CHEMISTRY
A EUROPEAN JOURNAL

Supporting Information

Kibdelones: Novel Anticancer Polyketides from a Rare Australian Actinomycete

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Supporting Information

Experimental Section

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 our (MST) database of 6,000 type microbial species, failed to identify these metabolites as belonging to a known or characterised microbial species.[1] 16S rRNA analysis identified MST-108465 as belonging to the Pseudonocardiaceae and having 98% identity with *Kibdelosporangium* spp. (aff *phillipinense*). Investigation of the metabolites produced by the known species of *Kibdelosporangium* found that they shared no common secondary metabolites with MST-108465. Accordingly, based on rRNA and metabolite data MST-108465 is regarded as a novel species, *Kibdelosporangium sp.* (MST-108465).

Mixed media fermentation of MST-108465. Kibdelosporangium sp. (MST-108465) was inoculated onto selection of solid and liquid media to explore the culture's metabolic diversity. The media selected were liquid media: (a) ISP2 liquid media and (b) rice flour liquid media and solid phase: (a) ISP2 agar and (b) barley grain. Each media was incubated at 28 °C for a range of times. The media were incubated at 28°C for times previously found to be optimal for other actinomycetes cultured under these conditions. At the conclusion of the incubation each treatment was processed as follows:

(1) The liquid media were centrifuged at 10,000 rpm for 30 min to pellet the mycelia (approximately, 10 to 15 mL). The supernatant was decanted as a separate fraction and the mycelia extracted with methanol to generate a mycelial extract. The decanted supernatant was concentrated *in vacuo* and/or freeze dried, then extracted with solvent to yield a supernatant extract.

(2) The solid phases were excised from the Petri plate and transferred to a flask or vial and extracted with methanol. The amounts of methanol varied with the nature of the media. Generally, a solid media was extracted 1 mL per 1 g with methanol by gentle mixing and shaking overnight. However, for grains, a larger volume (2 mL per g) was necessary to ensure coverage of the grain during extraction. The addition of nutrients during the fermentation resulted in an increase in production, as did an increase in media volume.

Fermentation of MST-108465 on barley grain. Of the fermentation media and conditions, barley grain gave the same distribution of kibdelone-like metabolites but in higher yields than that obtained on ISP2 agar. To isolate the remaining minor analogues a series of solid phase fermentations using barley was undertaken. Solid fermentations (7 x 500 g barley, incubated for 18 days at 28 °C) were extracted with methanol (MeOH) (7 x 1.5 L). The extracts were concentrated *in vacuo* and pooled to give an aqueous residue (2.6 L) and extracted with ethyl acetate (EtOAc) (2.6 L).

NMR analysis of kibdelones (^{1}H NMR and ^{13}C NMR; [D]$_6$DMSO, 600 MHz)

Table 1: NMR assignments for kibdelone B (**2**)

No.	^{1}H d_H (m, *J* (Hz))	^{13}C	COSY	HMBC
1		156.3		
2		117.9**		
3		182.2*		
4		140.7***		
5		112.1		
6		153.6		
7		108.9		
8		182.3		
9		118.3		
10	4.69 (m)	61.3	H-11, 10-OH	C-14, C-11, C-9, C-8
11	3.91 (m)	64.0	H-12a, H-10, 11-OH	
12a	2.24 (ddd, 13.1, 12.6, 4.7)	33.8	H-13, H-12b, H-11	C-11, C-10
12b	1.77 (brd, 13.1)		H-12a	C-14, C-13, C-10
13	4.68 (m)	65.0	H-12a, 13-OH	C-14, C-12, C-9, C-8
14		165.5		
15[O]				
16		150.2		
17		136.2		
18		140.3***		
19	2.87 (m)	21.5	H-20	C-21/C-18, C-17, C-5
20	2.63 (m)	18.4	H-19	C-21/C-18/C-4
21		140.7***		
22		178.7*		
23		138.4**		
24		105.9		
25		157.1		
26	3.02 (ddd, 11.0, 8.2, 8.0)	33.2	H-27	C-28, C-27, C-25, C-24
27	1.63 (m)	19.6	H-28, H-26	C-28, C-26, C-25
28	1.05 (t, 7.3)	13.9	H-27	C-27, C-26
17-OMe	3.84 (s)	61.4		C-17
NMe	3.65 (s)	33.2		C-1
6 OH	13.05 (s)			C-7, C-6, C-5
10 OH	5.03 (d, 4.7)		H-10	C-9, C-11, C-10
11 OH	4.76 (d, 6.3)		H-11	C-12, C-11, C-10
13 OH	6.02 (d, 6.0)		H-13	C-14, C-13, C-12

*Assignments maybe interchanged. ** Assignments are made in comparison with literature data for reported compounds and biosynthetic analogues from same culture. *** Assignments are based on HMBC correlations and the exact chemical shifts can be interchanged.

Table 2: NMR assignments for kibdelone C (**3**)

No.	^{1}H d_H (m, *J* (Hz))	^{13}C	COSY	HMBC
1		165.0		
2		108.8*		
3		152.6*		
4		116.8*		
5		114.7*		
6		152.3*		
7		108.6*		
8		182.4		
9		117.9		
10	4.70 (dd, 4.2, 3.8)	61.4	H-11, 10-OH, H-12b	C-14, C-12, C-11, C-9, C-8
11	3.93 (m)	64.1	H-12a, H-12b, H-10, 11-OH	
12a	2.24 (ddd, 13.0, 12.5, 4.2)	33.8	H-13, H-12b, H-11	C-11, C-10
12b	1.77 (brd, 13.0)		H-13, H-12a, H-11, H-10	C-14, C-13, C-10
13	4.67 (m)	65.1	H-12a, H-12b, 13-OH	C-14, C-12, C-10, C-9
14		164.9		
15[O]		-		
16		147.8**		
17		135.2		
18		141.3		
19a	2.24 (m)	23.09	H-20b	C-18
19b	2.14 (m)		H-20a	
20a	3.39 (brd, 13.0)	23.05	H-19b	C-18
20b	3.29 (brd, 13.0)		H-19a	C-21
21		137.4		
22		138.0		
23		124.1		
24		109.5		
25		140.6		
26	2.96 (dd, 8.2, 8.0)	31.8	H-27	C-28, C-27, C-24, C-25
27	1.63 (m)	20.3	H-28, H-26	C-28, C-26, C-25
28	1.04 (t, 7.2)	13.8	H-27	C-27, C-26
17-OMe	3.85 (s)	61.5		C-17
NMe	3.61 (s)	31.6		C-25, C-1
3 OH	13.99* (s)			C-4, C-3, C-2
6 OH	13.14* (s)			C-7, C-6, C-5
10 OH	4.95 (brs)		H-10	C-14, C-12, C-11, C-10, C-9
11 OH	4.74 (d, 6.3)		H-11	
13 OH	5.99 (d, 6.5)		H-13	C-14, C-13, C-12
22 OH	8.42 (s)			C-23, C-22, C-21

*The assignments for the chelated phenolic OH and the associated carbon assignments may interchange. ** Assignments are made in comparison with literature data for reported compounds and biosynthetic analogues from same culture.

Table 3: NMR assignments for dihydro kibdelone A (**10**)

No.	^{1}H d_H (m, *J* (Hz))	^{13}C*	HMBC
1		165.7	
2		108.7	
3		153.9	
4		114.7	
5		112.2	
6		154.9	
7		107.2	
8			
9		117.2	
10	4.70 – 4.80 (m)	63.8	
11	3.96 (m)	61.6	
12a	2.27 (ddd, 13.4, 12.2, 4.8)	33.7	C-10
12b	1.80 (brd, 13.4)		C-14, C-13, C-11
13	4.70 – 4.80 (m)	65.2	
14		165.4	
15[O]			
16			
17		132.7	
18		131.3	
19	8.03 (d, 9.3)	121.8	C-21, C-18, C-17, C-5
20	8.27 (d, 9.3)	125.4	C-22, C-21, C-18, C-4
21		129.1	
22		137.6	
23		120.6	
24		109.2	
25		139.9	
26	2.99 (brt, 8.1)	31.4	C-28, C-27, C-24, C-25
27	1.66 (m)	20.4	C-28, C-26, C-25
28	1.04 (t, 7.3)	13.7	C-27, C-26
17-OMe	4.02 (s)		C-17
NMe	3.65 (s)		C-25, C-1
3 OH	14.69 (s)		C-4, C-3, C-2
6 OH	14.11 (s)		C-7, C-6, C-5
10 OH	5.00 (d, 5.0)		C-9
11 OH	4.77 (d 6.2)		C-12, C-10
13 OH	6.06 (d, 6.3)		C-14, C-13
22 OH	9.23 (s)		C-23, C-22, C-21

* Assignments are based on gHMBC correlations.

Table 4: NMR assignments for kibdelone B-rhamnoside (**5**)

No.	^{1}H d_{H} (m, *J* (Hz))	^{13}C	COSY	HMBC
1		156.3**		
2		117.96**		
3		178.7**		
4		140.6**		
5		112.2		
6		153.6		
7		108.9		
8		182.19*		
9		117.86		
10	4.93 (ddd, 5.4, 4.2, 3.8)	57.7	H-11, 10-OH	C-14, C-12, C-9, C-8
11	4.01 (m)	68.1	H-12, H-10	C-10, C-1´
12a	2.35 (ddd, 13.1, 12.7, 4.6)	32.1	H-13, H-12b, H-11	C-11, C-10
12b	1.79 (brd, 12.7)		H-12a, H-11, H-13	C-14, C-13, C-11, C-10
13	4.72 (m)	64.8	H-12, 13-OH	C-14, C-11, C-9
14		165.3		
15[O]				
16		150.2**		
17		136.2		
18		140.3		
19a	2.89 (m)	21.5	H-20a, H-19b	C-21, C-20, C-18, C-5
19b	2.84 (m)		H-20b, H-19a	C-21, C-20, C-18, C-5
20a	2.68 (m)	18.4	H-20b, H-19a	
20b	2.61 (m)		H-20a, H-19b	C-21
21		140.8		
22		182.17*		
23		138.4**		
24		105.9		
25		157.1		
26	3.02 (m)	33.2	H-27	C-28, C-27, C-25, C-24
27	1.63 (m)	19.6	H-28, H-26	C-28, C-26, C-25
28	1.05 (t, 7.2)	13.9	H-27	C-27, C-26
17-OMe	3.85 (s)	61.5		C-17
NMe	3.65 (s)	33.2		C-25
6 OH	12.98 (s)			C-7, C-6, C-5
10 OH	5.19 (d, 5.4)		H-10	C-10, C-9
13 OH	6.12 (d, 6.3)		H-13	C-14, C-13
1´	4.87 (brs)	97.7	H-2´	C-11, C-5´, C-3´
2´	3.74 (dd, 4.2, 1.6)	70.4	H-3´, H-1´, 2-OH	C-4´, C-3´, C-1´
3´	3.49 (dd, 9.3, 6.2)	68.8	H-4´, H-2´, 3-OH	C-4´, C-2´
4´	3.22 (ddd, 9.3, 9.1, 5.8)	72.0	H-3´, 4-OH	C-6´, C-5´, C-3´, C-2´
5´	3.52 (dq, 9.1, 6.2)	70.6	H-6´	
6´	1.15 (d, 6.2)	17.9	H-5	C-5´, C-4
2´-OH	4.68 (d, 4.2)		H-2´	C-3´/C-2´, C-1´
3´-OH	4.53 (d, 6.2)		H-3´	C-4´, C-3´/C-2´
4´-OH	4.74 (d, 5.8)		H-4´	C-5´, C-4´, C-3´

*Assignments maybe interchanged. ** Assignments are made in comparison with literature data for reported compounds and biosynthetic analogues from same culture.

Table 5: NMR assignments for 13-oxo kibdelone A (**7**)

No.	^{1}H d_H (m, *J* (Hz))	^{13}C	COSY	HMBC
1				
2				
3				
4		134.2*		
5		113.3		
6				
7				
8				
9		125.0		
10	4.97 (dd, 5.5, 2.8)	61.7	H-11, 10-OH	C-14, C-12, C-9
11	4.14 (m)	65.8	H-12, H-10, 11-OH	
12a	3.05 (dd, 16.5, 11.9)	40.9	H-13, H-12b, H-11, H-10	C-13, C-11, C-10
12b	2.70 (dd, 16.5, 4.3)		H-12a, H-11	C-14, C-13, C-10
13	4.71 (m)	191.3	H-12a, 13-OH	
14		151.8		
15 [O]				
16				
17		133.4		
18		137.6*		
19	8.43 (d, 9.0)	125.5	H-20	C-21, C-17, C-5
20	8.11 (d, 9.0)	124.4	H-19	C-22, C-18, C-4
21		131.8		
22		181.1		
23				
24		105.6		
25		156.4		
26	3.03 (brt, 7.8)	32.8		C-28, C-27, C-25, C-24
27	1.65 (m)	19.3		C-28, C-26, C-25
28	1.06 (t, 7.3)	13.5		C-27, C-26
17-OMe	4.06 (s)	61.7		C-17
NMe	3.68 (s)	32.9		C-25/ C-1
6 OH	13.86 (s)			
10 OH	5.61 (d, 5.5)		H-10	C-10, C-9
11 OH	5.40 (d, 6.0)		H-11	C-12, C-11, C-10

* Assignments can be interchanged.

Table 6: NMR assignments for kibdelone analogue (**8**)

No.	^{1}H d_H (m, *J* (Hz))	^{13}C	COSY	HMBC
1		166.9		
2		106.0		
3		152.3		
4		132.3		
5		113.2		
6		154.0		
7		108.9		
8		182.3		
9		118.2		
10	4.70 (dd, 4.5, 3.8)	61.3	H-12b, H-11, 10-OH	C-14, C-12, C-8
11	3.92 (m)	64.0	H-12a, H-12b, H-10, 11-OH	
12a	2.24 (ddd, 13.1, 12.8, 4.6)	33.8	H-13, H-12b, H-11	C-11, C-10
12b	1.78 (brd, 13.1)		H-13, H-12a, H-11, H-10	C-14, C-13, C-10
13	4.68 (m)	65.0	H-12a, H-12b, 13-OH	C-11, C-9
14		165.2		
15[O]		-		
16		149.2*		
17		135.5		
18		**		
19a	2.34 (ddd, 15.3, 14.4, 3.8)	20.8	H-20b	
19b	3.30 (m)		H-20a	
20a	2.22 (brd, 15.3)	22.3	H-19b	
20b	3.30 (m)		H-19a	
21		110.9		
22		150.0		
23		135.2		
24		196.2		
25		92.8		
26	2.03 (dd, 12.0, 5.5)	40.0	H-27	C-27, C-25, C-24, C-23
27a	1.07 (m)	16.2	H-27b, H-28	
27b	0.92 (m)		H-27a, H-28	
28	0.81 (t, 7.2)	13.5	H-27 H-12a, H-12b	C-27, C-26
17-OMe	3.86 (s)	61.5		C-17
25-OMe	3.08 (s)	51.3		C-25
NMe	3.03 (s)	26.6		C-25, C-2, C-1
3 OH	13.60 (s)			C-4, C-3, C-2
6 OH	13.40 (s)			C-7, C-6, C-5
10 OH	5.01(d, 4.5)		H-10	
11 OH	4.76 (brd, 6.5)		H-11	C-12, C-11
13 OH	6.01 (d, 6.4)		H-13	C-14, C-12, C-11
22 OH	11.91 (s)			C-23, C-21

* Assignments are made in comparison with literature data for reported compounds and biosynthetic analogues from same culture. **Overlapping signals.

Interconversion of kibdelones

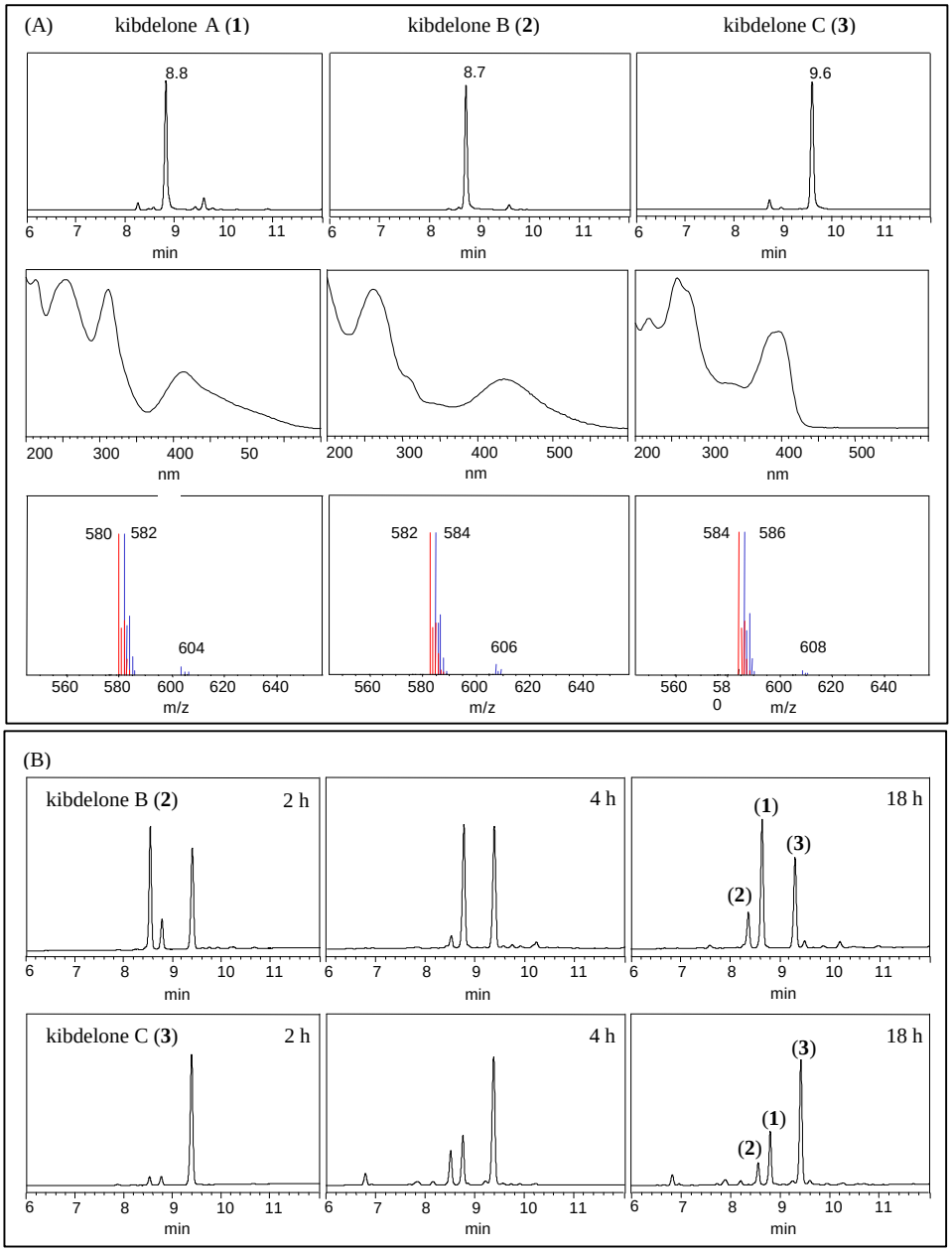

Figure 1: (A) HPLC-DAD-MS of kibdelones A-C (1-3) - displaying retention times, UV/vis and ESIMS spectra (ESI(+)MS in blue, ESI(-)MS in red). (B) Equilibration of 0.1 mg/mL of kibdelones B (2) and C (3) in MeOH in a sealed vial at 40 °C with monitoring (a) after 2 h, (b) after 4 h and (c) after 18 h.

Reference:

(1) Lacey E. and Tennant S.; *Microbiol. Australia*, 2003, *24*(3), 34-35.