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

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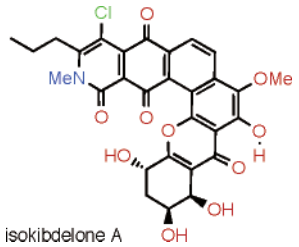
ABSTRACT



The isokibdelones are an unprecedented family of polyketides produced by an Australian isolate of a rare actinomycete, *Kibdelosporangium* sp. The structures of the isokibdelones were assigned by spectroscopic analysis and chemical interconversion. A proposed biosynthesis requires a novel *molecular twist* that generates an unprecedented heterocyclic system and differentiates the isokibdelones from their kibdelone co-metabolites. SAR analysis on the isokibdelones further defines the anticancer pharmacophore of these novel polyketides.

During an earlier investigation into cytotoxic, antibacterial, and nematocidal active metabolites from an Australian isolate of the rare actinomycete genus *Kibdelosporangium* (MST-108465), we described[1] the principle bioactive agents as a new class of heterocyclic polyketides, exemplified by kibdelone A (**1**). Having established the structures and investigated the biological properties of the kibdelones, our interest was drawn to a family of biosynthetically related co-metabolites, the isokibdelones. Our initial encounter with the isokibdelones occurred from a mixed media fermentation[1] that yielded isokibdelone A (**2**) at very low levels. Optimization studies revealed that *Kibdelosporangium* sp. (MST-108465) grown on wheat increased the production of **2** and produced detectable levels of additional metabolites believed to be isokibdelones. An EtOAc extract of a wheat fermentation (20 × 100 g wheat in 15-cm diameter Petri plates, incubated for 18 days at 28 °C) yielded isokibdelone A (**2**) (3.1 mg), isokibdelone A rhamnoside (**2a**) (3.3 mg), and isokibdelone B (**3**) (2.3 mg) and permitted detection of isokibdelone C (**4**). This report describes the production, isolation, characterization, and structure elucidation of the isokibdelones as a new family of heterocyclic polyketides. It also reaffirms the chemical equilibria that occurs in the kibdelone/isokibdelone family and extends an earlier kibdelone anticancer structure activity relationship (SAR) analysis to include the isokibdelones. The HRESI(+)MS data for isokibdelone A (**2**) displayed a pseudo molecular ion consistent with a molecular formula ($C_{29}H_{24}{}^{35}ClNO_{10}$, Δ mmu = −0.4) requiring 18 DBE. Cursory examination of the ESI(±)MS and NMR data suggested a very close structural similarity with the co-metabolite kibdelone A (**1**),[1] even though the UV spectra and HPLC retention times for

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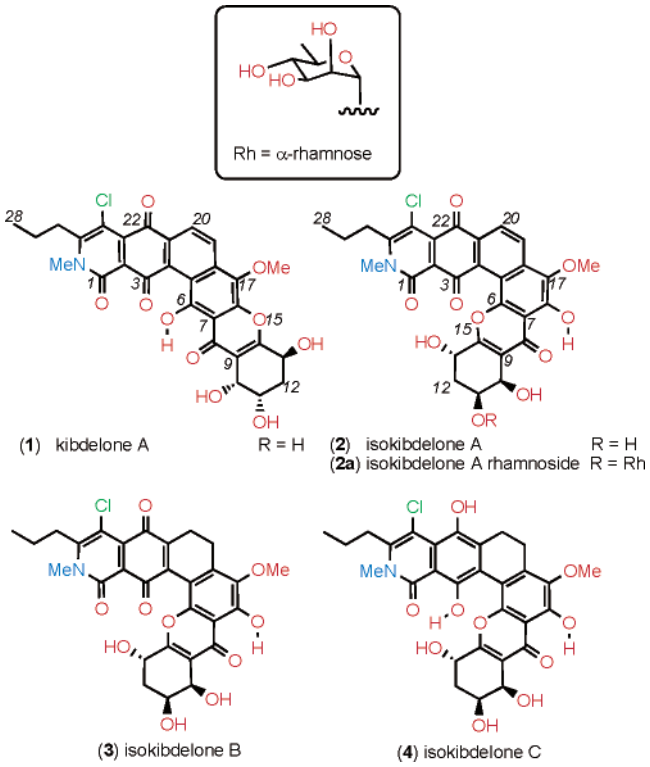

Figure 1. Kibdelone A and isokibdelones isolated from *Kibdelosporangium* sp.

1 and **2** differed significantly (see Figure 2). The ^{1}H NMR spectra of both **1** and **2** featured common *O*- and *N*-methyls, three hydroxy methines, a chelated phenol, an allylic/benzylic *n*-propyl, and a pair of ortho coupled aromatic protons, and the ^{13}C NMR data were remarkably similar. More specifically, the COSY NMR data for **2** displayed three isolated spin systems associated with an *n*-propyl side chain, two aromatic methines (ring C), and the three hydroxy methines connected to a diastereotopic methylene (ring F) (see Figure 3). Furthermore, gHMBC correlations listed in Table 1 permitted assembly of these spin systems into the structure fragments as shown, extending across rings A−F.

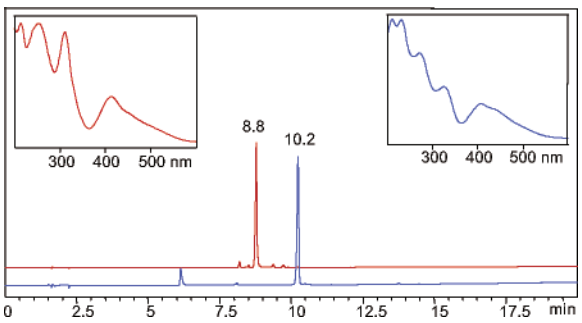

Figure 2. C_8 HPLC-DAD (254 nm) analysis of kibdelone A (**1**) (red) and isokibdelone A (**2**) (blue).

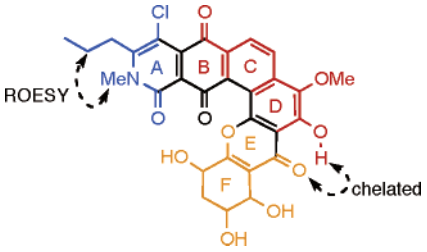

Figure 3. 2D NMR correlations used to confirm structure fragments in isokibdelone A (**2**).

A sequence of COSY and gHMBC correlations from C-8 to C-14 defined the structure fragment incorporating ring F. Attachment of O-15 to C-14 in this fragment was inferred

Table 1. NMR (600 MHz, d_6-DMSO) Data for Isokibdelone A (**2**)

no.	δ_C	δ_H (m, *J* (Hz))[a]	gHMBC
1	157.5		
2	121.7[b]		
3	180.7		
4	134.2		
5	110.8		
6	149.3[b]		
7	111.7		
8	182.2		
9	118.7		
10	61.6[c]	4.74 (m)	C-14, C-12, C-8
11	64.4	3.93 (brd, 12.6)	
12a	32.6	2.28 (ddd, 13.0, 12.6, 5.4)	C-13/C-10
12b		1.82 (brd, 13.0)	C-14, C-13/C-10
13	64.6[c]	4.74 (m)	C-11, C-9
14	165.6		
15[O]			
16	149.2		
17	135.7		
18	135.9		
19	125.5	8.36 (d, 9.0)	C-21, C-17, C-5
20	125.1	8.17 (d, 9.0)	C-22, C-18, C-4
21	130.8		
22	183.7		
23	138.4[b]		
24	106.9		
25	156.7		
26	33.1	3.06 (dd, 8.2, 8.4)	C-27, C-25, C-24
27	19.6	1.65 (m)	C-28, C-26
28	13.9	1.07 (t, 7.3)	C-27, C-26
O*Me*	60.7	3.98 (s)	C-17
N*Me*	33.7	3.74 (s)	C-1
10 OH		5.11 (brs)[c]	C-9
11 OH		4.88 (brs)	C-12, C-13/C-10
13 OH		5.43 (d, 5.0)[c]	C-12
16 OH		12.98 (s)	C-17, C-16, C-7

[a] Asssignments are made with the assistance of COSY correlations. [b] Assignments are made by comparison to known analogs. [c] ^{13}C assignments and some of the corresponding gHMBC signals may be interchanged as a result of overlapping proton signals.

from the ^{13}C NMR shift for C-14 (δ_C 165.6), and the deshielded character of C-8 (δ_C 182.2) identified it as a carbonyl. A second sequence of COSY and gHMBC correlations from C-24 to C-28 defined the structure fragment for an *n*-propyl side chain attached to a fully substituted double bond (δ_C 106.9 and 156.7). The deshielded ^{13}C NMR shift for C-25 (δ_C 156.7) supported attachment of the *N*-Me, while the shielded character of C-24 (δ_C 106.9) was consistent with chloro substitution. ROESY correlations from H_2-26 and H_2-27 to the *N*-Me confirmed the spatial proximity of these functionalities, and a gHMBC correlation from the *N*-Me to C-1 (δ_C 157.5) extended this structure fragment to include an amide/lactam moiety. The set of COSY and gHMBC correlations about H-19 and H-20 clearly defined the tetra-substituted ring C and positioned the quaternary C-22 and C-17 as shown. That C-22 was incorporated into a carbonyl (quinone) was apparent from the ^{13}C NMR shift (δ_C 183.7), and a gHMBC correlation from the *O*-Me to the deshielded C-17 (δ_C 135.7) positioned the *O*-Me moiety as shown. A set of gHMBC correlations from the chelated C-16 phenolic (δ_H 12.98) to C-17 (δ_C 135.7), C-16 (δ_C 149.2), and C-7 (δ_C 111.7) extended this structure fragment across rings B−D (see Figure 3).

Support for a ring B quinone (C-22 and C-3 carbonyls) was evident from the diagnostic upfield ^{13}C NMR resonance for the ring A lactam carbonyl (δ_C 157.5).[1] For example, in known compounds bearing a similar A/B ring system the ^{13}C NMR shift for C-1 is significantly influenced by the oxidation state of C-3, as reported for xantholipin[2] (δ_C 159.7) and actinoplanones (δ_C 163−166).[3] The ^{13}C NMR assignments for C-2, C-3, and C-23 in isokibdelone A (**2**) were attributed to resonances at δ_C 121.7, 180.7, and 138.4, respectively. These latter assignments were supported by excellent comparsions to the ^{13}C NMR data for kibdelone A (**1**) (δ_C 121.7, 183.4, and 138.4, respectively), confirming a common regiochemistry for the fusion between rings A/B.[1] The deshielded nature of the sole remaining unassigned carbon resonance (δ_C 149.3) required that it be substituted by oxygen and positioned at C-6, completing the fusion between rings D and E. This ring D/E linkage also explained the chelated character of the C-16 hydroxyl. A C-4 to C-5 closure of ring C completed the planar structure for **2**.

Assignment of relative stereochemistry to isokibdelone A (**2**) proved challenging as a result of overlapping ^1H NMR-(d_6-DMSO) resonances for H-10 and H-13 (δ_H 4.74 m). However, despite this impediment, values for $J_{11,12a}$ and $J_{12a,13}$ of 12.6 and 5.4 Hz clearly indicated pseudo diaxial and axial-equatorial relationships and required placement of the C-11 and C-13 hydroxyls in pseudo equatorial and axial orientations, respectively. Likewise lack of a significant value for $J_{10,11}$ ($W_{1/2}$ H-10/H-13 multiplet = 8.4 Hz) suggested placement of the C-10 hydroxyl in a pseudo axial orientation. Such a relative stereochemistry for **2** was consistent with that independently established for all kibdelone co-metabolites.[1] In an attempt to obtain additional evidence for this stereochemical assignment the ^1H NMR data for **2** was acquired in $CDCl_3$ (see Supporting Information). This data resolved resonances for H-10 (δ_H 5.04) and H-13 (δ_H 5.24) and provided an unambiguous sequence of COSY and gHMBC correlations from H-10 to H-13, consistent with the ring F to E regiochemistry as shown. Curiously, the ^1H NMR ($CDCl_3$) values for $J_{11,12a}$ and $J_{12b,13}$ of 5.5 and 9.0 Hz were no longer indicative of an H-11/H-12a pseudo diaxial and an H12b/H-13 pseudo diequatorial relationship, as previously determined from interpretation of the ^1H NMR (d_6-DMSO) data. This anomoly was readily reconciled by the proposition that ring F in **2** adopted alternate chair conformations in d_6-DMSO versus $CDCl_3$. Careful comparison of the full set of ^1H NMR *J* values for **2**, measured in both d_6-DMSO and $CDCl_3$, supported this proposal. In this manner the complete relative stereostructure for isokibdelone A (**2**) can be assigned as shown. The absolute stereochemistry of **2** remains unassigned at this time and will most likely require either the preparation of suitable crystalline derivatives for X-ray analysis, total asymmetric synthesis, or quantum mechanical calculations aimed at simulating optical properties.

The HRESI(+)MS data for isokibdelone B (**3**) displayed a pseudo molecular ion consistent with a molecular formula ($C_{29}H_{26}{}^{35}ClNO_{10}$, Δ mmu = 1.0) requiring 17 DBE and suggestive of a dihydro analogue of **2**. This was further confirmed by the replacement of ^1H NMR resonances for the ring C aromatic methines (H-19 and H-20) in **2** with resonances for two methylenes (δ_H 2.74, 2.63 and δ_H 2.97, 2.86) in **3**. A diagnostic gHMBC correlation from the chelated 16-OH to C-17, together with a detailed analysis of 1D and 2D NMR data (see Supporting Information), confirmed the relative stereostructure for isokibdelone B (**3**) as shown. Supportive of this assignment, on storage in MeOH at 40 °C the quinone **3** underwent a facile transformation over 24 h to a 1:10:1 equilibrium mixture attributed to isokibelones A (**2**), B (**3**), and C (**4**), respectively. The identity of **2** and **3** in this mixture was confirmed by HPLC-DAD-ESI(±) MS comparisons with authentic standards, while the identity of isokibdelone C (**4**) was inferred from its HPLC-DAD-ESI(±)MS characteristics and by consideration of the comparable equilibria known to occur between kibdelones A−C.[1] A plausible mechanism for the isokibdelone equilibrium is presented in Scheme 1. This mechanism draws on air oxidation, redox processes, and an orchestrated sequence of keto/enol transformations, which guide the equilibrium through a key quinone methide intermediate. It is our hypothesis that isokibdelone B (**3**) is suitably configured to undergo an acid-mediated double keto/enol tautomerization to yield an unstable quinone-methide intermediate, which can in turn undergo a third keto/enol tautomerization to yield the hydroquinone **5** in which ring C has aromatized. The proposed mechanism also explains the stability of isokibdelone A (**2**), insofar as aromatic stabilization of ring C in **5** ensures that the sequence of keto/enol transformations leading from **3** to **5** is irreversible and any redox transformation involving **2** and **5** would not lead to new chemical entities.

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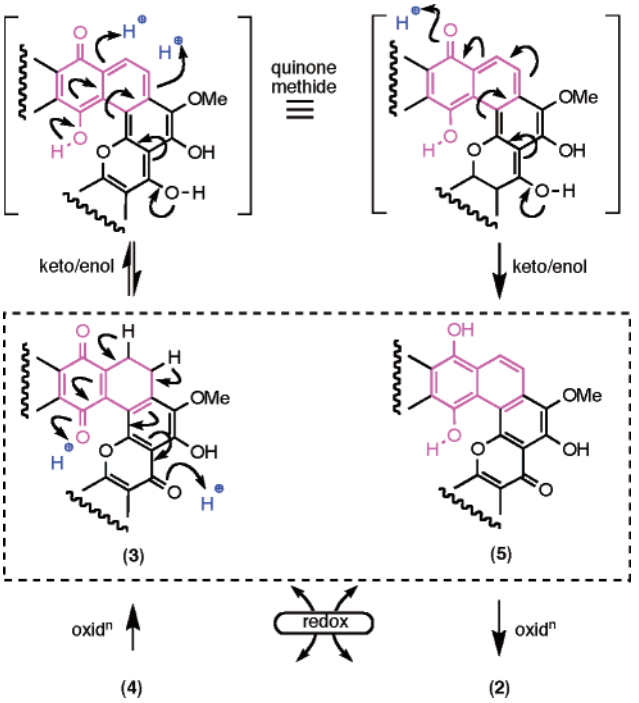
(3) Kobayashi, K.; Nishino, C.; Ohya, J.; Sato, S.; Mikawa, T.; Shiobara, Y.; Kodama, M. *J. Antibiot.* **1988**, *41*, 502−511.

Scheme 1. A Plausible Mechanism for the Equilibrium Interconversion of Isokibdelones A−C (**2**−**4**)



HRESI(+)MS analysis of the remaining isokibdelone discovered during this study, isokibdelone A rhamnoside (**2a**), confirmed a pseudo molecular ion consistent with a molecular formula ($C_{35}H_{34}{}^{35}ClNO_{14}$, Δ mmu = +0.3) requiring 19 DBE. Compound **2a** displayed NMR properties (see Supporting Information) almost identical to those observed for isokibdelone A (**2**), with additional resonances being attributed to a 6-deoxyhexapyranose residue. Comparison with NMR data reported for kibdelone B rhamnoside[1] defined the α-rhamnopyranosyl subunit in **2a**, and a gHMBC correlation (d_6-DMSO) from H-11 (δ_H 4.03) to C-1′ (δ_C 97.9) confirmed placement of the glycosidic linkage in ring F as shown. Arguments for assignment of relative stereochemistry followed those presented above for the aglycone **2**. A paucity of material precluded determination of absolute stereochemistry for the rhamnose residue.

Our discovery of the isokibdelones represented an ideal opportunity to expand structure activity relationship (SAR) investigations into this unique family of polyketides. Prior investigations[1] had confirmed that the kibdelones displayed potent and selective cytotoxicity against a range of human tumor cell lines, prompting the filing of a provisional patent and initiating ongoing in vivo and mode-of-action investigations. By comparison, this current study has revealed that the isomeric isokibdelones do not display significant antitumor activity. In the NCI 60 cell line panel the mean GI_{50} values for isokibdelones (isokibdelone A (**2**), 30 nM; isokibdelone A rhamnoside (**2a**), 350 nM; isokibdelone B (**3**), 710 nM) were 10−200 times less potent than those observed for kibdelone co-metabolites.

Of particular note, the isokibdelones feature an unprecedented polyketide heterocyclic skeleton that requires a new molecular twist to traditional polyketide biosynthesis. We propose that key transformations in the biosynthesis of kibdelones and isokibdelones follow a traditional polyketide cyclization pathway to yield rings A−E, with ring E adopting a quinone oxidation level. Oxidative cleavage of the C-15 to C-16 bond, via a lactone intermediate, would yield a transient hydroxy acid (C-16 phenol) that could reclose via phenolic displacement of formic acid to regenerate ring E as a xanthone. This biosynthetic conversion of a quinone to a xanthone has been previously documented.[4] We hypothesize that the biosynthesis of kibdelones and isokibdelones diverge at this key xanthone-forming step. Nucleophilic attack on C-14 by an adjacent C-16 phenol would eliminate formic acid and form the xanthone found in kibdelones. Alternatively, a 180° rotation about the C-7 to C-8 bond would permit the nucleophilic attack on C-14 by a C-6 phenol, displacing formic acid and forming the xanthone found in isokibdelones. This molecular twist in the isokibdelone biosynthesis is unprecedented and heralds a pathway to unexplored polyketide chemical space.

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Supporting Information Available: Full details of the collection and culturing of the *Kibdelosporangium* sp. and the isolation and spectroscopic characterization of compounds **2**, **3**, **4**, and **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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