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Kahakamides A and B, new neosidomycin metabolites from a marine-derived actinomycete

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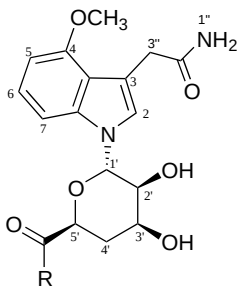
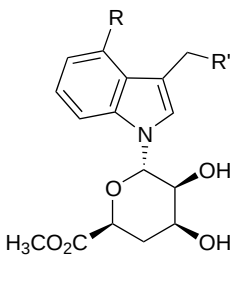
Abstract—Two new indole nucleosides kahakamides A (**1**) and B (**2**) were isolated from the actinomycete *Nocardiopsis dassonvillei*, obtained from a shallow water sediment sample collected on the island of Kauai, Hawaii. Compounds **1** and **2** are related to the neosidomycin antibiotics, a group of rare indole-*N*-glycosides, and their structures were determined using spectroscopic methods. Compound **1** exhibited antimicrobial activity toward the Gram positive bacterium *Bacillus subtilis*.

Marine microorganisms have been the topic of an increasing number of natural products investigations.[1] Marine bacteria, in particular, have received increased attention as potential sources of biologically active metabolites. Although largely ignored when compared to their terrestrial counterparts, bacteria isolated from both shallow and deep-water sediments have produced many biologically-active and/or structurally unique compounds.[2] We would now like to report the isolation and structure determination of two new compounds, kahakamides A (**1**) and B (**2**), from an actinomycete cultured from a shallow water marine-sediment sample. Compounds **1** and **2** were identified to be rare *N*-glycosyl indoles, a class of microbial metabolites represented previously by only two compounds, neosidomycin (**3**)[3] and SF-2140 (**4**).[4]



1 R = OCH_3
2 R = NH_2



3 R = H; R' = $CONH_2$
4 R = OCH_3; R' = CN

Isolate BH-609, cultured from sediments collected along the high tide line at Kahaka Beach Park, Kauai, was identified as *Nocardiopsis dassonvillei* on the basis of FAME analysis. The crude extract (800 mg), obtained by extraction of the fermentation broth with EtOAc, was subjected to Sephadex LH-20 (MeOH/$CHCl_3$) chromatography followed by silica gel flash chromatography, yielding purified compounds **1** (12 mg) and **2** (1 mg).

The UV spectrum of **1** displayed absorption maxima at 222, 266, 286, and 295 nm, characteristic of an indole ring. IR peaks at 3345, 1740, and 1665 cm^{-1} supported the presence of alcohol, ester, and amide functionalities. The EI mass spectrum of compound **1** did not show a M^+ ion; however, treatment with acetic anhydride/pyridine provided diacetyl derivative **5**, which yielded a molecular ion at m/z 462, requiring a molecular formula of $C_{22}H_{26}N_2O_9$ (462.1638 Δ 0.0 mmu).

The ^1H spectrum of **1** (Table 1) contained signals assignable to a 3,4-disubstituted indole ring system, a pair of exchangeable protons [6.92 (bs) and 6.78 (bs) ppm], an anomeric proton (H1′), three heteroatom substituted methines (H2′, H3′, and H5′), two diastereomeric methylenes (H4′ and H3″), and two hetero-substituted methyl groups. Diagnostic ^{13}C NMR signals (Table 1) included those assigned to both ester and amide carbonyls (172.4 and 173.1 ppm, respectively), as well as eight sp^2 carbons that belong to the indole ring.

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Table 1. NMR data for **1** and **2**[a]

Atom no.	**1**			**2**	
	^{13}C	^{1}H (mult., J (Hz))	HMBC correlations	^{13}C	^{1}H (mult., J (Hz))
2	122.9	7.21 (s)	H1′, H3a″, H3b″	123.5	7.17 (s)
3	109.4		H2, H3a″, H3b″	109.5	
3a	117.6		H2, H3a″, H3b″, H5, H7	117.7	
4	154.0		H5, H6, ArOMe	154.1	
5	100.0	6.52 (d, 7.6)	H7	100.2	6.51 (d, 7.8)
6	122.3	7.06 (t, 7.9)		122.4	7.02 (t, 8.0)
7	104.0	7.30 (d, 8.1)	H5	104.5	7.23 (d, 8.3)
7a	138.8		H2, H6, H1′	138.5	
1′	78.0	6.11 (d, 9.2)	H2, H3′, H5′	80.7	5.99 (d, 7.0)
2′	68.5	3.94 (d, 9.2)	H1′	68.3	4.00 (t, 5.4)[b]
3′	67.0	4.07 (br s)	H1′, H5′	66.5	4.06 (m)
4′α	33.5	2.15 (dd, 13.2, 6.8)	H2′, H5′	32.1	2.18 (dt, 13.9, 5.9)
4′β		2.30 (dd, 13.2, 2.6)			2.02 (ddd, 13.9, 5.9, 3.5)
5′	69.2	4.46 (d, 6.8)	H1′, H4α′	70.5	3.99 (m)[b]
6′	172.4		H4′α, COOMe	173.2	
1″ NH_2		(a) 6.78			(a) 6.78
		(b) 6.92			(b) 6.92
2″	173.1			173.2	
3″	33.8	(a) 3.56 (d, 16.0)		33.8	3.55 (d, 15.8)
		(b) 3.61 (d, 16.0)			3.64 (d, 15.8)
ArOMe	55.1	3.81 (s)		55.2	3.80 (s)
COOMe	51.5	3.69 (s)			
$CONH_2$					(a) 7.12
					(b) 7.13

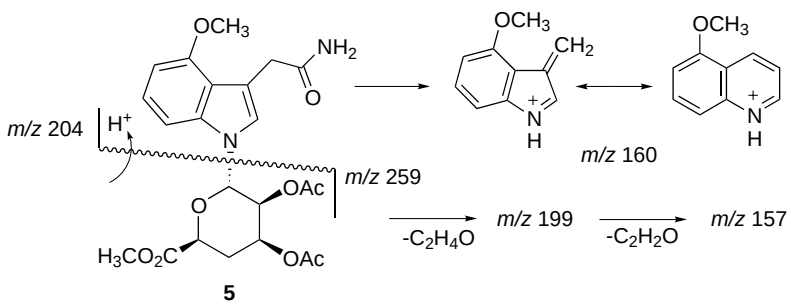
[a] Recorded in DMSO-d_6.
[b] Assignments may be reversed.

The structure of the indole subunit was confirmed using a HMBC experiment. Specifically, quaternary carbon C4 showed 3-bond coupling to the methoxy protons at δ 3.81 as well as to H6. Likewise, quaternary carbon C3a showed 3-bond correlations to both the isolated aromatic singlet H2 and the diastereomeric methylene protons H3″, while quaternary carbon C7a showed coupling to both H2 and H6, thus establishing the structure of the indole subunit.

Stepwise coupling was observed in the COSY experiment from H1′ through H5′, allowing construction of the glycosidic subunit found in **1**, and HMBC correlations between C6′ and both H4′β and the methyl group at δ 3.69 established the position for the attachment of the ester. The position of the glycosidic linkage was also established using the HMBC data. In particular, C7a and C2 both showed 3-bond correlations to anomeric proton H1′, while C1′ showed a 3-bond correlation to the aromatic proton H2. Furthermore, in the ^{13}C NMR spectrum, the anomeric carbon was observed at δ 78.0, which is characteristic of a N–C–O glycoside linkage.[5] These results firmly establish that the glycosidic moiety is linked to the N-atom of the indole ring.

The atomic connectivity proposed for compound **1** was further supported by the fragmentation observed for compound **5** in the EIMS (see Scheme 1). As previously observed for diacetylneosidomycin,[3] cleavage of the glycosidic bond leads to daughter ions at m/z 259 and 204. In addition, further fragmentation yielded ions at m/z 199 (100), 160 (79), and 157 (77), which were also analogous to those observed previously.[3]

The relative stereochemistry of **1** was proposed based on analyses of the ^{1}H–^{1}H scalar couplings (see Table 1) and through a comparison of the NMR data with those reported for neosidomycin[3] and SF-2140.[4] The large coupling between H1′ and H2′ requires both the indole



Scheme 1. Mass spectral fragmentation of diacetylkahakamide A (**5**).

and the C2′-OH to be in equatorial positions, while the weak coupling between H2′ and H3′ (only seen in COSY spectrum) requires the C3′-OH to be axial. Small coupling constants observed between H4′β (equatorial) and both H3′ and H5′, together with the W-coupling observed between H3′ and H5′ only in the COSY spectrum, require the ester group at C5′ to be axial. It has been argued[4,6] that the sugar units in both neosidomycin (**3**) and SF-2140 (**4**) adopt a twist-boat conformation when dissolved in acetone-d_6 or $CDCl_3$. While the coupling constants recorded for kahakamide A (**1**) dissolved in DMSO-d_6 are similar to those reported for compounds **3** and **4**, the W-coupling observed between H3′ and H5′ better supports a chair conformation, as was observed in the crystal structure of SF-2140.[4] The absolute configuration is drawn simply to be consistent with those determined for compounds **3** and **4** and was not been determined experimentally.

As with compound **1**, the EI mass spectrum of kahakamide B (**2**) did not show a M^+ ion; nevertheless, the NMR spectral data (see Table 1) was very similar to that observed for **1**. Two obvious differences in the ^1H NMR data were that the signal assigned to the methoxy group at δ 3.69 was missing and that two new signals assignable to amide protons were observed at δ 7.2 and 7.3. In addition, a significant chemical shift difference was observed for H5′. To account for these differences, kahakamide B (**2**) is proposed to differ from kahakamide A (**1**) simply by the substitution of a primary amide at C5′ for the ester group found in kahakamide A (**1**). Confirmation of the proposed structure was obtained by treatment of **1** with NH_4OH (2 h, 110°C), yielding a product whose ^1H NMR spectrum matched that of **2**.

Compound **1** was antimicrobial showing slight inhibition of *Bacillus subtilis* in a disk-diffusion assay. Compound **2** was not tested due to insufficient sample size.

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