Isokibdelones: Novel Heterocyclic Polyketides from a *Kibdelosporangium* sp.

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Supporting Information-Experimental section

General Experimental. Details are as reported previously.¹

Fungal Collection and Identification. MST-108465 was isolated from a soil sample, collected from a timber woolshed 15 km north of Port Augusta in South Australia in 1996. Analysis of the non-polar secondary metabolites produced by the culture identified the presence of a series of metabolites with unusual UV spectra. A comparison of the UV spectra against a library of 1,500 microbial metabolites failed to identify any of the metabolites as known. A more extensive search of the UV spectra using extracts derived from Microbial Screening Technology's database of 6,000 type microbial species, failed to identify these metabolites as belonging to a known or characterized microbial species.² 16S rRNA analysis identified MST-108465 as belonging to the Pseudonocardiaceae and having 98% identity with *Kibdelosporangium* spp. (aff. *phillipinense*). MST-108465 was found to share no common secondary metabolites with any known species of *Kibdelosporangium*. Accordingly, based on rRNA and metabolite data MST-108465 is regarded as a novel species, *Kibdelosporangium* sp. (MST-108465).

Culturing and Processing.

Mixed media fermentation of MST-108465: Fermentation details are similar to those reported for the kibdelones.¹

Fermentation of MST-108465 on wheat grain: In the mixed media fermentation, wheat was found to afford a different distribution of kibdelone-like metabolites to that observed on ISP2 agar. MST-108465 was recultured on wheat in an effort to produce sufficient amounts of these metabolites to enable characterization. The wheat fermentation (20 x 100 g wheat in 15 cm diameter Petri plates, incubated for 18 days at 28 °C) was extracted with EtOAc (3L).

Isolation of isokibdelones

(1) *From mixed media culture:* The individual extracts from the fermentation were pooled to provide 4 L of methanolic extract. This extract was concentrated *in vacuo* to an aqueous residue (500 mL) that was diluted with H₂O to a final volume of 2 L. This cloudy solution was passed through two parallel C₁₈ solid phase extraction (SPE) cartridges (2 x 10 g, Varian HF C₁₈), eluting with 50% aqueous MeOH (2 x 80 mL each) followed by elution with 100% MeOH (2 x 80 mL each). HPLC and bioassay confirmed the presence of the activity and non-polar metabolites in the MeOH fraction. Partition of the pooled MeOH fraction (160 mL) with hexane removed non-polar fats (134 mg) yielding an enriched active methanol fraction (875 mg). The MeOH fraction was dissolved in a mixture of DMSO and methanol and fractionated by preparative HPLC (60 mL/min with gradient elution of 70% to 10% H₂O/MeCN over 20 min followed by MeCN for 10 min, through a 5 mm Platinum EPS C₁₈ 50 x 100 mm column). One hundred fractions were collected, concentrated *in vacuo* and combined on the basis of analytical HPLC analysis. A relatively non-polar pool of fractions (13.7 mg) was concentrated *in vacuo* and further fractionated by HPLC (2.5 mL/min gradient elution from 50% H₂O/MeCN to 100% MeCN over 20 min through a Phenomenex LUNA C₁₈ 5µm (2) 250 x 10 mm column), to yield isokibdelone A (**2**) (2.1 mg, 0.24 % yield). (NOTE: calculation of yield is based on the crude extract weight after hexane partition)

(2) *From the Wheat grain fermentation:* The EtOAc extract was washed with water and the organic layer concentrated *in vacuo* to yield a crude extract (1.94 g). The crude extract was dissolved in methanol, defatted with hexane and concentrated *in vacuo* to yield an enriched extract (1.44 g). The extract was subjected to preparative HPLC (60 mL/min with gradient elution of 70% to 10% H₂O/MeCN over 20

minutes followed by MeCN for 10 min, through a 5 mm Platinum EPS C₁₈ 50 x 100 mm column). One hundred fractions were collected, concentrated *in vacuo* and combined on the basis of analytical HPLC analysis.

Two fractions containing “*kibdelone-like*” metabolites were identified by a combination of UV, ESI(±)MS and ¹H NMR spectroscopy and were subjected to multiple serial fractionation by HPLC (2.0 mL/min gradient elution from 50-60% H₂O/MeCN to 20% H₂O/MeCN or 100% MeCN over 20 min through a C₈ 5µm Zorbax-StableBond 250 x 9.4 mm column). This gave, in relative order of elution, isokibdelone A rhamnoside (**2a**; 3.3 mg 0.23%), isokibdelone B (**3**; 0.6 mg 0.04%), and isokibdelone A (**2**) (3.1 mg, 0.22%). All yields are calculated relative to defatted enriched fraction (1.44 g). Isokibdelone C (**4**) was detected as minor constituents during the above purification process. The structure for **4** was proposed on the evidence based on LC-DAD-MS analysis and by comparison to the biosynthetically related kibdelone¹ and isokibdelone analogues.

Isokibdelone A (2). Orange amorphous solid; $[\alpha]_D +133^\circ$ (c 0.015, CHCl_3); UV (EtOH) λ_{max} ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) 442 (sh), 404 (11700), 319 (16300), 271 (26600), 239 (27600) nm; ^1H NMR and ^{13}C NMR (CDCl_3 , 500 MHz and 100 MHz respectively); ESI(+)MS m/z 582.0/584.0 $[\text{M}+\text{H}]^+$ and ESI(-)MS m/z 580.1/582.0 $[\text{M}-\text{H}]^-$; HRESI(+)MS 604.0982 ($[\text{M}+\text{Na}]^+$, $\text{C}_{29}\text{H}_{24}^{35}\text{ClNO}_{10}\text{Na}$ requires 604.0986).

Table 1: NMR (CDCl_3 , 500 MHz) assignments for isokibdelone A (2)

No.	^1H δ_{H} (m, J (Hz))	^{13}C	COSY	HMBC
1		158.6		
2		#		
3		183.5 ^b		
4		135.3 ^c		
5		111.5		
6		150.0 ^a		
7		112.2		
8		183.4 ^b		
9		116.5		
10	5.04 (brd, 3.0)	66.9	H-11, 10-OH	C-14, C-9
11	4.39 (brdd, 5.5, 3.0)	66.5	H-12a, H-12b, H-10	C-10, C-9
12a	2.74 (ddd, 14.0, 6.5, 5.5)	33.9	H-13, H-12b, H-11	C-14, C-13, C-11, C-10
12b	1.82 (ddd, 14.0, 9.0, 2.0)		H-13, H-12a, H-11	C-13
13	5.24 (ddd, 9.0, 6.5, 5.0)	62.8	H-12a, H-12b, 13-OH	
14		166.9		
15[O]				
16		149.7		
17		136.6		
18		135.3 ^c		
19	8.45 (d, 9.0)	126.3	H-20	C-21 C-17 C-5
20	8.23 (d, 9.0)	125.3	H-19	C-22 C-18 C-4
21		131.4		
22		181.1		
23		138.8 ^a		
24		108.9		
25		155.9		
26	3.08 (dd, 8.5, 8.0)	33.8	H-27	C-28, C-27, C-25, C-24
27	1.74 (m)	20.4	H-28, H-26	C-28, C-25
28	1.21 (t, 7.5)	14.2	H-27	C-27, C-26
17-OMe	3.98 (s)	61.1		C-17
NMe	3.74 (s)	33.6		C-1
10 OH	4.88 (brs)		H-10	C-10
11 OH				
13 OH	5.92 (d, 5.0)		H-13	C-12, C-13
16 OH	12.68 (s)			C-17, C-16, C-7

^aAssignments are made in comparison with literature data for reported compounds and biosynthetic analogues from same culture. ^{b, c}Assignments can be interchanged. # ^{13}C not observed due to overlapping signals.

Isokibdelone B (3). Orange amorphous solid $[\alpha]_D +82^\circ$ (c 0.02, CHCl_3); UV (EtOH) λ_{max} ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) 583 (sh), 447 (3700), 326 (sh), 266 (sh), 244

(9500), 203 (13700) nm; ^1H NMR and ^{13}C NMR (d_6 -DMSO, 600 MHz and 150 MHz respectively), see Table 8; ESI(+)-MS m/z 584.0/586.2 $[\text{M}+\text{H}]^+$ and ESI(-)-MS m/z 582.1/584.0 $[\text{M}-\text{H}]^-$; HRESI(+)-MS 606.1133 ($[\text{M}+\text{Na}]^+ \text{C}_{29}\text{H}_{26}^{35}\text{ClNO}_{10}\text{Na}$ requires 606.1143).

Table 2: NMR (d_6 -DMSO, 600 MHz) assignments for isokibdelone B (**3**)

No.	^1H δ_{H} (m, J (Hz))	^{13}C	COSY	HMBC
1		157.0		
2		119.2 ^a		
3		181.8 ^{a,b}		
4		141.3 ^{a,c}		
5		106.8 ^a		
6		148.3 ^a		
7		109.1		
8		182.4		
9		116.8		
10	4.66 (brd, 3.3)	57.9	H-12b, H-11, 10-OH	C-14, C-13, C-12, C-9, C-8
11	3.86 (ddd, 12.5, 3.3, 3.2)	68.7	H-12a, H-12b, H-10	
12a	2.20 (ddd, 13.2, 12.5, 4.5)	30.9	H-13, H-12b, H-11,	C-13, C-10
12b	1.75 (brd, 13.2)		H-13, H-12a, H-11	C-14, C-13, C-10
13	4.48 (brd, 4.5)	64.8	H-12	C-14
14		165.4		
15[O]				
16		154.8		
17		139.7		
18		139.45 ^{a,c}		
19	2.74 (m)	18.2	H-20	
	2.63 (m)			
20	2.97 (m)	21.3	H-19	
	2.86 (m)			
21		139.4 ^{a,c}		
22		181.1 ^{a,b}		
23		137.8 ^{a,c}		
24		108.9		
25		157.5		
26	3.05 (ddd, 8.3, 8.2, 2.6)	33.2	H-27	C-28, C-27, C-25, C-24
27	1.64 (m)	19.6	H-28 H-26	C-28, C-26, C-25
28	1.06 (t, 7.3)	13.9	H-27	C-27, C-26
17-OMe	3.83 (s)	60.2		C-17
NMe	3.71 (s)	33.5		C-1
10 OH				
11 OH				
13 OH	5.45 (brs)		H-13	
16 OH	13.32 (s)			C-17, C-16, C-7

^a Assignments are made in comparison with literature data for reported compounds and biosynthetic analogues from same culture. ^{b,c} values may be interchanged

Isokibdelone A rhamnoside (2a). Orange amorphous solid $[\alpha]_{\text{D}} +60^\circ$ (c 0.015, CHCl_3); UV (EtOH) λ_{max} ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) 442 (sh), 410 (6700), 271 (17400), 229 (23200), 210 (25000) nm; ^1H NMR and ^{13}C NMR (d_6 -DMSO, 600 MHz and 150 MHz respectively); ESI(+)-MS m/z 728.2/730.3 $[\text{M}+\text{H}]^+$,

582.2/584.2 (M-rha+H) and ESI(-)MS m/z 726.4/728.0 [M-H]⁻; HRESI(+)MS 750.1569 ([M+Na]⁺, C₃₅H₃₄³⁵ClNO₁₄Na requires 750.1566).

Table 3: NMR (d_6 -DMSO, 600 MHz) assignments for isokibdelone A-rhamnoside (**2a**)

No.	¹ H δ_H (m, J (Hz))	¹³ C	COSY	HMBC
1		157.6		
2		121.7 ^a		
3		183.4 ^a		
4		134.2		
5		110.8		
6		149.3 ^a		
7		117.7		
8		182.2		
9		118.2		
10	4.97 (brs)	57.9	H-12b, H-11, 10-OH	C-14, C-11, C-9, C-8
11	4.03 (ddd, 12.5, 3.2, 3.1)	68.7	H-10, H-12a, H-12b	C-1'
12a	2.43 (ddd, 13.3, 12.5, 5.2)	30.9	H-13, H-12b, H-11	C-11
12b	1.86 (brd, 13.3)		H-13, H-12a, H-11	C-14, C-13, C-11, C-10
13	4.76 (dd, 5.2, 5.0)	64.2	H-12	C-14
14		165.4		
15[O]				
16		149.1		
17		135.9		
18		135.6		
19	8.35 (d, 9.0)	125.5	H-20	C-21, C-17, C-5
20	8.16 (d, 9.0)	125.1	H-19	C-22, C-18, C-4
21		130.8		
22		180.6		
23		138.4 ^a		
24		107.0		
25		156.7		
26	3.06 (dd, 8.3, 8.2)	33.1	H-27	C-28, C-27, C-25, C-24
27	1.65 (m)	19.7	H-28 H-26	C-28, C-26, C-25
28	1.07 (t, 7.3)	13.9	H-27	C-27, C-26
17-OMe	3.98 (s)	60.7		C-17
NMe	3.74 (s)	33.7		C-1
10 OH	5.28 (brs)		H-12	
11 OH				
13 OH	5.52 (d, 5.0)		H-13	C-12
16 OH	12.92 (s)			C-17, C-16, C-7
1'	4.91 (brs)	97.9	H-2'	C-5', C-3'
2'	3.75 (dd, 1.5, 3.0)	70.4	H-3', H-1'	C-4', C-3
3'	3.54 (dd, 3.0, 9.5)	70.6	H-4', H-2'	C-4'
4'	3.24 (dd, 9.5, 9.3)	72.1	H-5', H-3'	C-6', C-5', C-3', C-2'
5'	3.52 (brd, 9.3)	68.9	H-6', H-4	C-4'
6'	1.18 (d, 6.3)	17.9	H-5'	C-5', C-4'

^a Assignments are made in comparison with literature data for reported compounds and biosynthetic analogues from same culture.

Biological activity

Anticancer assays were carried out as detailed previously for the kibdelones.¹ Procedures for antimicrobial, nematocidal and NS-1 cytotoxicity have been described previously.³

Reference:

1. Ratnayake, R.; Lacey, E.; Tennant, S.; Gill, J. H.; Capon, R. J. *Chem. Eur. J.* **2006** accepted.
2. Lacey, E.; Tennant, S. *Microbiol. Australia* **2003**, *24*, 34-35.

3. Capon, R. J.; Ratnayake, R.; Stewart, M.; Lacey, E.; Tennant, S.; Gill, J. H. *Org. Biomol. Chem.* **2005**, 3, 123-129.