Amphimedosides, 3-Alkylpyridine Glycosides from a Marine Sponge *Amphimedon* sp.

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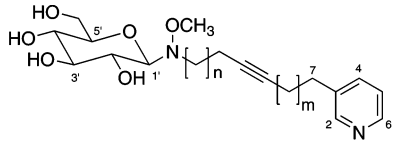
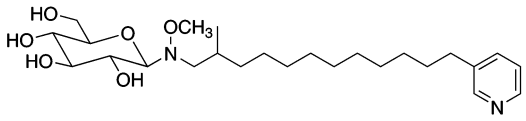
Five 3-alkylpyridine alkaloids, amphimedosides A−E (**1**−**5**), have been isolated from a marine sponge *Amphimedon* sp. The structures of **1**−**5** have been determined by analysis of NMR and FABMS data and chiral GC analyses of the acid hyrolyzates. In particular, the site of glycosylation in **1** was confirmed by the $^{1}H-^{15}N$ HMBC experiment, and the location of the double bond in **5** was assigned on the basis of tandem FABMS data. Amphimedosides are the first examples of β-D-glucosylated 3-alkylpyridine alkaloids and exhibited cytotoxic activities comparable with those of the nonglycosylated congeners.

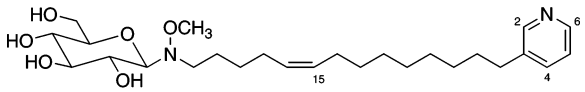
A unified view of the biogenesis of diverse structural arrays of sponge-derived alkaloids such as halitoxins,[1] haliclamines,[2] sarains,[3] manzamines,[4] petrosins,[5] xestospongins,[6] papuamines,[7] and niphatynes[8] was provided by the seminal proposal of Baldwin and Whitehead.[9] These compounds could be formed through 3-alkylpyridine derivatives that were formed by condensations of one or more molecules of acrolein, α,ω-alkandial, and ammonia. The relevant biosynthetic pathways appear to be widely distributed among sponges of the order Haplosclerida.[10] Although glycosylation of secondary metabolites has frequently been observed in nature,[11] none of the above-mentioned alkaloids have been found to be glycosylated.[12] In this paper we describe the first glycosylated 3-alkylpyridine alkaloids, amphimedosides A−E (**1**−**5**), from a marine sponge *Amphimedon* sp.



amphimedoside A (**1**): m = 3, n = 9
amphimedoside B (**2**): m = 3, n = 7
amphimedoside C (**3**): m = 1, n = 9



amphimedoside D (**4**)



amphimedoside E (**5**)

The sponge was extracted with MeOH, EtOH, and acetone, and the combined extracts were subjected to solvent partitioning. The resulting $CHCl_3$ layer was separated by normal and reversed-phase chromatographies to afford amphimedosides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**).

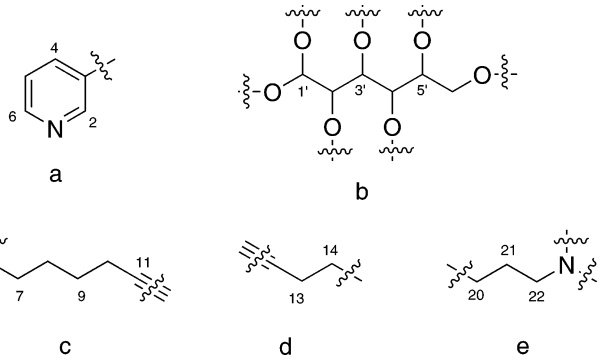


Figure 1. Partial structures of **1** as assigned by the COSY data.

The molecular formula of amphimedoside A (**1**) was determined to be $C_{28}H_{46}N_2O_6$ on the basis of HRFABMS. The ^{1}H NMR spectrum exhibited a methylene envelope (δ 1.27−1.40), nine methylenes (δ 1.35, 1.43, 1.50, 1.58, 1.74, 2.11, 2.17, 2.66, and 2.84/3.03), one oxygenated methylene (δ 3.67 and 3.82), four oxygenated methines (δ 3.20, 3.29, 3.37, and 3.44), one anomeric proton (δ 4.01), one methoxyl (δ 3.62), and four aromatic protons (δ 7.35, 7.69, 8.35, and 8.38). Additionally, the ^{13}C NMR spectrum exhibited a pair of nonprotonated sp carbons (δ 80.6 and 81.4) and an sp^2 (δ 140.2) carbon.

By interpretation of COSY and HMQC data, five substructures, **a**−**e**, were established (Figure 1). Four mutually coupled aromatic protons comprised a 3-substituted pyridine (substructure **a**). Starting from the C-1′ anomeric carbon (δ_C 94.4, δ_H 4.01), the connectivity of five contiguous oxygenated methines and one oxygenated methylene from C-1′ to C-6′ was defined as substructure **b**. Substructure **c** (C-7 to C10) was determined as four contiguous methylenes in which C-7 was allylic and C-10 was propargylic on the basis of ^{1}H and ^{13}C chemical shifts. Substructure **d** consisted of the C-13 and C-14 methylenes. The chemical shift of C-13 (δ 19.5) indicated that this carbon was also propargylic. Substructure **e** consisted of three contiguous methylenes (C-20−C-22), in which C-22 was attached to nitrogen as indicated by the characteristic chemical shift values (δ_C 54.2; δ_H 2.84/3.03). Both H_2-14 and H_2-20 were correlated to signals in the methylene envelope (δ_H 1.27−1.40).

Further structure analysis was carried out by interpretation of the HMBC spectrum. The presence of a 3-substituted pyridine ring was confirmed by the HMBC correlations H-2/C-3, H-4/C-2, H-5/C-3, and H-6/C-2, whereas C-7 in substructure **c** was connected to the pyridine ring as shown by the HMBC cross-peaks H-7/C-2, H-7/C-3, and H-7/C-4. Substructures **c** and **d** were connected through the acetylene (C-11 and C-12) on the basis of HMBC

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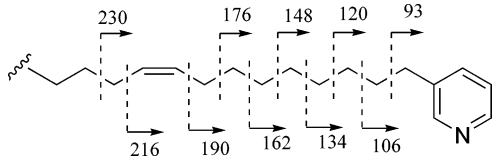

Figure 2. Tandem FABMS analysis of **5**.

correlations H-9/C-11, H-10/C-11, H-13/C-12, and H-14/C-12. Substructure **b** was a hexapyranosyl moiety, as shown by the HMBC correlation H-5′/C-1′. Considering the magnitudes of the coupling constants ($J_{H-1'/H-2'}$ = 8.9 Hz, $J_{H-2'/H-3'}$ = 8.9 Hz, $J_{H-3'/H-4'}$ = 8.9 Hz, $J_{H-4'/H-5'}$ = 8.9 Hz, $J_{H-5'/H-6'b}$ = 2.0 Hz, $J_{H-5'/H-6'a}$ = 5.2 and 11.9 Hz), the sugar was assigned as β-glucopyranose. A pair of HMBC cross-peaks (H-22/C-1′ and H-1′/C-22) indicated glycosylation of the nitrogen connected to C-22 in substructure **e**. $^1H-^{15}N$ HMBC correlations (23-OMe/N-23, H-1′/N-23, and H-22/N-23) established that the *O*-methyl group (δ_C 63.1, δ_H 3.62) and the glucosyl moiety were attached at N-23. To fulfill the molecular formula, a $(CH_2)_5$ unit was required between substructure **d** and substructure **e**.

Amphimedoside B (**2**) had a molecular formula of $C_{26}H_{42}N_2O_6$, which was smaller than that of **1** by a C_2H_4 unit. The ^1H NMR spectrum of **2** was very similar to that of **1** except for the integration of the methylene envelope (δ_H 1.27−1.35) (Table S1). All the 2D NMR data of **2** were indistinguishable from that of **1**. From these data, **2** was shown to have two less methylene carbons in the long alkyl chain of **1**.

Amphimedoside C (**3**) was isomeric with **2**. The ^1H NMR spectrum of **3** indicated the presence of the 3-substituted pyridine, an N-linked β-glucosyl moiety, a triple bond, and an *O*-methyl group. The sugar residue was again assigned as β-glucopyranose on the basis of the NMR data (Table S2). Fortuitously, the position of the triple bond was determined to be between C-9 and C-10 on the basis of the HMBC cross-peaks (H-7/C-9, H-8/C-9, H-11/C-10, and H-12/C-10). Therefore, amphimedoside C (**3**) was a positional isomer of **2** in the acetylenic bond.

Amphimedoside D (**4**) had the molecular formula of $C_{25}H_{44}N_2O_6$, as established by the HRFABMS. The NMR data (Table S3) revealed the structural similarity of this compound to **1**−**3**. The significant differences lay in the absence of the acetylenic signals and the presence of one methyl signal (δ_C 18.9; δ_H 0.99, d). The position of the branched methyl group was determined to be at C-17 on the basis of COSY correlations between the methyl doublet and H-17, which was in turn correlated with the deshielded H_2-18 methylene protons. The sugar residue was also assigned as β-glucopyranose on the basis of the NMR data.

Amphimedoside E (**5**) had a molecular formula of $C_{26}H_{44}N_2O_6$, which was larger than that of **2** by H_2. The NMR data (Table S4) indicated that the triple bond in **2** was replace by a double bond. The sugar moiety was again assigned as β-glucopyranose on the basis of the NMR data, and the position of the double bond was determined by tandem FABMS (Figure 2). The *Z* configuration of the double bond was assigned on the basis of the carbon chemical shifts of the allylic carbons, δ 28.0 each for C-14 and C-17.[13]

The glucose residue in **1**−**5** was determined to be D by chiral GC analyses of the methanolysis products. Amphimedosides **1**−**5** showed moderate cytotoxicity against P388 murine leukemia cells [IC_{50} values (μg/mL) of **1**, **2**, **3**, **4**, and **5** are 11, 11, 5.0, 0.45, and 2.2, respectively].

A number of 3-alkylpyridines have been isolated from marine sponges of the family Niphatidae.[8] The majority of these metabolites possess a methoxyamino group at the ω-end of the alkyl chain. Amphimedoside C (**3**) is the β-D-glucopyranoside of niphatesine H,[14] whereas amphinedoside D (**4**) is the β-D-glucopyranoside of hachijodin D (cribochaline A).[15,16] The aglycones of amphimedosides A, B, and E have not been reported. Amphimedosides possess cytotoxic activity almost comparable with those of nonglucosylated 3-alkylpyridines[15]; hence, the N-glucosylation neither enhances nor diminishes the cytotoxic activity of the aglycone.

Experimental Section

General Experimental Procedures. NMR spectra were recorded either on a JEOL A600 or A500 NMR spectrometer. ^1H and ^{13}C NMR chemical shifts were referenced to the solvent peaks, δ_H 3.30 and δ_C 49.0 for CD_3OD. FAB mass spectra were measured on a JEOL JMX-SX102/SX102 mass spectrometer.

Collection and Isolation. The sponge *Amphimedon* sp. (collection number of Zoological Museum, University of Amsterdam, ZMAPOR19092) was collected by hand using scuba at a depth of 20

Table 1. NMR Spectral Data for Amphimedoside A (**1**) in CD_3OD

position	δ_C	δ_H (mult, J = Hz)	COSY	$^1H-^{13}C$ HMBC	$^1H-^{15}N$ HMBC
1					
2	150.1	8.38 (d, 1.6)	H-4	C-3, 4, 6, 7	N-1
3	140.2				
4	138.3	7.69 (dt, 1.8, 8.0)	H-5	C-2, 6, 7	
5	125.3	7.35 (dd, 4.9, 7.6)	H-4, 6	C-3, 6	N-1
6	147.6	8.35 (dd, 1.2, 4.5)	H-5	C-2, 4, 5	N-1
7	33.4	2.66 (t, 7.8)	H-8	C-2, 3, 4, 8, 9	
8	31.5	1.74 (m)	H-7, 9	C-7, 9, 10	
9	29.7	1.50 (m)	H-8, 10	C-7, 8, 10, 11	
10	19.3	2.17 (m)	H-9, 13	C-8, 9, 11, 13	
11	80.6				
12	81.4				
13	19.5	2.11 (m)	H-10, 14	C-12, 14	
14	30.0	1.43 (m)	H-13	C-12, 13	
15−19	28−31	1.27−1.40			
20	28.5	1.35 (m)	H-21		
21	28.6	1.58 (m)	H-20, 22	C-20, 22	N-23
22a	54.2	2.84 (m)	H-21, 22b	C-21, 24	N-23
22b		3.03 (m)	H-21, 22a	C-21, 24	N-23
23					
1′	94.4	4.01 (d, 8.9)	H-2′	C-22, 2′, 3′	N-23
2′	71.7	3.44 (t, 8.9)	H-1′, 3′	C-1′, 3′, 4′	N-23
3′	79.6	3.37 (t, 8.9)	H-2′, 4′	C-2′, 4′	
4′	71.3	3.29 (t, 8.9)	H-3′, 5′	C-2′, 3′, 6′	
5′	79.8	3.20 (m, 2.0, 5.2, 8.6)	H-4′, 6′a	C-1′, 4′	
6′a	62.9	3.67 (dd, 5.2, 11.9)	H-5′, 6′b	C-5′	
6′b		3.82 (dd, 2.0, 11.9)	H-6′a	C-3′, 4′, 5′	
23-OMe	63.1	3.62 (s)			N-23

m off Hachijo Island (33°7′ N, 139°41′ E) and kept frozen at −20 °C until used. The sample (1.7 kg, wet weight) was extracted with MeOH (3 L × 2), EtOH (3 L × 1), and acetone (3 L × 1). The extracts were combined, evaporated in vacuo, and partitioned between H_2O and Et_2O. The Et_2O layer was partitioned between 90% MeOH and *n*-hexane, and the 90% MeOH layer was diluted with H_2O to adjust the concentration of MeOH to 60% and extracted with $CHCl_3$. The $CHCl_3$ layer was subjected to ODS flash chromatography with increasing amounts of MeOH in water. The fraction eluted with MeOH−H_2O (9:1) was separated by SiO_2 flash chromatography using $CHCl_3$ with increasing amounts of MeOH. The fraction eluted with $CHCl_3$−MeOH (9:1) was purified by ODS-HPLC with MeOH−H_2O (7:3) to afford 0.9 mg of amphimedoside C (**3**). Another HPLC fraction was further purified by Phenylhexyl-HPLC with MeCN−H_2O (38:62) to give 1.7 mg of amphimedoside B (**2**). The other fraction from the above-mentioned ODS-HPLC was purified by ODS-HPLC with MeCN−50 mM NH_4OAc (45:55) followed by C_{30}-HPLC with MeOH−50 mM NH_4OAc (75:25) to yield 28.1 mg of amphimedoside A (**1**). A fraction from the ODS-HPLC with MeCN−50 mM NH_4OAc was further purified by Phenylhexyl-HPLC with MeOH−50 mM NH_4OAc (75:25) to afford 22.7 mg of amphimedoside D (**4**) and 2.1 mg of amphimedoside E (**5**).

Amphimedoside A (1): NMR data, see Table 1; FABMS (positive, NBA matrix) *m*/*z* 507 $[M + H]^+$, 529 $[M + Na]^+$; HRFABMS (positive, PEG 500 in glycerol matrix) *m*/*z* 507.3436 (calcd for $C_{28}H_{47}N_2O_6$, 507.3429).

Amphimedoside B (2): NMR data, see Table S1; FABMS (positive, NBA matrix) *m*/*z* 479 $[M + H]^+$, 501 $[M + Na]^+$; HRFABMS (positive, PEG 500 in glycerol matrix) *m*/*z* 479.3157 (calcd for $C_{26}H_{43}N_2O_6$, 479.3116).

Amphimedoside C (3): NMR data, see Table S2; FABMS (positive, NBA matrix) *m*/*z* 479 $[M + H]^+$, 501 $[M + Na]^+$; HRFABMS (positive, PEG 500 in glycerol matrix) *m*/*z* 479.3124 (calcd for $C_{26}H_{43}N_2O_6$, 479.3116).

Amphimedoside D (4): NMR data, see Table S3; FABMS (positive, NBA matrix) *m*/*z* 469 $[M + H]^+$, 491 $[M + Na]^+$; HRFABMS (positive, PEG 500 in glycerol matrix) *m*/*z* 469.3303 (calcd for $C_{25}H_{45}N_2O_6$, 469.3272).

Amphimedoside E (5): NMR data, see Table S4; FABMS (positive, NBA matrix) *m*/*z* 481 $[M + H]^+$, 503 $[M + Na]^+$; HRFABMS (positive, PEG 500 in glycerol matrix) *m*/*z* 481.3248 (calcd for $C_{26}H_{45}N_2O_6$, 481.3272); FABMS/MS, specific prominent peaks, *m*/*z* 272, 258, 244, 230, 216, 190, 176, 162, 148, 134, 120, 106, and 93.

Determination of the Absolute Stereochemistry of the Glucose Residue.[17] A portion of each compound (0.1 mg, respectively) was dissolved in 10% HCl−MeOH and kept at 100 °C for 2 h. The solvent was removed with a stream of nitrogen, and the residue was further dried in vacuo. The products were dissolved in a mixture of CH_2Cl_2 (50 μL) and trifluoroacetic anhydride (50 μL) and kept at 100 °C for 10 min. The reaction mixture was dried and redissolved in CH_2Cl_2 (50 μL) and subjected to GC analysis: stationary phase, Chirasil-L-Val (25 m × 0.25 mm, i.d.); detection, FID; initial temperature 50 °C for 6 min, final temperature 160 °C for 1 min, temperature raised at 4 °C min^{-1}. Retention times: L-Glu (19.3 and 24.5 min), D-Glu (19.5 and 24.8 min); product peaks (19.5 and 24.8 min). The assignment of peaks was confirmed by co-injection with the standard.

Cytotoxicity Test. P388 murine leukemia cells were cultured in RPMI 1640 medium supplemented with 100 μg/mL kanamycin sulfate, 2 mmol/L L-glutamine, 10% fetal bovine serum, and 10 mg/mL 2-hydroxyethyl disulfide at 37 °C under an atmosphere of 5% CO_2. To each well of the 96-well microplates, which contained the P388 murine leukemia cells in culture medium (10 000 cells/mL), was added 100 μL of test solution, and the plates were incubated at 37 °C for 96 h. After addition of 50 μL of MTT saline solution (1 mg/mL) to each well, the plates were incubated for another 3 h. The mixtures were centrifuged, and the supernatants were removed. The precipitates were dissolved in DMSO, and UV absorption at 510 nm was measured with a microplate spectrophotometer.

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Supporting Information Available: Tables of ^1H and ^{13}C chemical shifts for **2**−**5** and FABMS and 2D NMR spectra of **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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