New Macrocyclic Spermine (Budmunchiamine) Alkaloids from *Albizia gummifera*: With Some Observations on the Structure−Activity Relationships of the Budmunchiamines

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The CH_2Cl_2 extract of the stem bark of *Albizia gummifera* yielded four macrocyclic spermine alkaloids (budmunchiamines), three of them being new analogues. On the basis of spectral analysis and comparison with related compounds they were identified as budmunchiamine G (**1**) and the new analogues budmunchiamine K (**2**), 6′ξ-hydroxybudmunchiamine K (**3**), and 9-normethylbudmunchiamine K (**4**). The four isolated alkaloids and other related budmunchiamines isolated from *Albizia schimperana* were all active against two Gram-positive and two Gram-negative bacteria at MIC levels below 80 μg mL^{-1}, and showed toxicity to brine shrimp larvae (with LC_{50} values below 100 μg mL^{-1}). The negative impact of side chain hydroxylation and *N*-demethylation on both measures of biological activity was shown to be considerable.

Albizia gummifera (J. F. Gmel.) C. A. Sm. (Fabaceae, Mimosoideae) is used in Kenyan traditional medicine; the stem bark being employed in the preparation of medicines for the treatment of coughs, gonorrhea, and fever.[1] The plant has also been indicated for use for the treatment of skin diseases, malaria, and stomach pains.[1] Except for the treatment of wounds, where there is a direct topical application of the powdered bark to the site of infection, all medicinal preparations are made by boiling the bark in water. Because of the ethnomedical uses of this plant, we undertook an investigation for antibacterial compounds contained in the bark.

The only previous report on this species had indicated the presence of an unknown saponoside,[2] while the isolation of triterpenes,[3] flavonoids,[4] and macrocyclic spermine-based alkaloids[5−8] (the budmunchiamines) have been reported from other *Albizia* species. As a result of our investigation, we have obtained four alkaloids, which were identified as budmunchiamine K (**1**), 6ξ-hydroxybudmunchiamine K (**2**), budmunchiamine G (**3**),[6] and 9-normethylbudmunchiamine K (**4**). All except **3** appear to be new analogues**.** Previous work[9] had revealed that the budmunchiamines isolated from *Albizia amara* exhibited considerable biological activity, including interaction with calf thymus DNA and cytotoxicity on cultured mammalian cell lines.

Further to the identification of the alkaloids, we have undertaken some simple biological testing using the budmunchiamines reported here and from *Albizia schimperana*.[8] Examination of antibacterial activity against both Gram-negative and Gram-positive bacteria and toxicity to brine shrimp nauplii revealed that the occurrence of side-chain oxidation and variation in *N*-methylation patterns on the macrocycle have considerable impact on bioactivity.

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Table 1. ^{13}C NMR Chemical Shift Data for Compounds **1**−**4**

	1	**2**	**3**	**4**
2	172.9	172.9	172.0	173.0
3	37.3	37.4	39.4	38.7
4	61.5	61.5	56.0	61.8
5-N(Me)	35.4	35.7		37.3
6	52.0	51.5	46.8	49.5
7	26.7	25.8	26.2	24.6
8	55.0	54.5	57.4	45.4
9-N(Me)	43.4	42.5	43.4	
10	56.8	56.7	56.8	48.1
11	24.8	24.4	25.2	26.0
12	23.7	23.3	25.6	25.4
13	56.7	56.3	57.7	57.3
14-N(Me)	42.8	42.5	42.8	40.6
15	56.3	55.8	54.8	55.5
16	27.7	27.6	26.5	28.4
17	38.1	37.8	37.8	37.0
1′	29.6	29.9a	33.3	29.9a
2′	27.8	27.4	26.1	27.2
3′	30.0[a]	29.8[a]	29.9[a]	29.8[a]
4′	29.9[a]	29.7[a]	29.8[a]	29.8[a]
5′	29.9[a]	37.9[b]	29.8[a]	29.8[a]
6′	29.9[a]	72.2	29.8[a‘]	29.7[a]
7′	29.9[a]	37.7[b]	29.7[a]	29.7[a]
8′	29.8[a]	29.6[a]	29.7[a]	29.6[a]
9′	29.8[a]	29.6[a]	29.6[a]	29.5[a]
10′	29.8[a]	29.8[a]	29.6[a]	29.6[a]
11′	29.6[a]	29.8[a]	32.1	29.4[a]
12′	29.6[a]	29.8[a]	22.9	29.7[a]
13′	32.2	32.0	14.3	32.1
14′	22.9	22.9		22.9
15′	14.4	14.3		14.3

[a] Values in the same column may be interchanged. [b] Values in the same column may be interchanged.

Results and Discussion

Compound **1** gave a molecular formula of $C_{31}H_{64}N_40$ based on the HREIMS. The *J*-modulated ^{13}C-NMR spectrum (Table 1) showed resonances attributable to the following groups: one $C-CH_3$ (δ 14.4); three $N-CH_3$ (δ 35.4, 43.4, and 42.8); five $N-CH_2$ (δ 52.0, 55.0 56.8, 56.6, and 56.3); two $C-CH_2$, one adjacent to an amide nitrogen atom [$CONH-CH_2$ (δ 38.1)] and the other next to amide carbonyl carbon atom [CH_2CONH (δ 37.3)]; one

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Table 2. ^{1}H NMR Chemical Shift Data for Compounds **1**−**4** (*J* values, in Hz, in parentheses)

	1	2	3	4
1-H	8.62 br s	8.42 br s	8.40 br s	8.12 bs
3	2.24, 2.38 m	2.24, 2.38 m	2.20, 2.30 m	2.22−2.46 m
4	2.83 m	2.88 m	3.09 m	3.04 m
5-Me	2.19 s	2.22 s		2.23 s
6	2.65 dt (7)	2.66 dt (7)	2.82 m	2.85 m
	2.42 m	2.43 m	2.82 m	2.38 m
7	1.63 m	1.70 m	1.73 m	1.76 m
8	2.40 m	2.53−2.64 m	2.45 −2.50 m	3.02, 2.82 m
9-Me	2.24 s	2.35 s	2.26 s	
10	2.31−2.38 m	2.40−2.52 m	2.38−2.42 m	3.02, 2.65 m
11	1.50 m	1.54 m	1.52 m	1.85 m
12	1.53 m	1.59 m	1.54 m	1.71, 1.65 m
13	2.42−2.50 m	2.50−2.54 m	2.34−2.41 m	2.40−2.45 m
14-Me	2.19 s	2.23 s	2.23 s	2.19 s
15	2.37 m	2.41−2.46 m	2.41−2.47 m	2.42, 2.75 m
16	1.65 m	1.67 m	1.68 m	1.76, 1.62 m
17	3.33 dt (14,6)	3.38 dt (14,7)	3.35 m	3.56 m
		3.30 dt (14,7)		3.12 m
1′	1.15 m	1.15 m	1.20 m	1.15 m
	1.52 m	1.52 m		
2′-4′	1.26	1.26	1.26	1.25
5′	1.26	1.44 m	1.26	1.25
6′	1.26	3.59 m	1.26	1.25
7′	1.26	1.44 m	1.26	1.25
8′-11′	1.26	1.26	1.26	1.25
12′	1.26	1.26	1.30 m	1.25
13′	1.26	1.26	0.88 t (7)	1.25
14′	1.30 m	1.30 m		1.30 m
15′	0.89 t (7)	0.88 t (7)		0.88 t (7)

amide carbonyl (δ 172.9) and one N−CH (δ 61.5), with the rest of the resonances belonging to C−CH_2 most of them with resonances typical of an aliphatic chain (δ ca. 29). These resonances were comparable to those observed for 5,9,14 *N*-methylated macrocyclic spermine-derived alkaloids.[5−8] The ^{1}H-NMR spectral data (Table 2) also agreed with this hypothesis.

The EIMS of **1** showed a base peak at *m*/*z* 297 corresponding to the macrocycle fragment ($C_{16}H_{33}N_4O$), which is identical to that of budmunchiamine A (**5**). The molecular ion at *m*/*z* 508 was 56 mass units higher than that of **5**, and because the macrocycle was the same for both compounds, the difference must be attributable to four more methylene groups in the side chain of **1**. The MS displayed the normal aliphatic chain fragmentation pattern with initial loss of a terminal methyl group followed by successive loss of fragments attributable to $[Me(CH_2)n]^+$. Thus, from the MS of **1** the aliphatic side chain contained 15-carbon atoms, and the compound was identified as a novel alkaloid to which we assigned the trivial name budmunchiamine K (**1**). The 6′-hydroxy and normethyl derivatives of budmunchiamine K have previously been reported *from A. schimperana*.[8]

The *J*-modulated ^{13}C-NMR spectrum of **2** (Table 1) showed resonances comparable to those of **1**, the only difference being the presence of a carbinolic carbon signal (δ 72.2) and two deshielded *C*-methylene signals (δ 37.6, 37.7), attributable to the two methylenes attached to the carbinol group. The ^{1}H-NMR spectrum (Table 2) showed an oxymethine resonance at δ 3.59 (m). The HREIMS revealed the molecular ion at *m*/*z* 524 ($C_{31}H_{64}N_4O_2$) and again has a significant fragment at *mlz* 297 for the same macrocycle as in **1**. The fragmentation pattern of **2** displayed the loss of the terminal methyl group from the molecular ion followed by successive loss of the $[Me(CH_2)n]^+$ fragments up to the ion at *m*/*z* 397 (20.1%), due to loss of $[Me(CH_2)_8]^+$. After this ion, the succeeding incremental loss contained an oxygen atom, starting with ion at *m*/*z* 367, which is due to loss of $[Me(CH_2)_8CHOH]^+$. This fragmentation allowed placement of the hydroxyl group on C-6′ in the aliphatic chain, confirming that **2** was 6′ξ-hydroxybudmunchiamine K.

The *J*-modulated ^{13}C-NMR spectrum of **3** differed from the above in showing resonances attributable to only two N−CH_3 (δ 43.4, 42.8); while one of the five N−CH_2 appeared at δ 46.8 and the N−CH at δ 56.0**.** These data indicated the lack of the 5 N-methyl group, with consequent upfield shifts of C-4 and C-6**.** The ^{1}H-NMR spectrum confirmed only two N−CH_3 resonances (δ 2.26 and 2.23). The HREIMS revealed the molecular ion at *m*/*z* 466 ($C_{28}H_{58}N_40$), with a significant ion at *m*/*z* 283 for the 5-normethyl macrocycle. The macrocycle was linked to a side chain of 13-carbon atoms, identifying **3** as the known budmunchiamine G, previously isolated from *A. amara*.[6]

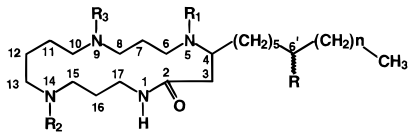
The *J*-modulated ^{13}C-NMR spectrum for compound **4** (Table 1) showed resonances generally typical of the budmunchiamines, with two N−CH_3 (δ 37.3, 40.6) and five N−CH_2 (δ 49.6, 45.5, 48.1, 57.3, 55.5), indicative of either a 9-normethyl or 14-normethyl macrocycle. The HREIMS indicated a molecular ion at *m*/*z* 494 ($C_{30}H_{62}N_4O$), with a significant peak at *m*/*z* 283 for the normethyl macrocycle, thus identifying **4** as either 9-normethylbudmunchiamine K or 14-normethylbudmunchiamine K.

The problem of assignment of the macrocycle substituents was overcome by use of HC−COBI[10] and HMBC[11] 2D heteronuclear correlation procedures. Key H−C direct correlations (from HC−COBI) included the multiplets at δ 3.56 and 3.12 with δ 37.0 (C-17), the N−CH_3 at δ 2.23 and 2.19 with δ 37.3 and 40.6, a multiplet at δ 3.04 with the methine carbon δ 61.8 (C-4), and resonances at δ 2.85 and 2.38 with the methylene at δ 49.5 and at δ 3.02 and 2.82 with the methylene at δ 45.4.

The HMBC spectrum (^2J and ^3J heteronuclear correlations) revealed that the multiplets at δ 3.56 and 3.12 (attributable to H-17) showed correlations to δ 28.4 (C-16, ^2J) and to δ 55.5 (C-15). The N−CH_3 signal at δ 2.19 correlated with the C-15 resonance and with a further methylene at δ 57.3 (C-13), so confirming the presence of a 14-N−CH_3. The other N−CH_3 singlet at δ 2.23 correlated with the methine δ 61.8 (C-4) and a methylene at δ 49.5 (C-6), hence confirming the position of the second N−CH_3 at N-5. The spectroscopic data therefore showed that **4** was 9-normethylbudmunchiamine K, which is novel.

The four budmunchiamines isolated from *A. gummifera* (**1**−**4**), together with five related compounds obtained from *A. schimperana* (**5**−**9**),[8] were tested against representative Gram-positive *(Bacillus subtilis, Staphylococcus aureus)* and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria. Using the simple disk diffusion assay method, all nine alkaloids exhibited zones of inhibition of at least 7-mm diameter against all the bacteria at a loading of 50 μg/disk (Table 3). None of the compounds was as active as the chloramphenicol standard. The 6′-hydroxy compounds (**2**, **6**, and **8**) were less active than the other alkaloids.

The results of minimum inhibitory concentration (MIC) studies on six of the alkaloids (the other three



	R	R_1	R_2	R_3	n
Budmunchiamine K (**1**)	H	Me	Me	Me	8
6'ξ-Hydroxybudmunchiamine K (**2**)	OH	Me	Me	Me	8
Budmunchiamine G (**3**)	H	H	Me	Me	6
9-Normethylbudmunchiamine K (**4**)	H	Me	Me	H	8
Budmunchiamine A (**5**)	H	Me	Me	Me	4
6'ξ-Hydroxybudmunchiamine C (**6**)	OH	Me	Me	Me	6
5-Normethylbudmunchiamine K (**7**)	H	H	Me	Me	8
6'ξ-Hydroxy-5-normethylbudmunchiamine K (**8**)	OH	H	Me	Me	8
14-Normethylbudmunchiamine K (**9**)	H	Me	H	Me	8

were not available in sufficient amounts) are also shown in Table 3 and confirm that the presence of the hydroxyl in the side chain leads to an appreciable reduction in antibacterial activity. By contrast, reduction of the degree of methylation on the macrocycle nitrogens from three to two did not cause a significant loss of antibacterial activity.

The potential cytotoxicity of the nine alkaloids was evaluated using the brine shrimp cytotoxicity assay (BSCA).[12,13] The results (Table 3) showed that the fully *N*-methylated budmunchiamines (**1**, **5**) without side chain hydroxylation were strongly cytotoxic (at doses of less than 10 μg mL^{-1}). Concentrations two to three times higher were required to obtain the same level of activity from the four alkaloids (**3**, **4**, **7**, and **9**) where one of the *N*-methyl groups had been lost. It did not appear that the position of *N*-demethylation was significant. Finally, the three 6′-hydroxylated compounds (**2**, **6**, and **8**) showed toxicity reduced by an order of magnitude. The toxicity of **3** and other budmunchiamines from *A. amara* on several mammalian cell lines has previously been reported.[9] Because compounds **1**, **2**, and **4** are similar to the budmunchiamines of *A. amara*, it is expected that they would show a parrallel toxicity to mammalian cell lines. The presence of a hydroxyl group on the side chain diminished both antibacterial activity and cytotoxicity. A similar trend of diminution of toxicity on mammalian cell lines was noted with budmunchiamines bearing carbonyl groups on the side chain.

As *A. gummifera* is used as a source of traditional medicine, the presence of cytotoxic budmunchiamines is obviously noteworthy. Because traditional medicine preparations from this plant were water based (with the exception of the topical use against wounds), budmunchiamines seem unlikely to be extracted in substantial quantities. However, this point needs to be clarified.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Mattson Genesis Series FT-IR spectrophotometer. Specific rotations $[\alpha]_D$ were determined at the sodium-D line using a Perkin-Elmer 241 polarimeter. MS were recorded on a high resolution electron impact mass spectrometer AEI-MS 902 double focusing instrument (direct probe insert at 70 eV). NMR spectra were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ as solvent (δ_H 7.27, δ_C 77.23).

Table 3. Antibacterial Activity and Brine Shrimp Lethality for a Series of Budmunchiamines

	antibacterial activity[a,b]				
compound	*S. aureus* disk (MIC)	*B. subtilis* disk (MIC)	*E. coli* disk (MIC)	*P. aeruginosa* disk (MIC)	BSCA
1	11 (15)	10 (19)	11 (24)	11 (28)	6.6
5	10 (18)	11 (15)	11 (25)	10 (30)	4.0
3	10 (18)	12 (24)	10 (30)	9 (35)	12.3
4	11	10	9	9	15.9
7	12 (15)	11 (20)	10 (27)	10 (32)	16.5
9	11	10	9	10	18.2
2	9 (30)	8 (42)	7 (64)	8 (75)	69.1
6	8 (35)	8 (45)	7 (72)	7 (84)	79.7
8	9	9	7	7	60.4
chloramphenicol	24 (7)	31 (7)	26 (12)	17 (26)	

[a] MIC and LC_{50} values are each in μg mL^{-1}. [b] Disk values are in mm at a concentration of 50 μg/disk.

Plant Material. The stem bark of *A. gummifera* was collected in July 1992 from Ruiti Forest, Meru District, Kenya. The plant was identified by the staff of the Botany Department, University of Nairobi, and the voucher specimens are on deposit at the departmental herbarium.

Test Organisms. The test organisms used were *Staphylococcus aureus* (NCTC 6751), *Bacillus subtilis* (NCTC 8326), *Escherichia coli* (NCTC 9001), and *Pseudomonas aeruginosa* (NCTC 6750). Media included Nutrient Broth E (Lab 68) and Nutrient Agar (Lab 8) from LAB M Laboratories, Lancashire, UK. Chloramphenicol disks were from Mast Laboratories Ltd., Merseyside, UK. Brine shrimp eggs were obtained from Interpet Ltd., Dorking, UK and commercial sea salt was from Rosewood Pet Products, Shropshire, UK.

Extraction of the Antibacterial Compounds. The powdered stem bark (500 g) was exhaustively extracted in a Soxhlet using petrololeum ether (bp 60−80 °C), then CH_2Cl_2, and finally MeOH. Each extract was dried *in vacuo* at 40 °C and then tested against the four bacteria using the disk diffusion assay method as described by Habtemariam *et al.*,[14] with chloramphenicol antibiotic disks as a positive control. The CH_2Cl_2 fraction showed highest activity against all the bacteria, and TLC analysis on Si gel using $CHCl_3$/Et_2NH (95:5) as the solvent revealed the presence of alkaloids (detected with Dragendorff's reagent).

The CH_2Cl_2 fraction (3.0 g) was then subjected to column chromatography on Si gel, eluting with $CHCl_3$ containing increasing amounts of Et_2NH (from 0 to 2%). Fractions of 100−150 mL were collected and monitored by TLC (same system as above). Fractions containing a single alkaloid were pooled appropriately, while fractions containing mixtures of alkaloids were further subjected to preparative TLC, with multiple developments using solvent systems composed of $CHCl_3$/Et_2-HN (125:1; 100:1, and 98:2). The major compound **1** (90 mg) was obtained pure direct from the column, while **2** (12 mg), **3** (15 mg), and **4 (11** mg) were obtained after preparative TLC.

Budmunchiamine K (1): obtained as yellow oil; $[\alpha]_D$ −9.3° (*c* 0.09, $CHCl_3$); IR ν max ($CHCl_3$) 3400, 2982, 2798, 1646 cm^{-1}; EIMS *mlz* $[M]^+$ 508 (10.7), 493 (5.6), 479 (18.2), 465 (53.1), 451 (24.0), 437 (38.8), 423 (26.9),

409 (23.5), 395 (33.1), 381 (15.7), 367 (21.1), 353 (18.4), 339 (23.3), 325 (22.3), 311 (26.9), 297 (100); HREIMS found 508.4996, calcd 508.4950 for $C_{31}H_{64}N_4O$.

6′ξ-Hydroxybudmunchiamine K (2): obtained as yellow oil; $[\alpha]_D$ −6.4° (*c* 0.07, $CHCI_3$); IR *ν* max 3400, 2928, 2797, 1649 cm^{-1}; EIMS *m/z* $[M]^+$ 524 (5.1), 509 (4.4), 495 (6.6), 481 (12.1), 467 (26.9), 453 (10.5), 439 (11.0), 425 (12.4), 410 (20.3), 397 (39.0), 367 (21), 353 (9.2), 339 (6.8), 325 (4.2), 311 (6.9), 297 (19.3); HREIMS found 524.5046, calcd 524.5029 for $C_{31}H_{64}N_4O_2$.

Budmunchiamine G (3): obtained as light yellow oil; $[\alpha]_D$ −5.1° (*c* 0.08, $CHCI_3$). IR *ν* max 3200, 2929, 2857, 1648 cm^{-1}. EIMS *m/z* $[M]^+$ 466 (3.5), 451 (9.6), 437 (3.9), 423 (7.1), 409 (11.0), 395 (10.7), 381 (17.1), 367 (6.0), 366 (9.0), 354 (7.8), 353 (10.4), 339 (6.5), 325 (5.0), 311 (3.3), 297 (10.4), 283 (31.6); HREIMS found 466.4615, calcd 466.4621 for $C_{28}H_{58}N_4O$.

9-Normethylbudmunchiamine K (4): obtained as yellow oil; $[\alpha]_D$ −1.8° (*c* 0.09, $CHCI_3$); IR *ν* max 3200, 2929, 2855, 2797, 1649 cm^{-1}; EIMS *m/z* $[M]^+$ 494 (1.0), 479 (2.0), 465 (7.0), 451 (7.1), 437 (4.0), 423 (5.2), 409 (3.0), 395 (4.4), 381 (5.1), 367 (4.0), 353 (5.0), 352 (11.0), 339 (4.3), 325 (6.2), 311 (5.1), 297 (7.0), 283 (22.3); HREIMS found 494.4773 calcd 494.4757 for $C_{30}H_{62}N_4O$.

Testing Alkaloids for Antibacterial Activity and Brine Shrimp Lethality. Compounds were evaluated for antibacterial properties using the MIC method as described by Dellar *et al.*[15] The BSCA was used exactly as previously described.[12,13]

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