Sparticolins A–G, Biologically Active Oxidized Spirodioxynaphthalene Derivatives from the Ascomycete *Sparticola junci*

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ABSTRACT: To explore the chemical diversity of metabolites from new species of Dothideomycetes, the ex-type strain of *Sparticola junci* was investigated. Seven highly oxygenated and functionalized spirodioxynaphthalene natural products incorporating carboxyalkylidene-cyclopentanoid (**1-4**), carboxyl-functionalized oxabicyclo[3.3.0]octane (**5-6**), and annelated 2-cyclopentenone/ δ -lactone (**7**) units, sparticolins A–G, were isolated from submerged cultures of the fungus. Their chemical structures including their relative (and absolute) configurations, were established through spectroscopic and X-ray crystallographic analyses. Sparticolin B (**2**) exhibited inhibitory activity against the Gram-positive bacteria *Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*, while sparticolin G (**7**) showed antifungal activities against *Schizosaccharomyces pombe* and *Mucor hiemalis*. All other sparticolins were only weakly active against *S. aureus* and also showed weak activities against the nematode *Caenorhabditis elegans*. Compounds **2** and **7** also showed moderate cytotoxic activities against seven mammalian cell lines.

Fungal metabolites have been shown to be a prolific source of novel molecules that can serve as lead candidates for development of pharmaceutical drugs, agrochemical pesticides and other products for the life science industry.^{1,2} However, the majority of the fungal species still remain untapped for secondary metabolite production, and in particular, newly discovered plant-associated Ascomycota may be a good source of novel chemistry. During a study of the mycobiota of the plant genera *Clematis* and *Spartium*, several new taxa of Dothideomycetes were discovered and their cultures were studied for secondary metabolite production.³ Several of these strains were subjected to intensive studies because their extracts showed interesting biological activities in various bioassays. We have recently reported a new terpenoid from a *Roussoella* sp. associated with *Clematis* from Thailand that can significantly inhibit biofilm formation by the human pathogenic bacterium *Staphylococcus aureus*.⁴ In the current study, a type strain of *Sparticola* (Sporormiaceae, Pleosporales)³ from the Spanish broom, *Spartium junceum* (Fabaceae) found in Italy³ was explored for its secondary metabolism and associated biological activity. The present paper reports the isolation, structure elucidation, and biological characterization of seven unprecedented polyketides from the mycelial cultures of *Sparticola junci* Phukhamsakda, Camporesi & K.D. Hyde and represent the first secondary metabolites of the genus *Sparticola*.

RESULTS AND DISCUSSION

The ethyl acetate extract obtained from the Q6½ medium⁵ was fractionated by repeated reversed-phase preparative and semi-preparative HPLC to afford compounds **1–7**.

The NMR spectroscopic data for sparticolins A–G (**1–7**) suggested that they are new members in the spirodioxynaphthalene natural products family. The common structural moiety present in sparticolins **1–7** is a 1,8-dioxynaphthalene linked to highly functionalized cyclopentanoid, or 5,6-dihydro-2*H*-pyran-2-one (in the case of compound **7**) substructures, and can be readily correlated to a spiroketal carbon resonating at approximately 96–113 ppm in the ¹³C NMR spectrum.⁶ The NMR spectroscopic data of the dioxynaphthalene moiety of all seven compounds were almost the same (Tables 1 and 2). Discussion of the structure elucidation of the 1,8-naphthalene moiety is provided for sparticolin A (**1**) to serve as a general example. Discussion of the structures of sparticolins B–G (**2–7**) focused on the remaining portions of each molecule, especially the relative configurations of derivatives possessing multiple chiral centers using 2D-ROESY.

Sparticolin A (**1**) was obtained as an amorphous powder and its molecular formula established as C₁₉H₁₄O₅ from HR-ESIMS, implying thirteen degrees of unsaturation.

Table 1. ¹H NMR Spectroscopic Data for Sparticolins A–G (1–7).

position	1 ^a	2 ^a	3 ^a	4 ^b	5 ^a	6 ^b	7 ^b
2	5.97, dd (6.2, 1.3)	7.44, d (6.3)	5.83, dd (6.0, 1.6)	4.51, ddd (5.1, 3.4, 0.8)	5.90, ddd (5.8, 1.0, 0.4)	5.91, dd (5.8, 0.9)	6.64, d (10.3)
3a	6.28, ddd (6.2, 2.3, 0.7)	6.59, dd (6.4, 0.6)	6.15, dd (6.0, 1.7)	2.30, ddd (13.5, 7.6, 3.5)	6.28, dd (5.8, 1.8)	6.27, dd (5.8, 1.8)	6.29, d (10.3)
3b				2.08, ddd (13.5, 7.1, 5.1)			
4	5.43, dd (1.9, 1.7)	-	4.59, dt (5.8, 1.7)	5.14, td (7.3, 2.3)	5.10, ddd (7.1, 1.9, 1.0)	5.10, ddd (7.1, 1.8, 1.0)	-
5	-	-	2.51 ddd (8.2, 6.9, 5.7)	-	3.18, td (9.1, 7.0)	3.19, td (9.1, 7.1)	-
6a	6.75, d (11.8)	7.09, d (11.9)	2.87, dddd (15.1, 8.4, 7.1, 1.6)	6.53, dd (11.7, 2.3)	1.83, m	1.82, m	3.38, dt (22.3, 2.3)
6b			2.75, m				3.02, dt (22.3, 2.3)
7	7.94, dd (15.4, 11.8)	8.61, dd (15.6, 11.8)	7.18, dt (15.5, 7.3)	7.89, dd (15.4, 11.7)	4.29, dtd (9.7, 6.5, 5.2)	4.29, ddt (9.8, 7.3, 5.4)	6.76, dt (7.3, 2.3)
8	6.04, d (15.5)	6.36, d (15.6)	6.04, dt (15.5, 1.5)	5.87, d (15.4)	2.54, d (6.6)	2.58, d (6.5)	6.41, d (7.3)
11	6.89, d (7.5)	6.99, d (7.6)	6.91, d (7.5)	7.01, d (7.5)	6.86, d (7.5)	6.92, d (7.6)	7.03, d (7.6)
12	7.40, dd (8.3, 7.5)	7.49, dd (8.4, 7.6)	7.41 dd (8.3, 7.5)	7.44 dd (8.5, 7.5)	7.42 dd (8.4, 7.5)	7.43 dd (8.3, 7.6)	7.49 dd (8.4, 7.6)
13	7.49, d (8.5)	7.61, d (8.4)	7.49, d (8.3)	7.52, d (8.5)	7.50, d (8.4)	7.50, d (8.3)	7.61, d (8.4)
14	-	-	-	-	-	-	-
15	7.49, d (8.5)	7.61, d (8.4)	7.49, d (8.3)	7.52, d (8.5)	7.50, d (8.4)	7.50, d (8.3)	7.61, d (8.4)
16	7.40, dd (8.3, 7.5)	7.49, dd (8.4, 7.6)	7.41 dd (8.3, 7.5)	7.44 dd (8.5, 7.5)	7.42 dd (8.4, 7.5)	7.43 dd (8.3, 7.5)	7.49 dd (8.4, 7.6)
17	6.89, d (7.5)	6.99, d (7.6)	6.89 d (7.5)	6.93, d (7.5)	6.92, d (7.5)	6.90, d (7.56)	7.00, d (7.6)
9-OMe	-		-	-	-	3.66, s	-

^a 500 MHz, MeOH-*d*₄; ^b 700 MHz, MeOH-*d*₄

Table 2. ¹³C NMR Spectroscopic Data* for Sparticolins A–G (1–7).

position	1 ^a	2 ^a	3 ^a	4 ^b	5 ^a	6 ^b	7 ^b
1	108.2, C	103.7, C	111.1, C	112.6, C	108.2, C	112.6, C	96.3, C
2	130.5, CH	150.8, CH	130.0, CH	73.3, CH	131.6, CH	131.7, CH	138.0, CH
3	141.3, CH	140.6, CH	142.5, CH	41.2, CH ₂	140.8, CH	140.8, CH	125.4, CH
4	73.0, CH	193.8, C	79.3, CH	69.3, CH	87.2, CH	87.4, CH	163.1, C
5	149.4, C	137.5, C	57.4, CH	150.4, C	52.6, CH	52.6, CH	89.2, C
6	128.6, CH	135.8, CH	31.0, CH ₂	129.6, CH	34.3, CH ₂	34.2, CH ₂	42.3, CH ₂
7	141.3, CH	138.2, CH	149.4, CH	141.2, CH	80.2, CH	80.1, CH	138.8, CH
8	126.6, CH	133.8, CH	123.8, CH	125.9, CH	41.2, CH ₂	41.1, CH ₂	128.8, CH
9	169.9, C	169.2, C	170.1, C	170.0, C	174.8, C	173.2, C	207.0, C
10	150.2, C	149.3, C	150.0, C	149.4, C	150.0, C	150.0, C	147.1, C
11	110.3, CH	110.8, CH	110.1, CH	110.5, CH	110.3, CH	110.3, CH	111.4, CH
12	128.6, CH	128.8, CH	128.6, CH	128.6, CH	128.6, CH	128.6, CH	128.9, CH
13	121.9, CH	122.6, CH	121.7, CH	121.8, CH	121.8, CH	121.8, CH	122.8, CH
14	135.9, C	135.8, C	136.0, C	135.9, C	136.1, C	136.2, C	135.8, C
15	121.9, CH	122.6, CH	121.7, CH	121.8, CH	121.7, CH	121.7, CH	122.8, CH
16	128.6, CH	128.8, CH	128.6, CH	128.5, CH	128.6, CH	128.6, CH	128.9, CH
17	110.2, CH	110.8, CH	109.9, CH	110.0, CH	110.1, CH	110.1, CH	110.9, CH
18	150.2, C	149.3, C	149.8, C	149.4, C	149.8, C	149.8, C	146.6, C
19	115.1, C	114.8, C	115.3, C	115.2, C	115.6, C	115.6, C	114.5, C
9-OMe	-	-	-	-	-	52.2	-

^a 125 MHz, MeOH-*d*₄; ^b 175 MHz, MeOH-*d*₄.

* Carbon multiplicities were deduced from HSQC –DEPT-135 spectra.

Figure 1. COSY (bold bonds), HMBC (red arrows), and ROESY (blue arrows) Correlations in Sparticolins A–G (**1–7**).

Detailed analysis of the ^1H , ^{13}C and HSQC-DEPT NMR spectroscopic data revealed the presence of a carboxylic acid carbon, a ketal carbon, five non-protonated aromatic carbons (two oxygenated and three non-oxygenated), six aromatic methines, five olefinic methines, and an oxygenated sp^3 methine carbon. In the ^1H - ^1H COSY spectrum of **1**, homonuclear coupling correlations of H-11 /H-17 (δ_{H} 6.89) with H-12/H-16 (δ_{H} 7.40), as well as correlations of H-13/H-15 (δ_{H} 7.49) with H-12/H-16 indicated the presence of two three-proton spin systems corresponding to the C-11 to C-13 and C-15 to C-17 substructures of **1** which displayed *ortho* coupling with J -values of 8.5 and 7.5 Hz. The HMBC correlations of H-11/H-17 with C-10/C-18 (δ_{C} 150.2) and C-19 (δ_{C} 115.1), and H-13/H-15 with C-14 (δ_{C} 135.9) and C-19 led to the attachment of both subunits at C-14 and C-19, suggesting the

presence of a naphthalene substructure. In addition, the chemical shifts of C-10 and C-18 were indicative of a 1,8-dioxynaphthalene fragment. The remaining part of **1** was constructed through analysis of COSY and HSQC-DEPT spectroscopic data revealing connectivities (bold line) between H-2 $\rightarrow$ H-3 $\rightarrow$ H-4 and H-6 $\rightarrow$ H-7 $\rightarrow$ H-8 (Figure 1a). HMBC correlations of H-2 (δ_H 5.97) and H-4 (δ_H 5.43) with dioxygenated C-1 (δ_C 108.2) and olefinic C-5 (δ_C 149.4) suggested a 1-ketal and 5-*exo*-alkylidene 2-cyclopenten-4-ol moiety, where the 1,8-dioxynaphthalene moiety should be connected to ketal C-1 through *spiro* annelation. Further HMBC correlations of H-6 (δ_H 6.75) with ketal C-1, alcohol C-4 (δ_C 73.0) and alkylidene C-5 and, of H-8 (δ_H 6.04) with the C-9 (δ_C 169.9) carboxylic acid carbon allowed the elucidation of the gross planar structure of **1** (Figure 1a). The geometric orientations of the protons in the $\Delta^{5(6)}$ and $\Delta^{7(8)}$ diene moiety were deduced to have *trans* relationships on the basis of their vicinal coupling constant ($J_{H-7, H-8} = 15.5$ Hz) and ROESY correlation. The $\Delta^{5(6)}$ configuration was established as *E* through a ROESY correlation between H-4 and H-7 giving rise to a *5E,7E* geometric configuration in the *exo*-alkylidene carboxylic acid moiety (Figure 1a). The absolute configuration at C-4 was deduced to be *S* on the basis of its single crystal X-ray diffraction data (Figure 2). Thus, the structure of **1** was assigned as (2*Z*,4*E*)-4-((*S*)-4-hydroxyspiro[cyclopentane-1,2'-naphtho[1,8-de][1,3]dioxin]-2-en-5-ylidene)but-2-enoic acid and hitherto assigned the trivial name, sparticolin A.

(a) (b)

Figure 2. (a) Single Crystal X-ray Absolute Stereostructure of **1** - monohydrate. (b) Hydrogen Bond Networks of **1** with Water in the Solid State Packing.

Sparticolin B (**2**) was isolated as a brown syrup. The HR-ESIMS of **2** showed a sodiated molecular ion peak at m/z 343.0754 $[M+Na]^+$, corresponding to the molecular formula $C_{19}H_{12}O_5$, indicating an index of hydrogen deficiency of fourteen. Detailed analysis of the NMR spectroscopic data (Tables 1 and 2) revealed that compound **2** has nearly the same planar structure as sparticolin A (**1**), differing only by replacement of the sp^3 oxygenated methine at C-4, with a conjugated ketone unit (δ_C 193.8). This observation was supported by an isolated H-2 $\rightarrow$ H-3 spin system deduced from the COSY spectrum, and HMBC correlations of H-2 (δ_H 7.44) and H-3 (δ_H 6.59) with the C-1 dioxygenated carbon (δ_C 103.7), ketone C-4, and C-5 alkylidene (δ_C 137.5) (Figure 1b). The relative configurations of the diene at C-5 and C-7 were deduced to be *E* based on coupling constant analysis and comparison to compound **1**. Thus, the structure of **2** was elucidated as shown, and the compound was assigned the name, sparticolin B.

Sparticolin C (**3**) was isolated as a light yellow syrup and its molecular formula was established as $C_{19}H_{16}O_5$ by HR-ESIMS in conjunction with the analysis of its 1H and ^{13}C NMR spectroscopic data (Tables 1 and 2). Its index of hydrogen deficiency was determined to be twelve. In addition to signals for a 1,8-dioxynaphthalene unit, detailed analysis of the 1H , ^{13}C , and HSQC-DEPT NMR data (Table 1 and 2) revealed the presence of additional signals due to one carboxylic acid, one doubly oxygenated non-protonated sp^3 carbon, four olefinic methines, one oxygenated methine, an additional sp^3 hybridized methine, and a methylene carbon. The COSY spectrum showed the presence of an eight-proton spin system (Figure 1c) corresponding to the C-2 to C-8 unit in **3**. The chemical shifts in the 1H and ^{13}C spectra along with the COSY NMR data revealed the loss of the typical alkylidenation at C-5

of the cyclopentenol moiety present in **1** and **2**. The gross structure of **3** was constructed based on the key HMBC correlations of H-5 and H₂-6 with C-1, and of H-7 and H-8 with carboxyl carbon C-9. The vicinal coupling constant ($^3J_{\text{H-4, H-5}} = 5.7\text{--}5.8\text{ Hz}$)⁷ between H-4 and H-5 revealed a *cis* configuration at C-4 and C-5 (Figure 1c). Thus, compound **3** was identified as shown and assigned the name, sparticolin C.

Sparticolin D (**4**) was obtained as a light yellow syrup and its molecular formula was established as C₁₉H₁₆O₆ based on HR-ESIMS data, implying twelve degrees of unsaturation. Examination of its ¹H and ¹³C NMR spectroscopic data (Tables 1 and 2) revealed the presence of a carboxylic acid, a ketal, four olefinic carbons (three of which were protonated), two oxygenated methines and a methylene carbon. Analysis of its COSY spectrum showed correlations for two spin systems, H-2 → H₂-3 → H-4 and the typical three-proton spin-system, H-6 → H-7 → H-8, which is also present in sparticolins A (**1**) and B (**2**) for the *exo*-alkylidene moiety (Figure 1d). Comparison of the ¹H and ¹³C NMR spectroscopic data (Tables 1 and 2) of **4** with those of **1–3** along with the COSY and HMBC data revealed the replacement of olefinic C-2 and C-3 by a carbinol, C-2 (δ_{C} 73.3) connected to a methylene, C-3 (δ_{C} 41.2). HMBC correlations of H-2 with C-1 and C-5, and H-4 with C-5 allowed the elucidation of a 1-dioxy-2,4-dihydroxycyclopentanoid substructure. The connection of the carboxylated *exo*-alkylidene to the latter substructure was established by HMBC correlations of H-6 with C-1 and C-5 (Figure 1d). The relative configuration of compound **4** was established through analysis of ¹H–¹H NMR coupling constants and ROESY data. The $^3J_{\text{H-2, H-3a}}$ coupling constant (~5 Hz) indicated that the 2-OH must adopt an equatorial orientation while the $^3J_{\text{H-3a/H-3b, H-4}}$ coupling constant (~7 Hz) suggested that the 4-OH is axially oriented.^{7a} This implies that **4** has a *trans* relative configuration for hydroxylated, C-2 and C-4. Strong ROESY correlations were also observed between H-2 and H-3b, and H-3a and H-4. In

addition, a ROESY correlation between H-4 and H-7 was also noted, pointing to a C-5 – C-6 olefin configuration similar to that of **1** and **2** (Figure 1d). Thus, **4** was established as (2*E*,4*E*)-4-((2*S**,4*S**)-2,4-dihydroxyspiro[cyclopentane-1,2'-naphtho[1,8-de][1,3]dioxin]-5-ylidene)but-2-enoic acid and assigned the name, sparticolin D.

Sparticolin E (**5**) was obtained as a reddish-brown syrup. Its molecular formula was determined to be C₁₉H₁₆O₅ by HR-ESIMS analysis reflecting twelve degrees of unsaturation, which was also supported by the ¹H and ¹³C NMR spectroscopic data (Tables 1 and 2). Apart from a set of ¹³C NMR chemical shifts characteristic of the 1,8-dioxynaphthalene moiety similar to **1-4**, the spectra of **5** showed additional signals for a carboxylic acid, a ketal, two olefinic methines, three *sp*³ methines (including two oxygenated functionalities), and two methylene carbons. The construction of the upper subunit was facilitated by analysis of ¹H-¹H COSY and HMBC spectra. Analysis of the COSY spectroscopic data led to the identification of a nine-proton spin system (Figure 1) corresponding to the C-2 – C-8 unit (Figure 1e). Key HMBC correlations of H-2, H-3, H-4, and H-5 with dioxygenated carbon C-1 allowed the elucidation of a C-4/C-5 disubstituted 2-cyclopentene moiety linked to 1,8-dioxynaphthalene through a spiroketal junction. Annulation of a tetrahydrofuran moiety at C-4 and C-5 in the cyclopentene substructure forming a 1-oxabicyclo[3.3.0]octane was established on the basis of HMBC correlations of H-4 with C-7, and that of H-7 with C-4. Finally, HMBC correlations of H-7 and H-8 with carboxylic acid C-9 completed the planar structure of sparticolin E (**5**) (Figure 1e). The *cis* relationship between H-4 and H-5 was depicted through a ~7 Hz vicinal coupling constant (³*J*_{H-4, H-5}).⁸ A ROESY correlation of H-7 with H-4 and H-5 enabled assignment of the relative configurations of the chiral carbons as 4*S**, 5*R**, and 7*S**. Thus, compound **5** was assigned the structure as shown.

Compound **6** (sparticolin F) was isolated as brownish solid. An HR-ESIMS analysis gave a molecular ion at *m/z* 339.1230 [*M* + *H*]⁺ which was consistent with the molecular formula

C₂₀H₁₉O₅, indicating twelve degrees of unsaturation. Analysis of the 1D and 2D NMR spectroscopic data of **6** showed structural and stereochemical similarities with sparticolin E (**5**) except for the appearance of an additional oxygenated methyl group resonating at δ_C 52.2. HMBC correlation of the singlet at δ_H 3.66 with carboxyl carbon C-9 indicated that **6** must be the methyl ester derivative of **5** (Figure 1f). This is supported by a 14 amu difference in their molecular masses.

Sparticolin G (**7**) was obtained as an optically active light yellow oil and its molecular formula was determined to be C₁₉H₁₂O₅ by HR-ESIMS analysis. This observation was consistent with the number of carbon signals (nineteen) observed in the ¹³C NMR spectra (Table 2) and was indicative of an index of hydrogen deficiency of fourteen. In addition to signals corresponding to a *spiro*-annelated 1,8-dioxynaphthalene moiety, detailed examination of the ¹H, ¹³C, and HSQC-DEPT NMR spectroscopic data (Tables 1 and 2) revealed additional resonances corresponding to chemical shifts of an ester carbon, a ketone, a ketal, four olefinic methines, an oxygenated non-protonated *sp*³ carbon and an *sp*³ methylene carbon. The COSY correlation and chemical shifts observed for H-2 (δ_H 6.64)/H-3 (δ_H 6.29), and H₂-6 (δ_H 3.02; δ_H 3.38)/H-7 (δ_H 6.76)/H-8 (δ_H 6.41) along with HMBC correlations of H-2 and H-3 with carboxyl ester C-4, and of H-7 and H-8 with ketone C-9 indicated the presence of α,β -unsaturated carbonyl moieties. Complete elucidation of the *spiro*-annelated δ -lactone/2-cyclopenten-1-one substructure was achieved via HMBC correlations of H-2 and H-3 with ketal carbon C-1 and oxygenated tertiary carbon C-5, of H₂-6 and H-7 with C-5, and of H₂-6 with C-1 (Figure 1f). The gross structure of **7** was elucidated by connecting the 1,8-dioxynaphthalene subunit to dioxygenated C-1, fashioning a second *spiro* junction. Thus, the structure of compound **7** was assigned as shown.

While most spirobisnaphthalenes derivatives have been isolated from fungi, several have been detected from plant sources.⁹ The first example of a nineteen carbon-containing *nor* derivative bearing a *spiro*-nonadiene skeleton, spiromamakone A, was isolated as an optically inactive compound from an unidentified non-sporulating endophytic fungus.¹⁰ The profound antimicrobial and cytotoxic activities of spiromamakone A sparked explorations especially in the fields of complex molecule synthesis^{11,12} and medicinal chemistry.¹³ An additional related structure, spiropressione A was reported from the Sporormiaceae fungus *Preussia* sp.¹⁴ However, it was later found to be identical to spiromamakone A through synthetic efforts.¹² To our knowledge, sparticolins A–F (**1–7**) are the first secondary metabolites reported from the genus *Sparticola*. The modification of moieties in the upper rim of each structure is unprecedented in polyketide metabolites, specifically among the spirodioxynaphthalene class of natural products.

Table 3. Antibacterial, antifungal, and nematocidal activities of **1–7**.

Tested organisms	Strain No.	MIC [$\mu\text{g/mL}$]							Reference <i>a</i>
		1	2	3	4	5	6	7	
Fungi									
<i>Candida albicans</i>	DSM 1665	–	–	–	–	–	–	33. 3	8.3 (N)
<i>Mucor hiemalis</i>	DSM 2656	–	16. 7	–	–	–	–	2.1 66.	4.2 (N)
<i>Pichia anomala</i>	DSM 6766	–	–	–	–	–	–	6 16.	8.3 (N)
<i>Rhodoturula glutinis</i>	DSM 10134	–	33. 3	–	–	–	–	7	1.0 (N)
<i>Schizosaccharomyces pombe</i>	DSM 70572	–	33. 3	–	–	–	–	8.3	8.3 (N)
Bacteria									
<i>Bacillus subtilis</i>	DSM 10	–	4.2	–	–	–	–	33. 3	2.4 (O)
<i>Chromobacterium violaceum</i>	DSM 30191	–	–	–	–	–	–	–	4.1 (O)
<i>Escherichia coli</i>	DSM 1116	–	–	–	–	–	–	–	33.3 (O)
<i>Micrococcus luteus</i>	DSM 1790	–	8.3	–	–	–	–	66. 6	4.2 (O)
<i>Mycobacterium smegmatis</i>	ATCC 700084	–	–	–	–	–	–	66. 6	4.1 (K)
<i>Pseudomonas aeruginosa</i>	DSM PA14	–	–	–	–	–	–	–	1.0 (G)
<i>Staphylococcus aureus</i>	DSM 346	66. 6	4.2	66. 6	66.6	66. 6	66.6	16. 7	2.1 (O)

Nematode	LD ₅₀ [µg/mL]								
<i>Caenorhabditis elegans</i>	-	50	50	25	50	50	12.5	nt	1.0 (I)

^a Positive drug controls: K = Kanamycin, N = Nystatin, O = Oxytetracyclin hydrochloride, I = Ivermectin. – No activity observed at concentrations of 66 μg/mL. nt: not tested

Sparticolins A–G (**1–7**) were evaluated for their antimicrobial, cytotoxic¹⁶ and nematicidal activities.¹⁷ All compounds showed weak inhibition against *Staphylococcus aureus* except Sparticolin B (**2**) which also exhibited inhibitory activity against other Gram-positive bacteria such as *Bacillus subtilis* and *Micrococcus luteus* and some fungal strains (Table 3).

Sparticolin G (**7**) showed broad spectrum antifungal activities with inhibitory activities against *Schizosaccharomyces pombe* and *Mucor hiemalis* with greater or equal potency compared to the positive control nystatin and showed weaker effects against *Candida albicans*, *Pichia anomala* and *Rhodotorula glutinis* as well as some Gram positive bacteria (Table 3). Compounds **1–5** showed very weak nematicidal activity, while compound **6** was moderately active against *C. elegans* and compound **7** was not tested due to insufficient material.

Interestingly, compound **2** and **7** also showed moderately strong cytotoxic activities against seven mammalian cell lines (Table 4). The presence of electrophilic α,β -unsaturated carbonyl functionalities such as a 2-cyclopentenone in **2** and a 2*H*-pyran-2-one/2-cyclopentenone hybrid in **7** may explain the observed biological activities.

Table 4 Cytotoxicity of **1–7** against mammalian cell lines.

Cell line	IC ₅₀ μM							
	1	2	3	4	5	6	7	Epothilon B
Mouse fibroblast L929	–	0.6	–	–	52.4	114.9	0.6	1.4×10^{-3}
HeLa cells KB3.1	–	0.8	40.1	–	61.7	70.7	1.2	8.9×10^{-5}
Human breast adenocarcinoma MCF-7	–	0.4	–	–	–	–	0.4	2.4×10^{-4}
Human lung carcinoma A549	–	2.2	–	–	–	–	1.6	6.9×10^{-5}

Human prostate cancer PC-3	–	0.9	–	–	–	–	0.4	1.6×10^{-3}
Ovarian carcinoma SKOV-3	–	0.6	–	–	–	–	0.3	2.8×10^{-4}
Squamous cell carcinoma A431	–	0.2	–	–	–	–	0.1	7.9×10^{-5}

In vitro cytotoxicity test of sparticolins A–G (**1–7**) against seven mammalian cell lines, with epothilon B as a positive control. Starting concentration for cytotoxicity assay was 300 µg/mL, substances were dissolved in MeOH (1 mg/ml). MeOH was used as negative control and showed no activity against the tested mammalian cell lines. Results were expressed as IC₅₀: half maximal inhibitory concentration (µM). Emdash (–): no inhibition.

EXPERIMENTAL SECTION

General Experimental Procedures. Specific optical rotations ($[\alpha]_D$) were measured on a Perkin Elmer 241 polarimeter in a 100×2 mm cell at 22 °C. UV–visible spectra were obtained on a Shimadzu UV-2450 spectrophotometer with 1 cm quartz cells. Nuclear magnetic resonance (NMR) spectra were acquired either on a Bruker AV II-500 MHz (¹H 500 MHz, ¹³C 120 MHz) spectrometer or Bruker Ascend 700 MHz spectrometer with 5 mm TXI cryoprobe (¹H 700 MHz, ¹³C 175 MHz). In all cases, spectra were acquired at 25 °C (unless otherwise specified) in solvents as specified in the text, with referencing to residual ¹H or ¹³C signals in the deuterated solvents. HRESI mass spectra were measured using an Agilent 1200 series HPLC-UV system in combination with an ESI-TOF-MS (Maxis, Bruker) [column 2.1 × 50 mm, 1.7 µm, C18 Acquity UPLC BEH (Waters), solvent A: 95% 5 mM ammonium acetate buffer (pH 5.5, adjusted with 1 M acetic acid) with 5% acetonitrile, solvent B: 95% acetonitrile with 5% 5 mM ammonium acetate buffer]. Elution was achieved using a gradient from 10% solvent B increasing to 100% solvent B within 30 min, maintaining 100% B for another 10 min, R_f = 0.3 mL min^{−1}, UV detection 200–600 nm].

Identity of the producer strain. The ascomycete *Sparticola junci* Phukhamsakda, Camporesi & K.D. Hyde, which represents the ex-type strain of the species, was isolated

from a dead branch of *Spartium junci* and its characteristics have previously been described in detail by Phukhamsakda et al.³ The holotype specimen and the ex-type culture are deposited at the culture collection of Mae Fah Luang University, Chiang Rai, Thailand, under the designation numbers MFLU 15-1405 and MFLUCC 15-0030, respectively. The GenBank accession number of the fungal barcode (5.8S gene region, the internal transcribed spacer ITS1 and ITS2) is provided as NR_154428.

Production, Extraction, Isolation, and Structure Characterization of 1–7. A 20-day old *Sparticola junci* mycelium grown on a yeast-malt glucose (YMG) agar plate (1% malt extract, 0.4% glucose, 0.4% yeast extract, 2% agar, pH 6.3) was used to prepare a seed culture. Five plugs of well-grown mycelium were inoculated in 500 mL Erlenmeyer flasks containing 200 mL of YMG media and incubated on a rotary shaker at 24 °C and 140 rpm for 30 days. The seed cultures were homogenized mechanically using an Ultra Turrax. Subsequently, 10 mL of seed culture were transferred to each of 30 x 500 mL sterilized Erlenmeyer flasks with 200 ml of Q6 ½ medium⁵ (6 L) and incubated on a rotary shaker (21 days, 24 °C, 140 rpm). Fermentation was terminated 6 days after glucose depletion (glucose strip test). The mycelia and supernatant were separated using vacuum filtration, and the mycelia were homogenized and extracted with acetone under ultrasonic conditions. The combined acetone extracts were evaporated in a rotary evaporator (40 °C), and the crude product was suspended in distilled water (300 mL) and extracted with an equal volume of ethyl acetate (5×). The aqueous layer was discarded and the organic phase was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to yield 2.2 g of crude ethyl acetate extract. The supernatant was mixed with Amberlite XAD-16 N (30 g per 1 L) and extracted with an equal volume of ethyl acetate (5×) according to a previously described procedure. The resulting ethyl acetate extracts were evaporated to dryness to give 800 mg of crude material. The combined crude extracts from the mycelia and broth yielded 3 g of a

brown syrup. The extracts were dissolved in methanol and filtered on a RP solid-phase cartridge (Strata-X 33 mm, polymeric reversed phase; Phenomenex Aschaffenburg, Germany) to yield 2.7 grams of crude material.

For the isolation of the compounds, preparative purifications were achieved on a preparative HPLC (Gilson, Middleton, WI, USA) equipped with a GX-271 Liquid Handler, a 172 DAD, and a 305 and 306 pump (with 50SC Piston Pump Head). A VP Nucleodur 100-10 C18 ec column (150 × 40 mm, 7 µm; Macherey-Nagel) was used as stationary phase. The mobile phase composed of deionized water (Milli-Q, Millipore, Schwalbach, Germany) with 0.05% trifluoroacetic acid (solvent A) and acetonitrile (AcCN, HPLC-grade) with 0.05% trifluoroacetic acid (solvent B). The crude extract (1.8 g) was initially purified by preparative HPLC using a linear gradient of 15% solvent B for 5 min, 15–100% solvent B for 40 min, and 100% solvent B for 15 min. The fractions were combined peak-wise, according to UV absorptions at 210, 254, and 350 nm to afford eighteen pooled fractions. Compound **1** (126 mg) eluted at $t_R = 25.5\text{--}26.2$ min from fraction thirteen. Fraction eight (16.6 mg) and fraction twelve (46.9 mg) were further purified using preparative RP-HPLC on a Gemini 10u C18 110A column (250 × 21.20 mm, 10 µm) with the following gradient; 40% to 100% of solvent B in 35 min, followed by 100% solvent B for 15 min. Compound **4** (3.6 mg) eluted at $t_R = 30\text{--}31$ min and compound **3** (4.1 mg) eluted at $t_R = 12\text{--}13$ min. Fraction fifteen (39.2 mg) was rechromatographed using preparative RP-HPLC (solvent system: 40% to 100% of solvent B in 20 min, followed by 100% solvent B for 10 min) to afford compounds **5** (6.1 mg, $t_R = 16.2\text{--}17.2$ min) and **6** (1.0 mg, $t_R = 20.5\text{--}21$ min). Fraction sixteen (33.6 mg) yielded compound **2** (2.1 mg, $t_R = 14\text{--}15$ min) and fraction eighteen (13.3 mg) yielded compound **7** (2.1 mg, $t_R = 14\text{--}15$ min) using preparative RP-HPLC employing a gradient of solvent B from 20% to 50% in 5 min, followed by 50 – 80 % B in 30 min, 80-100 % B in 5 min, and 100% solvent B for 10 additional minutes.

Sparticolin A (1): colorless crystals (MeOH-water); mp 186 °C (dec.); $[\alpha]_{\text{D}}^{25} -147$ (*c* 0.001, MeOH); UV (*c* 0.01, MeOH) λ_{max} ($\Delta\epsilon$) 225 (4.71), 258 (4.39), 300 (3.97) nm; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS m/z 345.0723 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{14}\text{O}_5\text{Na}$, 345.0733).

Sparticolin B (2): brown syrup; UV (*c* 0.01, MeOH) λ_{max} ($\Delta\epsilon$) 225 (4.67), 299 (4.26) nm; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS m/z 343.0574 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{12}\text{O}_5\text{Na}$, 343.0582).

Sparticolin C (3): light yellow syrup; $[\alpha]_{\text{D}}^{25} -201$ (*c* 0.001, MeOH); UV (*c* 0.01, MeOH) λ_{max} ($\Delta\epsilon$) 226 (4.63), 300 (3.78), 328 (3.52) nm; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS m/z 347.0881 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{16}\text{O}_5\text{Na}$, 347.0889).

Sparticolin D (4): light yellow syrup; $[\alpha]_{\text{D}}^{25} -287$ (*c* 0.001, MeOH); UV (*c* 0.01, MeOH) λ_{max} ($\Delta\epsilon$) 225 (4.64), 260 (4.23) nm; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS m/z 363.0834 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{16}\text{O}_6\text{Na}$, 363.0839).

Sparticolin E (5): reddish-brown syrup; $[\alpha]_{\text{D}}^{25} -115$ (*c* 0.001, MeOH); UV (*c* 0.01, MeOH) λ_{max} ($\Delta\epsilon$) 226 (4.63), 300 (3.78), 328 (3.53) nm; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS m/z 325.1066 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{17}\text{O}_5$, 325.1070).

Sparticolin F (6): brownish solid; $[\alpha]_{\text{D}}^{25} -157$ (*c* 0.001, MeOH); UV (*c* 0.01, MeOH) λ_{max} ($\Delta\epsilon$) 226 (4.67), 300 (3.83), 328 (3.59) nm; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS m/z 339.1229 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{19}\text{O}_5$, 339.1227).

Sparticolin G (7): light yellow oil; $[\alpha]_{\text{D}}^{25} -43$ (*c* 0.001, MeOH); UV (*c* 0.03, MeOH) λ_{max} ($\Delta\epsilon$) 224 (4.40), 296 (3.52) nm; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS m/z 321.0762 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{13}\text{O}_5$, 321.0757).

X-ray Crystallographic Analysis of Sparticolin A (1).¹⁸ Colorless crystals of **1** were obtained from a MeOH–H₂O solution. The monohydrate of **1** was analyzed with a Bruker D8 Venture diffractometer with a microfocus tube, Cu K α radiation ($\lambda = 1.54178$ Å). APEX3

was used for data collection, SAINT was used for cell refinement and data reduction, and SADABS was used for experimental absorption correction. The structure was solved by intrinsic phasing using SHELXT, while refinement was done by full-matrix least-squares on F^2 using SHELXL-2018/3.¹⁹⁻²¹ The hydrogen atoms were freely refined. The absolute configuration of **1** was solved using anomalous dispersion from Cu K α , resulting in a Flack parameter²² of 0.030(2) using Parsons' quotient method. Graphics were drawn using DIAMOND.²³ The structural data have been deposited in the Cambridge Crystallographic Data Center (CCDC No. 1915632).

Crystal data of **1**: C₁₉H₁₄O₅·H₂O, M = 340.32, orthorhombic system, space group $P2(1)2(1)2(1)$, $a = 5.5027(4)$ Å, $b = 7.9285(5)$ Å, $c = 36.706(2)$ Å, $V = 1601.42(18)$ Å³, $Z = 4$, $d_{\text{calc}} = 11.412$ mg/m³, crystal size 0.630 x 0.120 x 0.057 mm³, $F(000) = 712$, $\mu(\text{Mo K}\alpha) = 0.885$ mm⁻¹, $6.653^\circ < \theta < 80.377^\circ$. The 3909 measurements yielded 3318 independent reflections after equivalent data were averaged. The final refinement gave $R_1 = 0.0249$ and $wR_2 = 0.0649$ [$I > 2\sigma(I)$]. The absolute structure parameter was 0.03(2).

Cytotoxicity Assay. Cytotoxicity of **1–7** was tested against a panel of mammalian cell lines. The evaluation of in vitro cytotoxicity effects (IC₅₀) was performed with mouse fibroblast cell line L929 and mammalian HeLa KB3.1 cancer cells. Additionally, human ovarian carcinoma (SKOV-3), human prostate cancer (PC-3), human lung carcinoma tissue (A549), human skin squamous cell carcinoma (A431), and human breast adenocarcinoma (MCF-7) were evaluated for compounds **1–7**. The cell lines were cultured in DMEM (Gibco, ThermoFisher Scientific, Hilden, Germany) and MCF-7 in RPMI (Lonza, Cologne, Germany) media, all supplemented with 10% of fetal bovine serum (Gibco) under 10% CO₂ at 37 °C. The cytotoxicity assays were performed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) method in 96-well microplates (ThermoFisher

Scientific). Briefly, 60- μ L aliquots of serial dilutions from an initial stock of 1 mg/mL in MeOH of the test compounds were added to 120 μ L aliquots of a cell suspension (5.0×10^4 cells/mL) in 96-well microplates. After 5 days incubation, a MTT assay was performed, and the absorbance measured at 590 nm using an ELISA plate reader (Victor, PerkinElmer, Überlingen, Germany). The concentration at which the growth of cells was inhibited to 50% of the control (IC_{50}) was obtained from the dose response curves. Experiments were repeated three times. Epothilone B was used as positive control and the negative control was methanol.¹⁶

Antimicrobial Assay. Antimicrobial activities of **1–7** were evaluated in serial dilution assays against various fungal and bacterial strains as given in Table 3, using the broth microdilution method according to our previously described procedures.¹⁶ Gentamicin, kanamycin, and oxytetracyclin were used as positive controls against the Gram-positive and Gram-negative bacteria, respectively. Nystatin was used as a positive control against yeasts and filamentous fungi.¹⁶

Nematicidal Assay. The nematicidal activity of **1–7** against *Caenorhabditis elegans*, monoxenically grown on nematode agar (soy peptone 2 g, NaCl 1 g, agar 20 g, 1000 mL deionized water; adding 0.5 mL cholesterol (1 mg/mL EtOH), 1 mL 1 M $CaCl_2$, 1 mL 1 M $MgSO_4$, and 12.5 mL 40 mM potassium phosphate buffer after autoclaving; pH adjusted to 6.8) with living *E. coli* DSM498, at 20 °C for a week, was assessed according to our previously reported protocol with slight modifications. The nematodes were then washed from the plates with M9 buffer and the buffer diluted to 500 nematodes/mL. The assay was performed in 24-well microtiter plates at five different concentrations (100, 50, 25, 12.5, and 6.25 μ g/mL) of each compound. Ivermectin was used as positive control and methanol as

negative control. The plates were incubated at 20 °C in a shaker in the dark and nematocidal activity was recorded after 18 h of incubation and expressed as an LD₅₀.^{17, 24}

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at XXXXX;

Description and images of *Sparticola junci*, and 1D and 2D NMR spectra of **1–7** (PDF)

X-ray crystallographic data of **1** (CIF)

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Notes

The authors declare no competing financial interest.

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- (18) Crystallographic data of compound **1** have been deposited in the Cambridge Crystallographic Data Centre as CCDC 1915632. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif (or from the CCDC, 12 Union Road, Cambridge CB21EZ, U.K.; fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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