Dragmacidol A and dragmacidolide A from the Australian marine sponge *Dragmacidon australe*

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Abstract

Two new secondary metabolites, the xylytrythrin derivative dragmacidol A (**1**) and the steroid dragmacidolide A (**2**), were isolated from a specimen of the Australian marine sponge *Dragmacidon australe*, collected off the coast of the Whitsunday Islands in Queensland, Australia. Their structures were fully characterized via analysis of 1D/2D NMR and MS data. The proton-poor structure of **1** was also supported through successful ¹³C DFT-NMR calculations. The isolation of these two metabolites from this sponge genus is quite unusual, given that previous reports of *Dragmacidon* secondary metabolites have been dominated by indole and β -carboline alkaloids.

Keywords

Dragmacidon australe; natural products; dragmacidol A; dragmacidolide A; DFT-NMR.

1. Introduction

Marine sponges continue to yield new and unusual natural products.¹ The sponge genus *Dragmacidon* (Order: Halichondrida, Family: Axinellidae) has afforded a number of tryptophan-derived metabolites, including the bis-indole alkaloids dragmacidin (**3**),²⁻⁴ nortopsentin E (**4**),³⁻⁵ and the β -carboline alkaloids, dragmacidonamines A (**5**) and B (**6**) (Fig. 1).^{6,7} Recently, the unique nucleoside dragmacidoside (**7**) was also reported.⁸ In particular, the reported *Dragmacidon* alkaloids have complex architectures, and some have been shown to exhibit cytotoxicity towards various cancer cell lines.^{2,4,5}

Fig. 1 Structures of previously reported *Dragmacidon* metabolites: dragmacidin (**3**), nortopsentin E (**4**), dragmacidonamines A (**5**) and B (**6**), and dragmacidoside (**7**)

Dragmacidon, however, is a poorly investigated genus, with only the five new metabolites discussed above being reported to date. We therefore embarked on an investigation of a *Dragmacidon* specimen in order to gain more knowledge of its chemistry, including the discovery of new compounds of interest. The specimen examined in this study, *D. australe* (previously known as *Pseudaxinella australis* Bergquist, 1970), was collected by SCUBA off the coast of the Whitsunday Islands in Queensland, Australia. The MeOH/CH₂Cl₂ extract of a freeze-dried and ground specimen was chromatographed via RP-HPLC (MeOH/H₂O/0.1% TFA), affording the new compounds dragmacidol A (**1**) and dragmacidolide A (**2**) (Fig. 2).

Fig. 2 Chemical structures of dragmacidol A (**1**) and dragmacidolide A (**2**)

2. Results and discussion

Compound **1** (4.0 mg, 0.008% dry wt.) was obtained as an optically active yellow gum. HRESIMS analysis yielded a molecular formula of C₂₇H₁₈O₆, corresponding to 19 degrees of unsaturation. The ¹H NMR spectrum contained two exchangeable protons [δ_{H} 7.44 (br s, 1H), and δ_{H} 9.43 (br s, 1H)], two diastereotopic methylene doublets [δ_{H} 6.11/6.06 (2H)], and seven aromatic multiplets displaying second-order coupling effects [δ_{H} 6.56 (2H), 7.11 (2H), 7.16 (3H), 7.17 (2H), 7.44 (1H), 7.53 (2H) and 7.73 (2H)] (Table 1). A COSY experiment combined with ¹H NMR integration data confirmed the presence of three spin systems, corresponding to one di-substituted (δ_{H} 7.17, 6.56, ring B) and two mono-substituted

aromatic rings [(δ_{H} 7.44, 7.53, 7.73, ring A) and (δ_{H} 7.11, 7.16, 7.16, ring C)] (**Fig. 3**). These three rings accounted for 12 degrees of unsaturation.

Table 1
NMR data for dragmacidol A (**1**) in DMSO- d_6

Position	^1H , mult. (J in Hz) ^a	^{13}C , type ^b	COSY	HMBC	ROESY
2		176.2, C			
3		77.1, C			
3-OH	7.44, s			1''', 3, 3a	
3a		120.8, C			
4		121.2, C			
4a		142.2, C ^c			
6a	6.11, d (0.9)	101.4, CH ₂	6b	4a, 7a	
6b	6.06, d (0.9)		6a	4a, 7a	
7a		145.6, C ^c			
8		107.3, C			
8a		145.0, C			
1'		130.0, C			
2'/6'	7.73–7.74, m	129.4, CH	3', 5'	2', 4', 6', 8	3', 5'
3'/5'	7.53–7.56, m	128.5, CH	2', 4', 6'	1', 3', 5'	2', 4', 6'
4'	7.44–7.46, m	128.2, CH	3', 5'	2', 6'	3', 5'
1''		122.2, C			
2''/6''	7.17–7.18, m	131.0, CH	3'', 5''	4, 4''	3'', 5''
3''/5''	6.56–6.57, m	114.2, CH	2'', 6''	1'', 3'', 5''	2'', 4''-OH, 6''
4''		156.9, C			
4''-OH	9.43, s			3'', 5''	3'', 5''
1'''		138.6, C			
2'''/6'''	7.11–7.13, m	125.2, CH	3''', 5'''	2''', 3, 3''', 4''', 6'''	
3'''/5'''	7.16–7.17 ^d , m	127.9, CH	2''', 6'''	1''', 3''', 5'''	
4'''	7.16–7.17 ^d , m	127.7, CH		2''', 6'''	

^a ^1H NMR spectrum recorded at 600 MHz

^b ^{13}C NMR spectrum recorded at 125 MHz

^c ^{13}C NMR resonances are interchangeable

^d overlapping signals

Due to the presence of six chemically equivalent aromatic carbon atoms in rings A–C (**Fig. 3**), only 21 signals were observed in the ^{13}C NMR spectrum. This spectrum also revealed the presence of an ester (δ_{C} 176.2), an sp^3 hybridized oxygenated carbon (δ_{C} 77.1) and an acetal (δ_{C} 101.4). An additional six non-protonated sp^2 aromatic carbon resonances

(δ_C 145.0, 120.8, 121.2, 145.6, 142.2 and 107.3) were noted following HSQC data analysis. In order to account for further degrees of unsaturation, these six carbon atoms were proposed to be part of a hexa-substituted benzene system (ring D).

Fig. 3 COSY and key HMBC correlations for **1**

An HMBC correlation from an exchangeable proton δ_H 9.43 (4''-OH) to the carbon at δ_C 114.2 (C-3''/5'') indicated that ring B was *para*-hydroxylated (**Fig. 3**). The non-equivalent acetal protons at δ_H 6.06 (H-6a), and 6.11 (H-6b) both showed HMBC couplings to carbons resonating at δ_C 145.6 and δ_C 142.2 (C-4a/C-7a) of ring D, thus forming an additional five-membered ring (ring E). Rings A and B were each found to be bonded to ring D through HMBC analysis. This was deduced via a correlation from δ_H 7.73 (H-2') to δ_C 107.3 (C-8), and from δ_H 7.17 (H-2'') to δ_C 121.2 (C-4). A weak $^4J_{CH}$ correlation was observed from H-2'' to C-4a in the HMBC experiment conducted in CD_3OD , further confirming the position of ring B with respect to ring D. The exchangeable signal at δ_H 7.44 (3-OH) showed HMBC correlations to δ_C 77.1 (C-3), δ_C 138.6 (C-1''', ring C) and δ_C 120.8 (C-3a, ring D), linking rings C and D through the tertiary alcohol at C-3. While the remaining non-protonated sp^2 aromatic carbon at δ_C 145.0 (C-8a, ring D) did not couple to any protons in the HMBC experiment, the relative downfield shift suggested that it was oxygenated. The only oxygen atoms that remained to be assigned belonged to the ester (C-2, δ_C 176.2). Therefore, through a process of elimination, C-8a was proposed as the site of esterification. Finally, formation of a

lactone (ring F) between C-2 and C-8a, accounted for the remaining degree of unsaturation. Thus, the NMR and MS data strongly supported the proposed structure (**1**). However, owing to the inability to directly observe that C-8a was connected to C-3 and C-7a through the HMBC data, we sought further structural confirmation of **1**. Ideally, this confirmation would have been obtained through X-ray structure analysis. However, attempts to grow a crystal of **1** suitable for X-ray diffraction studies were unsuccessful, and we thus explored alternative methods of structure verification, including DFT-NMR analysis. Our group has previously had success calculating ^{13}C DFT-NMR chemical shifts for several marine natural products, with theoretical data in good agreement with the experimental chemical shifts.^{9,10}

Hence, compound **1** was subjected to molecular mechanics energy minimization and conformational searches using MMFFs.¹¹ All conformers resulting from this search identified within 20 kJ/mol of the global minimum energy structure were clustered by relative atomic coordinates. Following this, the lowest energy conformer of each cluster was then optimized using DFT with the B3LYP/6-311G(d,p) functional and basis set combination (see Supplementary data).¹²⁻¹⁴ Single-point calculations were determined using MPW1PW91/6-311+G(d,p), incorporating implicit PBF solvation which were performed on each DFT-optimized structure.¹⁵⁻¹⁷ This was followed by the calculation of NMR shielding constants via gauge including atomic orbitals (GIAO) methodology.¹⁵⁻¹⁷ Reference compounds with published experimental NMR data were subject to the same computational methodology and the results used to generate a linear regression model, which correlated observed chemical shifts with the calculated shielding constants.^{9,10,18} The multi-reference regression model was therefore used to scale calculated shielding constants to give ^{13}C NMR chemical shifts for **1**. The calculated NMR chemical shifts for dragmacidol A (see **Table 2**) were in very good agreement with the experimental chemical shifts.

Table 2Experimental and theoretical (DFT-NMR) ^{13}C NMR data for **1** in $\text{DMSO-}d_6$

Position	$^{13}\text{C}_{\text{exp.}}$	$^{13}\text{C}_{\text{theo.}}$	$ ^{13}\text{C}_{\text{exp.}} - ^{13}\text{C}_{\text{theo.}} $
2	176.2	174.2	2.0
3	77.1	78.7	1.6
3a	120.8	118.7	2.1
4	121.2	121.1	0.1
4a	145.6 ^a	143.7	1.9
6	101.4	98.4	3.0
7a	142.2 ^a	140.1	2.1
8	107.3	106.9	0.4
8a	145.0	143.3	1.7
1'	130.0	128.0	2.0
2'/6'	129.4	127.1	2.3
3'/5'	128.5	125.9	2.6
4'	128.2	125.7	2.5
1''	122.2	121.7	0.5
2''/6''	131.0	129.8	1.2
3''/5''	114.2	111.4	2.8
4''	156.9	153.0	3.9
1'''	138.6	135.1	3.5
2'''/6'''	125.2	123.6	1.6
3'''/5'''	127.9	125.7	2.2
4'''	127.7	126.0	1.7
MAD^b		2.0	
MD^c		3.9	

^a ^{13}C NMR resonances are interchangeable^b MAD = mean absolute deviation^c MD = maximum deviation

The greatest difference between calculated and experimental chemical shift data occurred for C-4'' (Δ 3.9 ppm), the phenolic carbon. However, the mean absolute deviation (MAD) for this compound was computed to be 2.0 ppm, well within the acceptable range (< 5.0 ppm) suggested previously.^{9,10,19-21} Based on this extensive analysis, we concluded that all ^{13}C NMR shifts had been assigned correctly and that the carbon-carbon bond connectivity was correct, and thus **1** was assigned to dragmacidol A. Whilst the specific rotation data for **1** identified that dragmacidol A had been purified as a single enantiomer (or at least an enriched enantiomeric mixture), the configuration at C-3 remains unassigned.

Dragmacidol A is related to previously published natural products that all possess the same carbon framework.²² For example, a number of compounds have been isolated from terrestrial macrofungi [*Phanerochaete (Peniophora) sanguinea*²³], including peniophorin (**8**)^{22,24}, xylerythrin (**9**)^{22,25}, 5-hydroxy-3,4,7-triphenyl-2,6-benzofurandione (**10**)²⁶ and xylerythrinin (**11**)^{22,27} (**Fig. 4**).

Fig. 4 Chemical structures for peniophorin (**8**), xylerythrin (**9**), 5-hydroxy-3,4,7-triphenyl-2,6-benzofurandione (**10**) and xylerythrinin (**11**)

These compounds are ultimately biosynthesized by reaction of three phenylpropane units (e.g. from phenylalanine or tyrosine).^{22,28,29} In the case of **1** (**Scheme 1**), two units of phenylpyruvic acid (**i**) could undergo a Claisen condensation to yield **ii**, which undergoes tautomerization to yield polyporic acid (**iii**).^{22,28,29} Enzymatic oxidative cleavage of **iii** could yield the *cis* 1,2-dicarboxylic acid (**iv**), which could subsequently rearrange to the *trans* 1,2-dicarboxylic acid (**v**). A pulvinic acid intermediate (**vi**) could potentially be formed through the lactonization of **v**.²² Addition of one unit of 4-hydroxypyruvic acid (enol form, **vii**) and decarboxylation would afford the xylerythrin-type skeleton, **viii**.²² Hydroxylation of **viii** to **ix** followed by acetal formation of **ix** could lead to dragmacidol A (**1**).

Scheme 1 Proposed biogenesis of drarmacidol A (**1**)

Compound **2** was also obtained as an optically active yellow gum following RP-HPLC of the crude extract. HRESIMS analysis yielded a molecular formula of $C_{31}H_{42}O_{10}$ (11 degrees of unsaturation). The 1H NMR spectrum revealed: three exchangeable protons (δ_H 7.39, 6.92, 6.33), one methyl doublet (δ_H 0.96), three methyl singlets (δ_H 2.01, 2.05, 2.20), one sp^2 methine singlet (δ_H 6.20), two mid-field methine signals (δ_H 4.99, 4.60), and a series of mid-field methylene signals (δ_H 4.81/4.63, 4.77/4.74, and 4.41/4.37) (**Table 3**). Furthermore, numerous methylene and methine signals were observed upfield between δ_H 1.20 and 2.97. The aliphatic nature of the molecule (as indicated by the 1H NMR spectrum) and number of carbon atoms (indicated by 2D NMR spectroscopy and HRESIMS) tentatively suggested the presence of a steroid (**Fig. 5**).

Fig. 5 COSY and key HMBC/ROESY correlations for **2**

Analysis of the HSQC and HMBC spectra revealed the ^{13}C NMR shifts of all 31 carbon atoms: four shifts were diagnostic of carbonyl groups (δ_{C} 199.8, 173.8, 170.7, 170.3), and at least five other shifts suggested oxygenated carbon atoms (δ_{C} 86.0, 80.3, 72.2, 66.3 and 61.8). Four steroidal spin systems were identified through COSY spectrum analysis. These included: a $\text{CH}_2\text{-CH}_2$ spin system (A), two $\text{CH}_2\text{-OH}$ spin systems (B and C), and an extended spin system (D). HMBC experiments established the presence of a conjugated enone through correlations from the proton singlet at δ_{H} 6.20 (H-4), to the methylene carbon at δ_{C} 35.2 (C-2), whose attached protons coupled to the carbonyl carbon at δ_{C} 199.8 (C-3).

Table 3
NMR data for dragmacidolide A (**2**) in pyridine-*d*₅

Position	¹ H, mult. (<i>J</i> in Hz) ^a	¹³ C, type ^b	HMBC	ROESY
1a	2.43, m	34.4, CH ₂	3, 10	1b, 2a, 19a, 19b
1b	1.66, ddd (4.6, 15.3, 14.6)		2, 9, 10, 19	1a, 2b, 9
2a	2.91, ddd (5.2, 15.3, 17.2)	35.2, CH ₂	1, 3	2a, 19a
2b	2.46, m		3, 10	1a, 1b, 2a
3		199.8, C		
4	6.20, s	128.3, CH	2, 6, 10	6
5		165.7, C		
6	4.60, br s	72.2, CH	8, 10	4, 7a, 7b, 6-OH, 8, 19a, 19b
6-OH	7.39, s			6
7a	2.25, m	39.4, CH ₂	5, 8	6
7b	1.32, m			6
8	2.76, dddd (3.6, 11.7, 11.7, 11.7)	30.7, CH		6, 15b, 18a, 18b, 19a, 19b
9	1.24, ddd (4.1, 11.7, 13.2)	52.6, CH	10, 19	1b, 8, 12
10		43.9, C		
11a	2.29, m	29.6, CH ₂	9, 12	12
11b	2.25, m		9, 12	12
12	4.99, dd (5.8, 11.4)	80.3, CH	11, 17, 18, 28	9, 11a, 11b, 14, 17, 21
13		50.6, C		
14	1.31, m	55.2, CH	8, 13, 15	12, 15a, 17
15a	1.78, m	25.0, CH ₂		15b
15b	1.53, m		14, 16	8, 15a, 18a, 18b
16a	2.13, m	30.6, CH ₂		
16b	1.74, m			
17	1.95, m	53.4, CH	12, 13, 16, 18, 20, 21	12, 14, 21
18a	4.81, d (12.5)	61.8, CH ₂	13, 14, 17, 30	8, 15b, 20, 21
18b	4.63, d (12.5)		13, 14, 17, 30	8, 15b, 20, 21
19a	4.41, d (10.7)	66.3, CH ₂	1, 5, 9, 10	1a, 2a, 6, 8, 19-OH
19b	4.37, d (10.7)		1, 5, 9, 10	1a, 2a, 6, 8, 19-OH
19-OH	6.33, s			19a, 19b
20	2.49, ddq (6.9, 10.7, 10.7)	39.2, CH		18a, 18b, 21, 22
21	0.96, d (6.9)	14.4, CH ₃	17, 20, 22	12, 18a, 18b, 20, 22, 27, 29
22	5.07, d (1.0)	86.0, CH	17, 20, 21, 23, 24	17, 20, 21, 27
23		162.3, C		
24		130.5, C		
25		173.8, C		
26a	4.77, d (13.0)	54.1, CH ₂	23, 24, 25	27
26b	4.74, d (13.0)		23, 24, 25	27
26-OH	6.92, s			
27	2.20, s	13.8, CH ₃	22, 23, 24	21, 22, 26a, 26b
28		170.3, C		
29	2.01, s	21.8, CH ₃	28	21
30		170.7, C		
31	2.05, s	21.4, CH ₃	30	

^a ¹H NMR spectra recorded at 900 MHz

^b ¹³C NMR shifts obtained from 2D NMR spectra (HSQC and HMBC)

HMBC correlations between δ_{H} 4.41/4.37 (H-19a/H-19b) and δ_{C} 34.4 (C-1), 165.7 (C-5) and 52.6 (C-9) linked spin system A, B and D through the quaternary carbon atom at δ_{C} 43.9 (C-10). Two of the carbonyl carbon atoms were connected to C-12 and C-18 through relevant long-range proton-carbon couplings. Specifically, HMBC crosspeaks were observed between

the protons δ_{H} 4.99 (H-12) and δ_{C} 170.3 (C-28), and δ_{H} 4.81/4.63 (H-18a/18b) and δ_{C} 170.7 (C-30) Methyl proton signals at δ_{H} 2.01 (H-29) and 2.05 (H-31) correlated to δ_{C} 170.3 (C-28) and 170.7 (C-30), respectively, in the HMBC spectrum, indicating the presence of two *O*-acetyl functionalities. The downfield methine doublet (δ_{H} 5.07, H-22) showed HMBC couplings to two unsaturated sp^2 alkene carbon atoms at δ_{C} 162.3 (C-23) and 130.5 (C-24). Spin system C was directly attached to this alkene through HMBC correlations between δ_{H} 4.77/4.74 (H-26a/H-26b) and C-23 (δ_{C} 162.3), C-24 (δ_{C} 130.5) and C-25 (δ_{C} 173.8). With one degree of unsaturation unaccounted for, a lactone connected to C-22 (δ_{C} 86.0) was proposed; this chemical shift was consistent with an oxygenated carbon atom. The NMR chemical shifts of this five-membered lactone were consistent with those reported for the butenolide (*S*)-(*E*) 3-(hydroxymethyl)-5-methoxy-4-methyl-5-(2-phenylethenyl)-furanone (**12**), which had been isolated from the terrestrial fungus *Favolaschia tonkinensis* (**Fig. 6**).³⁰ Therefore, the planar structure of dragmacidolide A was assigned as **2**.

Fig. 6 Selected ¹³C NMR data for the five-membered lactone of **2** (DMSO-*d*₆) and (*S*)-(*E*) 3-(hydroxymethyl)-5-methoxy-4-methyl-5-(2-phenylethenyl)-furanone (**12**, CDCl₃)

Through analysis of ROESY data, the relative configuration of **2** was determined to be 6*S*^{*}, 8*R*^{*}, 9*S*^{*}, 10*S*^{*}, 12*R*^{*}, 13*S*^{*}, 14*S*^{*} and 17*R*^{*}. Specifically, ROESY crosspeaks were observed between H-9 and H-12, H-12 and H-17, and H-17 and H-14, indicating that these protons were all on the same face of the steroid nucleus (**Fig. 5**). Additional crosspeaks between H-19 and H-8, H-19 and H-6, H-8 and H-6, as well as H-8 and H-18, supported the relative configurational assignment of **2** (shown in **Fig. 2**).

The relative configuration of C-20 and C-22 could not be assigned on the basis of the ROESY data since both H-21 and H-20 showed ROESY crosspeaks to H-18, indicating that free-rotation about this bond was possible.

3. Conclusions

Compounds **1** and **2** are the first report of non-alkaloid or non-nucleoside derived metabolites from the *Dragmacidon* genus. The fact that **1** is similar to known micro-fungal metabolites may suggest that a microbial symbiont is responsible for its production within the sponge. The proton poor structure of **1** was supported through a proposed biosynthesis that was consistent with the established secondary metabolism of related terrestrial fungal compounds, and was further corroborated through ^{13}C DFT NMR calculations.

4. Experimental section

4.1 General methods

UV spectra were recorded on a JASCO V-650 UV/VIS spectrophotometer. Optical rotations were recorded on a JASCO P-1020 polarimeter. All NMR spectra were recorded at 30 °C on a 500 or 600 MHz Unity INOVA spectrometer, or at 25 °C on a Bruker 900 MHz NMR spectrometer. The 600 and 900 MHz NMR spectrometer were each equipped with a triple-resonance cryo-probe. The ^1H and ^{13}C chemical shifts were referenced to the solvent peaks for DMSO- d_6 at δ_{H} 2.50 and δ_{C} 39.5, for CD $_3$ OD at δ_{H} 3.33 and δ_{C} 49.0 and for pyridine- d_5 at δ_{H} 8.71 and δ_{C} 149.9. LRESIMS were recorded on a Waters LCMS system equipped with a Luna analytical C $_{18}$ HPLC column (3 μm , 100 Å, 50 $\times$ 4.6 mm), a PDA detector, and a ZQ ESI mass spectrometer. HRESIMS was recorded on a Bruker Apex III 4.7 Tesla FT ion cyclotron resonance mass spectrometer. A Waters 600 controller/pump equipped with a Waters 996 PDA detector, a FLOM Gastorr 722 degasser and a Gilson 715 liquid handler

were used for all semipreparative HPLC work. Alltech Davisil C₁₈-bonded silica (35–75 µm, 150 Å) was used for preadsorption work. An Alltech stainless steel guard cartridge (10 × 30 mm) was used for packing the sponge extract that had been preadsorbed to the C₁₈-bonded silica. A ThermoElectron C₁₈ Betasil HPLC column (5 µm, 143 Å, 21.2 × 150 mm) was used for semipreparative work. All solvents used for extractions, chromatography, [α]_D, UV and MS were Lab-Scan HPLC grade. H₂O was Millipore Milli-Q PF filtered.

4.2 Animal material

The *D. australe* specimen was collected by SCUBA at a depth of 20 m from Round Reef (-19.9607, 149.6213) North-East of the Whitsunday Islands in Queensland, Australia in June of 1999. The sample was immediately frozen upon collection and transported to the Eskitis Institute, where it was freeze-dried, ground and stored as a dried powder in airtight containers in the dark in a temperature-controlled environment (~ 20 °C). A voucher specimen (QM G315273) has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia, and its description can be accessed at <http://bie.ala.org.au/species/Drarmacidon+australe#>.

4.3 Extraction and isolation

Freeze-dried and ground *D. australe* (52 g) was extracted with n-hexane (500 mL, 2 h), MeOH/CH₂Cl₂ (500 mL, 4:1, 1 h) and MeOH (600 mL, 17 h). The MeOH/CH₂Cl₂ and both MeOH extracts were combined and dried by rotary evaporation, yielding 2.1 g of crude sponge extract. This extract was pre-adsorbed onto C₁₈ silica (~1 g), packed into a stainless steel guard cartridge (10 × 30 mm) and attached in series to a C₁₈-bonded semipreparative HPLC column. Isocratic HPLC conditions of 90% H₂O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for the first 10 min, then a linear gradient to MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from the start of

the HPLC run. Based on the HPLC-generated UV data, fractions 5 to 10, and 20 to 55 were subjected to ^1H NMR spectroscopy and MS to ascertain the presence of compounds and their relative purity. Fraction 33 yielded dragmacidolide A (**2**, 0.8 mg, 0.0015% dry wt) and fraction 43 afforded dragmacidol A (**1**, 4.0 mg, 0.008% dry wt).

4.3.1 Dragmacidol A (1). Yellow gum; $[\alpha]_{\text{D}}^{25} +20$ (*c* 0.012, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (3.43), 324 (3.09) nm; ^1H and ^{13}C NMR data (DMSO- d_6), see Table 1; For further NMR data (CD $_3$ OD), see supplementary data; (+)-LRESIMS m/z 461 $[\text{M} + \text{Na}]^+$; (+)-HRESIMS m/z 461.1005 $[\text{M} + \text{Na}]^+$ (calcd. for C $_{26}$ H $_{18}$ O $_6$ Na, 461.1001).

4.3.2 Dragmacidolide A (2). Yellow/white gum; $[\alpha]_{\text{D}}^{28} +14$ (*c* 0.006, MeOH); UV (MeOH) λ_{max} (log ϵ) 275 (3.39) nm; ^1H and ^{13}C NMR data (pyridine- d_5), see Table 2; (+)-LRESIMS m/z 597 $[\text{M} + \text{Na}]^+$; (+)-HRESIMS 597.2693 $[\text{M} + \text{Na}]^+$ (calcd. for C $_{31}$ H $_{42}$ O $_{10}$ Na, 597.2670).

4.4 Computational procedures

All input structures were subject to geometry optimization in MacroModel using the MMFFs with H $_2$ O solvation, with default parameters and convergence criteria used unless otherwise stated. A conformational search was performed on each structure using the mixed MCMM/low-mode search with 1000 steps per rotatable bond and an energy window of 21 kJ mol $^{-1}$ for retention of conformers. The conformers were subjected to conformational clustering, and the lowest energy conformer from each cluster was taken for further studies. In total, five structurally distinct low-energy conformers were selected for more rigorous computational analysis. These conformers were subject to further gas-phase geometry optimization in Jaguar using the B3LYP functional and 6-311G(d,p) basis set. A fine integration grid with accurate SCF convergence criteria was employed, and all geometry-

optimized structures were characterized as true minima by the absence of imaginary vibrational frequencies at the stationary point. NMR shielding constants were calculated using the GIAO method as implemented in Jaguar using single-point MPW1PW91/6-311+G(d,p) calculations with the PBF implicit solvation model (DMSO). A multi-reference linear regression model was generated using the same computational methodology as described above for a range of commercially available compounds with experimental NMR shift data reported in DMSO-*d*₆. The theoretical shifts reported are the Boltzmann-weighted average of values calculated for each conformer using the regression model.

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Supplementary data

Supplementary data (including 1D and 2D NMR spectra of **1** and **2**, further NMR data of **1** recorded in CD₃OD, and additional computational data) associated with this article can be found at <http://dx.doi.org/>

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Graphical abstract