\text{-}3}$ = 5.1 Hz; $J_{4\text{-}5ax}$ = 9.9 Hz in C_5D_5N] and spatial proximities indicated by NOE enhancements [H-2ax/H-4; H-5ax/OH-3] appeared all explicable positioning H-3 and H-4 in equatorial and axial orientations, respectively; thus indicating their *cis* (3*S*,4*R* or 3*R*,4*S*) relationship.

Having completed the gross structural determination of **6**, we repeated for simplactone B (**7**) all the MS, IR, and NMR experiments. The analysis of the obtained results (reported in the Experimental Section) conduced to the same gross structure as **6**, and so we concluded that simplactone B (**7**) must differ only for the absolute configuration of one of the stereogenic centers C-3 and C-4. Indeed, compound **7** possessed the *trans* (3*S*,4*S*/3*R*,4*R*) relative stereochemistry, in a stable conformation with both substituents in equatorial position, as evidenced by the coupling constant pattern of H-3 and H-4 [$J_{2ax\text{-}3}$ = 10.1 Hz; $J_{3\text{-}4}$ = 10.1 Hz; $J_{4\text{-}5ax}$ = 10.5 Hz in $CDCl_3$] and by the diagnostic spatial couplings of H-3 with H-5ax and of H-2ax with H-4.

The absolute configurations of the diastereomeric lactones **6** and **7** have been determined by application of the modified Mosher method for secondary alcohols.[6] Treatment of two aliquots of **7** with (-) and (+) MTPA chlorides in dry pyridine at room temperature readily afforded **7a** (*S* ester) and **7b** (*R* ester), respectively, whose Δδ (*S*-*R*) values (see Experimental Section) pointed to the *R* configuration at C-3 of **7**, and, consequently, to the 3*R*,4*R* absolute stereochemistry of simplactone B. On the contrary, when we treated compound **6** with MTPA chlorides, in the same conditions, no reaction was observed, probably due to the steric hindrance around the axial hydroxyl group. However, after addition of DMAP, and carrying out the reaction at 70 °C overnight, **6a** (*S* MTPA ester) and **6b** (*R* MTPA ester) were obtained in reasonable yields. Application of the Mosher model to the measured Δδ (*S*-*R*) values indicated the *R* configuration at C-3, thus assigning to **6** the 3*R*,4*S* stereochemistry.

Simple lactone compounds are quite uncommon among *Plakortis* secondary metabolites, the only representative being, to our knowledge, a series of γ-lactone derivatives found in *Plakortis lita*.[7] On the other hand, some interesting δ-lactones, structurally similar to simplactones, have been isolated from marine and terrestrial natural sources, and many of them are endowed with peculiar bioactivities. Significant examples comprise (i) homomevalonolactone (**9**),[8] an isomer of simplactones, potential inhibitor of insect juvenile hormone biosynthesis, (ii) compound **10**, isolated from *Seridium unicorne* possessing antifungal activity;[9] and (iii) malyngolide (**11**), a potent antibiotic from the green alga *Lyngbia majuscula*.[10]



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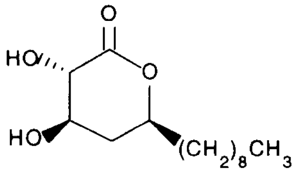

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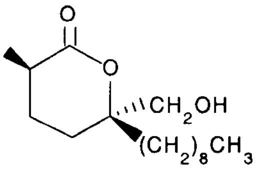

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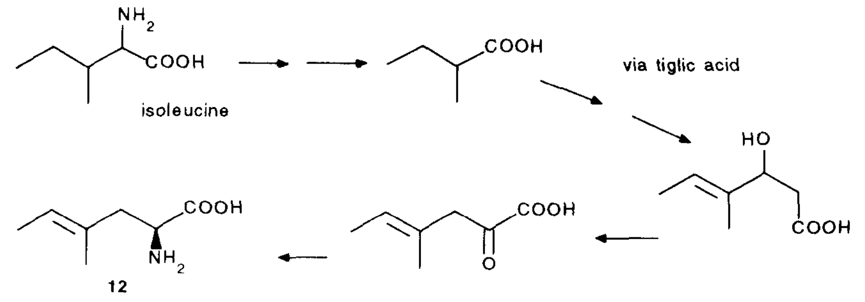
We have evaluated both simplactones A and B for in vitro cytotoxic activity against WEHI 164, and only a very mild cytotoxicity (IC_{50} ca. 20 µg/mL) was detected. Furthermore, simplactones proved to be inactive as antibiotic agents in tests against Gram positive and Gram negative bacteria, and against the fungus *Aspergillus niger*. Comparing the chemical structure of simplactones with those of compounds **10** and **11**, we should conclude that probably the presence of a long alkyl chain is one of the requirements for the antibiotic activity of δ-lactones.

The novel amino acid **8**, which appears structurally related to simplactones, has been also found among the components of fraction A_2. Compound **8**, was isolated as a colorless solid (mg 6.5), $[\alpha]_D$ -2, and its molecular formula was determined as $C_7H_{13}NO_2$ from HR-ESMS [calcd. for ($M+H^+$): *m/z* 144.1025; found *m/z* 144.1035]. The amino acidic nature of **8** was initially deduced by positive reaction with ninhydrin and by the absorption peak in the IR (KBr) spectrum at ν_{max} 1740 cm^{-1}, typical value of amino acid carboxylate stretching. The ^1H NMR (CD_3OD) spectrum of **8**, studied with the aid of a COSY spectrum, showed the presence of two separate spin systems (CH_2CH_3 and CH_2CH groups, respectively), in addition to two 1H broad singlets resonating at δ 4.84 and 5.09, respectively. These last protons must be linked to the same sp^2 carbon at δ 108.5, as revealed by inspection of the HMQC spectrum of **8**. In addition, the ^{13}C NMR spectrum contained two quaternary sp^2 carbons at δ 154.3 and 180.5, respectively, the latter resonance being attributable to the carboxylic group.

The above MS and NMR data, corroborated by HMBC correlation peaks between C-5 and H_2-7 and between C-7 and H_2-3, appeared sufficient to unambiguously define the gross structure of **8** as depicted in figure. Furthermore, the NOE contacts of the singlet at δ 5.09 with H_2-3 and of that at δ 4.84 with H_2-5 were used to assign the NMR resonances of the sp^2 methylene protons. Finally, absolute configuration at the chiral center C-2 has been deduced as *L* (*S*) by the CD spectrum in water (λ_{max} 212 nm; $\Delta\varepsilon + 0.41$) of **8**: when no unusual conformational constraints and no other interfering chromophores are present in the molecule, a positive sign of the Cotton effect is indicative of *L* stereochemistry at the aminoacidic center.[11]

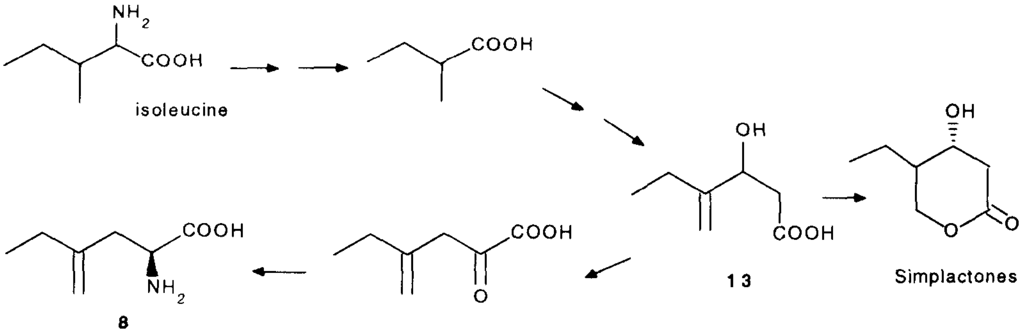
Compound **8**, evaluated for in vitro cytotoxic activity against WEHI 164, exhibited a moderate cytotoxicity at 72 h (IC_{50} 15 µg/mL), possibly acting as an antimetabolite.

Unusual amino acids have already been found among sponge metabolites; however, most of them only as constituents of complex molecules such as cyclic peptides or diketopiperazines. On the other hand, an inspection of literature revealed that a natural compound structurally similar to **8**, namely its isomer 2-amino-4-methylhex-4-enoic acid (**12**), was isolated some years ago from a terrestrial source, the fresh mature seeds of *Aesculus californica*.[12] By using ^{14}C-labelled precursor feeding, Fowden and coworkers have unambiguously demonstrated that biosynthesis of **12** starts from isoleucine and then follows the pattern of reactions summarized in Scheme 1.[13]



Scheme 1. Demonstrated biogenetic pathway of compound **12**

Most likely the isomeric amino acid **8** originates by the very similar pathway shown in Scheme 2, which differs only for positioning of the alkyl chain double bond. Simplactones, which possess the same carbon chain of compound **8**, could be reasonably generated by the cyclization of the postulated intermediate **13** (Scheme 2).



Scheme 2. Hypothesized biogenesis of simplactones and of aminoacid **8**

EXPERIMENTAL SECTION

General Methods. Optical rotations were measured in $CHCl_3$ or in MeOH on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (λ = 589 nm) and a 10-cm microcell. IR (KBr) spectra were recorded on a Bruker model IFS-48 spectrophotometer. UV spectra were obtained in CH_3CN and H_2O using a Beckman DU70 spectrophotometer. CD spectrum was obtained on a JASCO 500A polarimeter. Low and high resolution ESMS and EI (70 or 45 eV) mass spectra were performed on a VG Prospec (FISONS) mass spectrometer. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Bruker AMX-500 spectrometer; chemical shifts are referenced to the residual solvent signal (for $CDCl_3$: $\delta_H = 7.26$, $\delta_C = 77.0$; for CD_3OD: $\delta_H = 3.34$, $\delta_C = 49.0$; for C_5D_5N: $\delta_H = 7.55$). Homonuclear ^1H connectivities were determined by using COSY experiments. One bond heteronuclear ^1H-^{13}C connectivities were determined with HMQC pulse sequence using a BIRD pulse of 0.50 s before each scan in order to suppress signals originating from protons not directly bound to ^{13}C (interpulse delay set for $^1J_{CH} = 130$ Hz). During the acquisition time, ^{13}C broad band decoupling was performed using the GARP sequence. Two and three bond ^1H-^{13}C connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 9.0 Hz. Medium-pressure liquid chromatographies (MPLC) were performed using a Büchi 861 apparatus with RP18 and SiO_2 (230-400 mesh) stationary phases. High performance liquid chromatography (HPLC) separations were achieved on a Beckman apparatus equipped with RI detector and LUNA Si60 (250 x 4 mm) or reverse phase (C_{18}, 250 x 4 mm) columns.

Collection, extraction and purification. A specimen of *Plakortis simplex* was collected in Summer 1992 along the coasts of Little San Salvador Island, and identified by Prof. M. Pansini (Università di Genova). A voucher specimen has been deposited at the Istituto di Zoologia, Università di Genova, Italy, with the ref. n° 2006. The organism was immediately frozen after collection and kept frozen until extraction, when the sponge (57 g, dry weight after extraction) was homogenized and extracted with methanol (4 x 500 mL) and with chloroform (4 x 500 mL). The methanol extract was initially partitioned between H_2O and *n*-BuOH and then the organic phases were combined and concentrated *in vacuo* affording 29.3 g of a brown-colored viscous oil. This was subjected to chromatography on a column packed with RP18 silica gel and eluted with H_2O/MeOH 9:1 (A_1), H_2O/MeOH 7:3 (A_2), H_2O/MeOH 4:6 (A_3), H_2O/MeOH 2:8 (A_4), and H_2O/MeOH 1:9 (A_5). Combined fractions A_4 and A_5 (13.3 g) were further chromatographed by MPLC (SiO_2 230-400 mesh; solvent gradient system of increasing polarity from *n*-hexane to EtOAc/MeOH 1:1). Fractions eluted with *n*-hexane/EtOAc 7:3 and 8:2 were separately rechromatographed by HPLC (eluent *n*-hexane/EtOAc 94:6, flow 1.0 mL/min) affording plakortones B (**1**, 1.0 g), C (**2**, 4.2 mg), and D (**3**, 9.2 mg), and the novel plakortones E (**4**, 1.2 mg) and F (**5**, 1.3 mg) in a pure state. Fraction A_2 was separated by MPLC (SiO_2 230-400 mesh; solvent gradient system of increasing polarity from EtOAc to MeOH) and fractions eluted with EtOAc were rechromatographed by HPLC (eluent *n*-hexane/EtOAc 2:8, flow 0.6 mL/min) and furnished simplactone A (**6**, 4.6 mg) and B (**7**, 1.3 mg). Fractions eluted with EtOAc/MeOH 6:4 were purified by reverse phase HPLC (eluent H_2O/MeOH 7:3, flow 0.5 mL/min) yielding 6.5 mg of the novel amino acid **8**, in the pure state.

Plakortone E (**4**). Colorless oil. $[\alpha]_D^{25}$ -10 (c = 0.001 in $CHCl_3$); IR (KBr) ν_{max} 1786, 1679, 1631, 1480 cm^{-1}; UV (CH_3CN) λ_{max} 214, 230 nm. ^1H and ^{13}C NMR ($CDCl_3$): see Table 1. EIMS (45 eV) *m/z* 294 (M), 265 (M – CH_2CH_3), 183 (base peak, M – side chain: C_8H_{15}); HREIMS: *m/z* 294.2204, calcd. for $C_{18}H_{30}O_3$, 294.2195; *m/z* 183.1026, calcd. for $C_{10}H_{15}O_3$ *m/z* 183.1021. HMBC and ROESY correlations: see fig. 1.

Plakortone F (**5**). Colorless oil. $[\alpha]_D^{25}$ -11 (c = 0.001 in $CHCl_3$); IR (KBr) ν_{max} 1780, 1674, 1466 cm^{-1}; UV (CH_3CN) λ_{max} 220, 227 nm. ^1H and ^{13}C NMR ($CDCl_3$): see Table 1. HMBC ($CDCl_3$): H-3/C-1; H_2-2/C-4; H_2-5/C-20; H_2-5/C-18; H_2-7/C-18; H_2-7/C-6; H-8/C-6; H_3-21/C-4. ROESY ($CDCl_3$): H-3/H_2-20; H-3/H-7a. EIMS (45 eV) *m/z* 338 (M), 309 (M – CH_2CH_3), 183 (base peak, M – side chain: $C_{11}H_{23}$); HREIMS: *m/z* 338.2826, calcd. for $C_{21}H_{38}O_3$, *m/z* 338.2821.

Simplactone A (**6**). Colorless amorphous solid. $[\alpha]_D^{25}$ -3 (c = 0.002 in $CHCl_3$); IR (KBr) ν_{max} 3420, 2925, 1718, 1467, 1233 cm^{-1}. ^{13}C NMR ($CDCl_3$): δ 170.2 (s, C-1); 69.0 (d, C-3); 68.1 (t, C-5); 42.4 (d, C-4); 38.2 (t, C-2); 21.6 (t, C-6); 11.2 (q, C-7). ^1H NMR ($CDCl_3$): δ 4.49 (H-5eq, dd, *J* = 11.4, 4.4 Hz), 3.98 (H-3, overlapped), 3.97 (H-5ax, overlapped), 2.88 (H-2eq, dd, *J* = 17.5, 5.8 Hz), 2.55 (H-2ax, dd, *J* = 17.5, 5.5 Hz), 1.84 (OH-3, bd, *J* = 4.1 Hz), 1.78 (H-4, m), 1.65 (H-6a, m), 1.36 (H-6b, m), 1.01 (H_3-7, t, *J* = 7.5 Hz). ^1H NMR (C_5D_5CN): 4.68 (H-5eq, dd, *J* = 11.4, 4.0 Hz), 4.28 (H-3, m), 4.13 (H-5ax, dd, *J* = 11.4, 9.8), 3.26 (H-2eq, dd, *J* = 17.0, 5.1 Hz), 3.03 (H-2ax, dd, *J* = 17.0, 5.3 Hz), 2.14 (OH-3, bs), 2.08 (H-4, m), 1.80 (H-6a, m), 1.45 (H-6b, m), 1.01 (H_3-7, t, *J* = 6.3 Hz). EIMS (70 eV) *m/z* 144 (M), 126 (M – H_2O), 111 (M – H_2O - CH_3); 96 (base peak, M – H_2O - CH_3CH_3); HREIMS: *m/z* 144.0793, calcd. for $C_7H_{12}O_3$; found *m/z* 144.0786.

Simplactone B (**7**). Colorless amorphous solid. $[\alpha]_D^{25}$ -6 (c = 0.001 in $CHCl_3$); IR (KBr) ν_{max} 3415, 2925, 1717, 1467, 1232 cm^{-1}. ^{13}C NMR ($CDCl_3$): δ 170.3 (s, C-1); 68.5 (d, C-3); 67.1 (t, C-5); 42.4 (d, C-4); 37.5 (t, C-2); 20.6 (t, C-6); 11.8 (q, C-7). ^1H NMR ($CDCl_3$): δ 4.36 (H-5ax, dd, *J* = 11.2, 10.5 Hz), 4.25 (H-5eq, dd, *J* = 11.2, 4.6 Hz), 4.21 (H-3, t, *J* = 10.1), 2.72 (H-2eq, overlapped), 2.71 (H-2ax, overlapped), 1.87 (H-4, m), 1.68 (OH-3, bd, *J* = 2.8 Hz), 1.47 (H-6a, m), 1.36 (H-6b, m), 1.00 (H_3-7, t, *J* = 7.3 Hz). EIMS (70 eV) *m/z* 144 (M), 126 (M – H_2O), 111 (M – H_2O - CH_3); 96 (base peak, M – H_2O - CH_3CH_3); HREIMS: *m/z* 144.0793, calcd. for $C_7H_{12}O_3$; found *m/z* 144.0782.

Preparation of MTPA esters of compounds **6** and **7**. Simplactone B (**7**) (0.9 mg) was dissolved in 120 μL of dry pyridine and divided into two aliquots. One of these was treated with 10 μL of (-) MTPA chloride, and the other with the same amount of (+) MTPA chloride. Both reaction mixtures were stirred at r.t., overnight, and, after removal of the solvent, they were purified by HPLC (SI60 column, eluent *n*-hexane/EtOAc 9:1), affording (*S*) MTPA ester **7a** (0.3 mg) and (*R*) MTPA ester **7b** (0.3 mg), respectively. Simplactone A (**6**) (1.2 mg) was dissolved in 160 μL of dry pyridine and divided into two aliquots. To the first one 20 μL of (-) MTPA chloride and a spatula tip of DMAP were added; the second one was treated with the same amount of (+) MTPA chloride and with DMAP. The reaction mixtures were stirred at 70 °C, 15 h, and, after HPLC purification (SI60 column, eluent *n*-hexane/EtOAc 9:1), (*S*) MTPA ester **6a** (0.4 mg) and (*R*) MTPA ester **6b** (0.4 mg), respectively, were obtained.

Compound **6a**. [(*S*) MTPA ester derivative of **6**]. Amorphous solid. (KBr) ν_{max} 1745, 1687, 1540 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.35 and 7.45 (MTPA phenyl protons, m), 5.16 (H-3, m), 4.34 (H-5a, dd, *J* = 12.5, 5.8), 4.02 (H-5b, dd, *J* = 12.5, 4.3), 3.69 (MTPA $OC\underline{H}_3$, s), 2.97 (H-2a, dd, *J* = 17.1, 9.1 Hz), 2.50 (H-2b, dd, *J* = 17.1, 5.4 Hz), 1.94 (H-4, m), 1.48 (H-6a, m), 1.34 (H-6b, m), 0.94 (H_3-7, t, *J* = 7.3 Hz). FABMS (glycerol matrix, positive ions) *m/z* 361 $[M+H]^+$. *Compound* **6b**. [(*R*) MTPA ester derivative of **6**]. Amorphous solid. (KBr) ν_{max} 1744, 1687, 1540 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.35 and 7.55 (MTPA phenyl protons, m), 5.29 (H-3, m), 4.36 (H-5a, dd, *J* = 12.0, 5.0), 4.17 (H-5b, dd, *J* = 12.0, 4.2), 3.64 (MTPA $OC\underline{H}_3$, s), 2.83 (H-2a, dd, *J* = 17.3, 8.8 Hz), 2.29 (H-2b, dd, *J* = 17.3, 5.0 Hz), 1.98 (H-4, m), 1.56 (H-6a, m), 1.36 (H-6b, m), 0.98 (H_3-7, t, *J* = 7.0 Hz). FABMS (glycerol matrix, positive ions) *m/z* 361 $[M+H]^+$. *^1H NMR chemical shift differences* (δ_{6a} - δ_{6b}): + 0.14 ppm (H-2a), + 0.21 ppm (H-2b), - 0.13 ppm (H-3), - 0.04 ppm (H-4), - 0.02 ppm (H-5a), - 0.15 ppm (H-5b), - 0.02 ppm (H-6a), - 0.08 ppm (H-6b), - 0.04 ppm (H_3-7).

Compound **7a**. [(*S*) MTPA ester derivative of **7**]. Amorphous solid. (KBr) ν_{max} 1743, 1690, 1535 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.35 and 7.45 (MTPA phenyl protons, m), 5.34 (H-3, t, *J* = 9.5), 4.11 (H-5a, dd, *J* = 11.5, 10.0), 4.05 (H-5b, dd, *J* = 11.5, 4.6), 3.68 (MTPA $OC\underline{H}_3$, s), 2.86 (H_2-2, m), 2.00 (H-4, m), 1.50 (H-6a, m), 1.40 (H-6b, m), 0.93 (H_3-7, t, *J* = 7.3 Hz). FABMS (glycerol matrix, positive ions) *m/z* 361 $[M+H]^+$. *Compound* **7b**. [(*R*) MTPA ester derivative of **7**]. Amorphous solid. (KBr) ν_{max} 1744, 1692, 1535 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.35 and 7.55 (MTPA phenyl protons, m), 5.34 (H-3, t, *J* = 9.8), 4.11 (H-5a, dd, *J* = 11.5, 10.0), 4.05 (H-5b, dd, *J* = 11.5, 4.5), 3.64 (MTPA $OC\underline{H}_3$, s), 2.85 (H_2-2, m), 2.01 (H-4, m), 1.51 (H-6a, m), 1.41 (H-6b, m), 0.94 (H_3-7, t, *J* = 7.3 Hz). FABMS (glycerol matrix, positive ions) *m/z* 361 $[M+H]^+$. *^1H NMR chemical shift differences* (δ_{7a} - δ_{7b}): + 0.01 ppm (H_2-2), - 0.01 ppm (H-4), - 0.02 ppm (H-6a), - 0.01 ppm (H-6b), - 0.01 ppm (H_3-7).

Compound **8**. Colorless solid. $[\alpha]_D^{25}$ -2 (c = 0.005 in MeOH); IR (KBr) ν_{max} 3450, 3050, 2125, 1740 cm^{-1}. CD (H_2O): λ_{max} 212 nm; Δε + 0.41. ^{13}C NMR (CD_3OD): δ 180.5 (C-1); 154.3 (C-4); 108.5 (C-7); 73.4 (C-2); 44.2 (C-3); 22.7 (C-5); 12.7 (C-6). ^1H NMR (CD_3OD): δ 5.09 (H-7a, br.s), 4.84 (H-7b, br.s), 4.43 (H-2, dd, *J* =

9.4, 4.2 Hz), 2.45 (H-3a, dd, J = 15.1, 4.0 Hz), 2.34 (H-3b, J = 15.1, 8.8 Hz), 2.16 (H-5a, m), 2.09 (H-5b, m), 1.10 (H_3-6, t, J = 7.3 Hz). HRESMS: calcd. for $C_7H_{14}NO_2$ ($M+H^+$): *m/z* 144.1025; found *m/z* 144.1035.

Cytotoxicity assay. WEHI 164 (1×10^4 cells) have been plated on 96-well plates in 50 µL and allowed to adhere at 37°C in 5% CO_2/95% air for 2 h in Dulbecco's modified Eagle's medium. The medium was then replaced with 50 µL of fresh medium, and 50 µL of 1:4 v/v serial dilution of test compounds **1-8** were added and the cells were incubated for 72 h. The cell viability was assessed through the MTT conversion assay. After incubation, 25 µL of MTT (5 mg/mL) were added to each cell, and the cells were incubated for additional 3 h. After this time, the cells were lyzed and the dark blue crystals solubilized with 100 µL of a solution containing 50% v/v SDS with an pH adjusted at 4.5. The optical density (OD) of each cell was measured with a microplate spectrophotometer equipped with a 620-nm filter. The viability of cell line in response to treatment with each compounds **1-8** was calculated as % dead cells = 100 - (OD treated/OD control) x 100. The results are expressed in the text as IC_{50} (the concentration that inhibited the cell growth by 50%).

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