Triterpenoidal Alkaloids from *Buxus natalensis* and Their Acetylcholinesterase Inhibitory Activity

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Acetylcholinesterase (AChE) inhibition-directed phytochemical studies on the methanolic extract of *Buxus natalensis*, collected in South Africa, resulted in the isolation of 12 compounds: O^2-natafuranamine (**1**), O^{10}-natafuranamine (**2**), cyclonataminol (**3**), 31-demethylbuxaminol A (**4**), buxaminol A (**5**), buxafuranamide (**6**), buxalongifolamidine (**7**), buxamine A (**8**), cyclobuxophylline K (**9**), buxaminol C (**10**), methyl syringate (**11**), and *p*-coumaroylputrescine (**12**). Compounds **1**−**4** were new alkaloids, and compound **5** was isolated for the first time as a natural product. Their structures were elucidated with the aid of extensive NMR and mass spectroscopic studies. Compounds **1** and **2** are members of a rarely occurring class of *Buxus* alkaloids, having a tetrahydrofuran ring incorporated in their structures. Compounds **1**−**12** exhibited strong to moderate AChE inhibitory activity.

The genus *Buxus* is a rich source of alkaloids comprised of a steroid-triterpenoid pregnane-type 9β,10β-cycloartenol system (with C-4 methyls) and a degraded C-20 side chain.[1−4] Phytochemical studies on various plants of this genus, including *Buxus sempervirens*, *B. papillosa*, *B. microphylla*,, *B. hildebrandtii*, and *B. hyrcana*, resulted in the isolation of over 200 triterpenoidal alkaloids. Some of these alkaloids have been reported to exhibit antimalaria, antituberculosis, anti-HIV, and anticancer activities and have been used to treat skin infections, rheumatism, and heart disorders.[5−8] *B. natalensis*, native to South Africa, has not been previously chemically investigated. A crude extract of this plant was reported to exhibit antimicrobial activity,[9] and the plant is used to enhance memory of elderly people by traditional healers in the local tribes of South Africa.

Acetylcholine serves as a neurotransmitter in the central and peripheral nervous system in signal transduction across synapses. Acetylcholinesterase (AChE) stops these functions of acetylcholine by hydrolyzing it into choline and acetic acid.[10] Its deficiency has been reported to be associated with Alzheimer's disease (AD). Enhancement of acetylcholine level in the brain is considered to be one of the most effective approaches for treating AD.[10,11] The level of acetylcholine in the brain can be increased by using potent AChE inhibitors. AChE inhibitors also prevent pro-aggregating activity of AChE leading to the deposition of β-amyloid plaques, another cause of AD.[12,13] Four AChE inhibitors, tacrine, donepezil, galanthamine, and rivastigmine, approved by the FDA, are used in clinical practices.[14] All of these inhibitors have limited effectiveness and a number of side effects.[15,16] For instance, tacrine shows hepatotoxic liability and rivastigmine has a short half-life. For these reasons, interest in AChE inhibitors has increased in the past few years.

We are involved in discovering new lead compounds exhibiting anti-AChE, antiglutathione *S*-transferase, anti-α-glucosidase, antioxidant, antileishmanial, and antimicrobial activities from medicinally important plants.[17−20] In these efforts, we collected *B. natalensis* from South Africa on the basis of reported aforementioned ethnomedicinal properties and discovered AChE inhibitory activity of the crude methanolic extract of this plant (IC_{50} 28 μg/mL). AChE inhibition-directed fractionations of this methanolic extract afforded 12 compounds: O^2-natafuranamine (**1**), O^{10}-natafuranamine (**2**), cyclonataminol (**3**), 31-demethylbuxaminol A (**4**), buxaminol A (**5**), buxafuranamide (**6**), buxalongifolamidine (**7**), buxamine A (**8**), cyclobuxophylline K (**9**), buxaminol C (**10**), methyl syringate (**11**), and *p*-coumaroylputrescine (**12**). Compounds **1**−**4** were isolated and characterized for the first time, while **5** was isolated for the first time as a natural product. Compounds **1**−**12** were all identified using NMR and mass spectroscopic methods and were evaluated for their potential to inhibit the activity of AChE. In this article, the isolation and structure elucidation of compounds **1**−**12** and their AChE inhibitory activity data are described.

Results and Discussion

Compound **1**, O^2-natafuranamine, was isolated as a yellow gum. Its UV spectrum showed maximum absorption at 224 nm, indicating the presence of a benzamide chromophore.[21] The IR spectrum displayed intense absorption bands at 3418 (OH), 1729 (ester C=O), and 1662 cm^{-1} (amide C=O). The high-resolution electron-spray ionization mass spectrum (HRESIMS) of **1** gave a molecular ion peak at *m/z* 592.3517 ($C_{35}H_{48}N_2O_6$). The EIMS of **1** exhibited ions at *m/z* 72 and 105 indicating the presence of an *N*,*N*-dimethyl amino group at C-20 and a benzamide moiety at C-3, respectively.[22]

The ^{1}H NMR spectrum of **1** featured singlets at δ 0.77, 1.12, and 0.90 due to the C-18, C-30 and C-32 methyl groups, respectively. A doublet at δ 0.85 (3H, J = 6.4 Hz) was assigned to the C-21 secondary methyl group. The acetyl methyl protons resonated as a singlet at δ 1.91, while the $N(CH_3)_2$ protons appeared as a 6H singlet at δ 2.30. The C-31 methylene protons resonated as a 2H singlet at δ 3.86. This signal was resolved into a set of two AB doublets at 3.84 (J = 8.9 Hz) and 3.97 (J = 8.9 Hz), when the ^{1}H NMR spectrum was acquired in acetone-d_6. The C-2 methine proton resonated at δ 4.29, while the C-1 methine proton appeared at δ 3.28 (J = 2.2 Hz). The C-11 olefinic proton resonated as a multiplet at δ 5.14. A downfield aliphatic one-proton multiplet at δ 5.20 was due to the C-6 methine proton, geminal to an acetoxy group. The C-16 methine proton, geminal to an OH group, resonated at δ 4.16. A 3H multiplet resonating at δ 7.41−7.46 was ascribed to H-3′/H-5′ and H-4′, while 2H doublet at δ 7.76 (J = 7.3 Hz) was assigned to H-2′/H-6′.

The COSY-45° spectrum of **1** showed the presence of five isolated spin systems; "a" to "e" (Figure 1). Spin system "a" represented the phenyl moiety. Spin system "b" was comprised of a fragment of coupled H-1 to amidic NH protons, and H-1 (δ 3.28) showed vicinal coupling with H-2 (δ 4.29), which in turn exhibited

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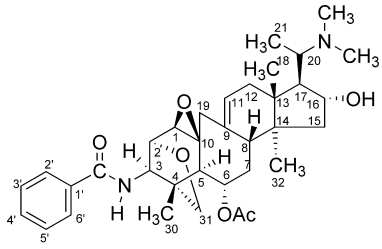

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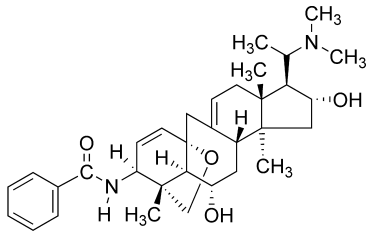

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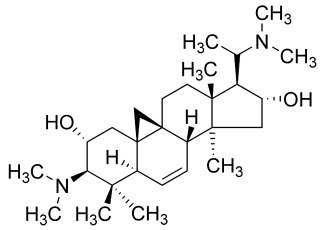

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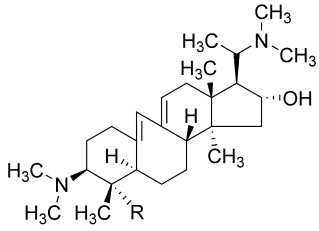

4 R = H

5 R = CH_3

cross-peaks with H-3 (δ 4.48). The latter showed vicinal coupling with amidic NH (δ 7.73). Similarly, other spin systems "c" to "e" were traced by the interpretation of COSY-45° and TOCSY spectra. The ^{13}C NMR and DEPT spectra indicated the presence of seven CH_3, five CH_2, 15 CH, and eight quaternary carbons in compound **1**. Complete ^{1}H and ^{13}C NMR chemical shift assignments of **1** are shown in Table 1. The HMBC spectrum of **1** showed couplings of H_2-31 with C-2 (δ 72.0), C-4 (43.2), and C-5 (δ 55.8). H-2 showed cross-peaks with C-1 (δ 64.4), C-10 (δ 59.4), and C-31 (δ 83.8). These HMBC data suggested that C-31 was bonded to C-2 via an ether linkage. Important HMBC interactions of **1** are shown in

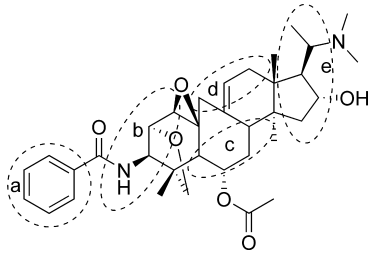

Figure 1. Spin systems "a−e" of **1**, obtained from COSY-45° and TOCSY spectra.

Table 1. ^{1}H and ^{13}C NMR Assignments for O^2-Natafuranamine and O^{10}-Natafuranamine in $CDCl_3$[a]

	O^2-natafuranamine (**1**)		O^{10}-natafuranamine (**2**)	
position	$\delta_{C(DEPT)}$	δ_H (J in Hz)[b]	$\delta_{C(DEPT)}$	δ_H (J in Hz)[b]
1	64.4, CH	3.28, d (2.2)	131.3, CH	5.74, d (9.5)
2	72.0, CH	4.29, m	129.5, CH	6.06, dd (9.5, 3.8)
3	57.3, CH	4.48, m	55.0, CH	4.52, m
4	43.2, C		46.1, C	
5	55.8, CH	2.25, m	60.8, CH	1.60, m
6	75.4, CH	5.20, m	75.5, CH	3.81, m
7	35.7, CH_2	1.76, m, 1.62, m	38.7, CH_2	1.85, m, 1.60, m
8	42.5, CH	2.40, m	43.4, CH	1.93, m
9	135.4, C		133.6, C	
10	59.4, C		80.3, C	
11	122.4, CH	5.14, m	124.8, CH	5.40, br s
12	36.7, CH_2	2.01, m, 1.78, m	37.1, CH_2	2.02, m, 1.81, m
13	44.2, C		43.8, C	
14	47.5, C		47.9, C	
15	42.7, CH_2	1.97, m, 1.43, m	42.5, CH_2	1.92, m, 1.40, m
16	77.4, CH	4.16, m	77.4, CH	4.09, m
17	56.2, CH	1.83, m	56.0, CH	1.83, m
18	16.2, CH_3	0.77, s	16.8, CH_3	0.66, s
19	44.1, CH_2	2.33, d (15.6), 2.82, d (15.3)	44.6, CH_2	2.69, m
20	62.9, CH	2.69, m	63.7, CH	2.69, m
21	10.3, CH_3	0.85, d (6.4)	10.5, CH_3	0.90, s (6.8)
30	22.0, CH_3	1.12, s	22.0, CH_3	1.36, s
31	83.8, CH_2	3.86 (s)	78.2, CH_2	3.69, d (8.7), 3.60, d (8.7)
32	18.4, CH_3	0.90, s	18.7, CH_3	0.92, s
O*C*NH	167.5, C		166.7, C	
OCN*H*		7.73, d (10.6)		7.27, d (7.6)
$N(CH_3)_2$	44.7	2.30, s	44.5	2.28, s
O*C*OCH$_3$	169.6, C			
OCO*CH$_3$*	21.8, CH_3	1.91, s		
1′	134.7, C		134.5, C	
2′	127.1, CH	7.76, d (7.3)	127.3, CH	7.85, d (7.1)
3′	128.8, CH	7.41, m	128.9, CH	7.44, m
4′	131.9, CH	7.46, m	131.8, CH	7.48, m
5′	128.8, CH	7.41, m	128.9, CH	7.44, m
6′	127.1, CH	7.76, d (7.3)	127.3, CH	7.85, d (7.1)

[a] Multiplicity was determined by DEPT spectra. [b] ^{1}H NMR data of **1** in acetone-d_6 and pyridine-d_5 as well as ^{1}H NMR data of **2** in pyridine-d_5 are provided in the Supporting Information.

Figure 2. The ^{1}H NMR spectrum of **1** was recorded in pyridine-d_5, and chemical shift values of H-2 and H_2-31 remained unaffected in this spectrum, further confirming the presence of an ether linkage between C-2 and C-31. It has been reported that a pronounced shift of ~0.2 ppm was observed in the case of protons adjacent to an OH group, when the ^{1}H NMR spectrum was recorded in pyridine-d_5.[23] Furthermore, the ^{1}H NMR spectrum of **1** (C_5D_5N, 400 MHz) also indicated the presence of an OH group at C-16, as this proton exhibited a pronounced downfield shift from δ 4.16 ($CDCl_3$) to δ 4.41 (C_5D_5N). The chemical shift value of H-1 also remained unaffected in this ^{1}H NMR spectrum, confirming the presence of an epoxide ring at C-1/10. Most of the resonances in the ^{1}H and ^{13}C NMR spectra of **1** were similar to those of O^2-buxafuramine,[1] further suggesting that **1** had a structure similar to that of O^2-buxafuramine. The NOESY spectrum of **1** suggested the α-orientation for H-1, H-3, H-5, H_3-32, and H-17 and the β-orientation for H-2, H-6, H-8, H-30, and H-16.[24] The *S* configuration of C-20 was assigned on the basis of ^{1}H and ^{13}C NMR chemical shift comparisons of C-20 with other reported *Buxus* alkaloids.[21,22,24] α-Orientation of the oxygenated C-31 methylene group was assumed from the X-ray structure of another compound of this series.[25,26] These spectral studies led us to propose structure **1** for this novel compound.

Compound **2**, O^{10}-natafuranamine, was isolated as a yellow solid. Its UV and IR spectra were similar to those of compound **1**, with the exception that the IR spectrum of **2** did not exhibit an absorption band due to an ester carbonyl group. The HREIMS of **2** showed

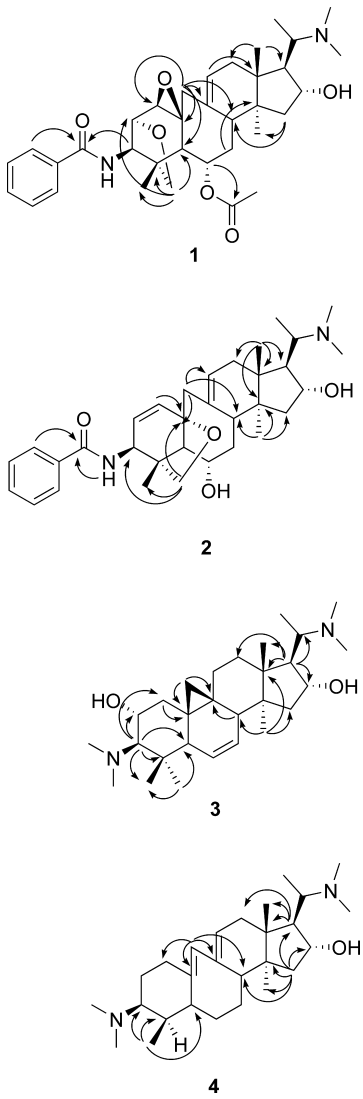

Figure 2. Important HMBC interactions of compounds **1**−**4**.

the molecular ion peak $[M + H]^+$ at *m/z* 535.3523 ($C_{33}H_{47}N_2O_4$). The ^{1}H NMR spectrum ($CDCl_3$) of **2** showed olefinic signals δ 5.74 and 6.06 due to H-1 and H-2, respectively. These protons exhibited vicinal couplings in the COSY spectrum; H-2 showed cross-peaks with H-3 (δ 4.52), which in turn showed ^{1}H−^{1}H spin correlations with the amidic *N*H (δ 7.27). The C-31 methylene protons resonated as a set of two AB doublets at δ 3.69 and 3.60 (J = 8.8). The H-1 proton resonated as a doublet, indicating the presence of a quaternary C-10 (at δ 80.3) in the ^{13}C NMR spectrum. The HMBC spectrum of **2** showed couplings of H_2-31 with C-3 and C-10. The chemical shifts of H_2-31 remained unaffected in the ^{1}H NMR spectrum, recorded in pyridine-d_5. A combination of ^{1}H,^{13}C, COSY and HMBC data indicated an ether linkage between C-31 and C-10. The C-6 methine proton, geminal to an OH, resonated at δ 3.81. The remaining ^{1}H and ^{13}C NMR data of compound **2** were similar to those of **1**, indicating that the rest of the structure of **2** was identical to that of **1**. Complete ^{1}H and ^{13}C NMR assignments of **2** are shown in Table 1. Important HMBC interactions of **2** are shown in Figure 2. The ^{1}H and ^{13}C NMR data of **2** were nearly identical to those of O^{10}-buxafuramine,[1] confirming that compound **2** had a similar structure. The NOESY spectrum of **2** suggested the same configuration as in compound **1** and other *Buxus* alkaloids reported in the literature.[1] Thus, the structure of compound **2** was determined to be as shown, and it was named O^{10}-natafuranamine.

Table 2. ^{1}H and ^{13}C NMR Assignments for Cyclonataminol and 31-Demethylbuxaminol A[a]

	cyclonataminol A (**3**)		31-demethylbuxaminol A (**4**)	
position	$\delta_{C(DEPT)}$	δ_H (J in Hz)[b]	$\delta_{C(DEPT)}$	δ_H (J in Hz)
1	39.7, CH_2	1.86, m, 1.55, m	40.1, CH_2	2.07, m
2	66.2, CH	3.75, m	23.1, CH_2	1.85, m, 1.24, m
3	78.0, CH	2.09, m	68.3, CH	2.19, m
4	43.7, C		42.9, CH	1.24, m
5	49.1, CH	1.92, m	48.4, CH	1.79, m
6	126.3, CH	5.56, m	34.8, CH_2	2.40, m, 1.23, m
7	129.3, CH	5.44, m	26.0, CH_2	1.62, m, 1.25, m
8	43.5, CH	2.53, m	49.8, CH	2.19, m
9	20.2, C		138.5, C[b]	
10	20.2, C		138.5, C[b]	
11	25.0, CH_2	1.83, m, 1.42, dd (3.5, 4.3)	129.5, CH	5.52, br s
12	31.9, CH_2	1.65, m, 1.30, m	39.1, CH_2	2.05, m, 1.95, m
13	45.4, C		43.4, C	
14	49.9, C		46.4, C	
15	41.7, CH_2	1.98, m, 1.24, m	43.7, CH_2	2.03, 1.51, dd (6.2, 6.3)
16	79.3, CH	4.09, m	72.4, CH	4.43, m
17	56.9, CH	1.80, m	52.9, CH	1.83, m
18	19.3, CH_3	0.88, s	17.5, CH_3	0.91, s
19	18.8, CH_2	0.73, d (4.1), −0.13, d (4.1)	127.7, CH	5.87, s
20	62.8, CH	2.63, m	57.6, CH	3.00, m
21	10.3, CH_3	0.86, d (6.6)	9.6, CH_3	0.92, d (6.4)
30	27.3, CH_3	1.07, s	16.1, CH_3	1.05, d (6.6)
31	15.6, CH_3	0.89, s		
32	18.6, CH_3	0.94, s	17.8, CH_3	0.66, s
$N_a(CH_3)_2$	43.5, CH_3	2.23	40.3, CH_3	2.27, s
$N_b(CH_3)_2$	43.5, CH_3	2.54	40.3, CH_3	2.24, s

[a] Multiplicity was determined by DEPT spectra. [b] Signals were overlapping.

Compound **3** was obtained as a yellow, amorphous solid. The UV spectrum of **3** showed the terminal absorption indicating the lack of a conjugated π system. Its IR spectrum displayed intense absorption bands at 3435 (OH) and 1552 (C=C) cm^{-1}. The HREIMS of **3** showed the molecular ion peak at *m/z* 444.3708 corresponding to the molecular formula $C_{28}H_{48}N_2O_2$. The EIMS of **3** displayed a base peak at *m/z* 72 and another ion at *m/z* 71, revealing *N*,*N*-dimethyl amino groups at C-20 and C-3, respectively.[22] The ^{1}H NMR spectrum of **3** showed four methyl singlets (δ 0.88, 1.07, 0.89, and 0.94) due to C-18, C-30, C-31, and C-32, respectively. A set of two AB doublets, integrating for one proton each, at δ −0.13 and 0.73 (J = 4.1 Hz) suggested the presence of a 9(10→19) cyclopropyl ring system in **3**. It has been reported that *Buxus* alkaloids containing a C-6/C-7 double bond show an upfield resonance for one-half of the AB doublets due to the C-19 methylene protons, while the other half is embedded in the methyl/methylene regions.[27] The ^{1}H NMR spectrum of **3** indicated two vinylic protons, H-6 (δ 5.56) and H-7 (δ 5.44). H-6 showed COSY interactions with H-5 (δ 1.92) and H-7. The latter showed cross-peaks with H-8 (δ 2.53). The H-6 (δ 5.56) exhibited HMBC interactions with C-5 (δ 49.1) and C-7 (δ 129.3), and H-7 (δ 5.44) showed cross-peaks with C-5 (δ 49.1), C-6 (δ 126.3), and C-8 (δ 43.5). A combination of COSY and HMBC data confirmed the presence of a Δ^{6-7} double bond. The C-2 methine proton resonated at δ 3.75 and showed COSY interactions with the C-1 methylene (δ 1.86 and 1.55) and the C-3 methine (δ 2.09) protons. H-2 exhibited HMBC interactions with C-1 (δ 39.7) and C-3 (δ 78.0), indicating an OH group at C-2. Important HMBC interactions of **3** are shown in Figure 2. Complete ^{1}H and ^{13}C NMR chemical shift assignments of **3** are shown in Table 2. The NOESY spectrum of **3** showed the same configuration at all chiral centers as those of compounds **1** and **2**. Thus, structure **3** was proposed for this new alkaloid, and it was named cyclonataminol.

31-Demethylbuxaminol A (**4**) was isolated as a colorless, amorphous solid. The UV spectrum showed absorption maxima at 238 and 245 nm with shoulders at 231 and 254 nm, consistent with the presence of a 9(10→19) *abeo*-diene system.[1,28] Its IR spectrum indicated an OH group (3301 cm^{-1}) and a C=C bond (1551 cm^{-1}). The HREIMS provided the molecular formula $C_{27}H_{46}N_2O$ (M^+ *m/z* 414.3614). The ^1H NMR spectrum of **4** exhibited two three-proton singlets (δ 0.91 and 0.66) due to the C-18 and C-32 methyl protons, respectively. The C-21 and C-30 methyl protons resonated at δ 0.92 (J = 6.4 Hz) and 1.05 (J = 6.6 Hz), respectively. H_3-21 showed vicinal couplings with the C-20 methine proton (δ 3.00), which in turn showed cross-peaks with H-17 (δ 1.83). The latter displayed vicinal couplings with H-16 (δ 4.43). The C-30 methyl protons exhibited COSY-45° interactions with H-4 (δ 1.24). The latter showed cross-peaks with H-3 and H-5. The long-range couplings of H-5 with C-3, C-4, C-6, C-10, C-19, and C-30 were observed in the HMBC spectrum (Figure 2), and H_3-30 exhibited HMBC interactions with C-3, C-4, and C-5. The NMR data suggested that **3** was a C-31-demethyl derivative of buxaminol A. The mass spectrum of **3** also showed fragments and losses expected for a demethyl analogue of buxaminol A. The NOESY spectrum revealed α-orientations for H-4, H-5, H-17, and H_3-32. In compound **4**, H-8, H-16, H_3-18, and H_3-30 were assigned β-orientations based on NOE data, consistent with those of compounds **1**−**3** and other *Buxus* alkaloids.[1,28,29] Complete ^1H and ^{13}C NMR assignments for compound **4** are shown in Table 2. On the basis of these studies, structure **4** was proposed, and it was named 31-demethylbuxaminol A.

Eight known natural products, buxaminol A (**5**), buxafuranamide (**6**), buxalongifolamidine (**7**), buxamine A (**8**), cyclobuxophylline K (**9**), buxaminol C (**10**), methyl syringate (**11**), and *p*-coumaroylputrescine (**12**), were also isolated in this study. The ^1H and ^{13}C NMR, UV, IR, and mass spectra of compounds **5**−**12** were identical to those reported in the literature.[30−37] This is the first report on the isolation of compound **5** as a natural product. Previously compound **5** was synthesized by carrying out *N*-methylation of the C-20 amino functionality of buxaminol E.[30] The complete NMR and mass spectral data of **5** are listed in the Experimental Section, as they were not previously reported in the literature. Compound **7** was previously purified from *B. longifolia*,[31] alkaloids **6**, **8**, and **9** were isolated were *B. papillosa*,[32−34] and compounds **10** and **11** were obtained previously from *B. sempervirens*.[35,36] Compound **12** was previously reported from *Nicotiana tabacum*.[37] Compounds **1**, **2**, and **6** are members of a rarely occurring class of *Buxus* alkaloids, and only six compounds of this series have so far been reported in the literature.[1]

Compounds **1**−**12** were evaluated for their potential to inhibit the activity of AChE. The bioactivity results are shown in Table 3. Compounds **1**, **2**, and **6** exhibited significantly higher AChE inhibitory activity compared to the rest of the isolates, with IC_{50} values of 3.0, 8.5, and 14.0 μM, respectively. The activity of compound **1** was close to that of huperzine, a standard AChE inhibitor. Previously, we reported O^6-buxafurandiene and 7-deoxy-O^6-buxafurandiene exhibiting anti-AChE activity with IC_{50} values of 17 and 13 μM, respectively.[38] Both of these compounds contain an ether linkage between C-6 and C-31. The bioactivity data of compounds **1**, **2**, **6**, O^6-buxafurandiene, and 7-deoxy-O^6-buxafurandiene suggest that this bioactivity might be due to the presence of a tetrahydrofuran group in their structures. Furthermore, the location of the ether bond does not play a role in this bioactivity. The higher potency of **1** was hypothesized due to the presence of an epoxy functionality at C-1 and C-10. Further studies on structure−activity relationships of compound **1** are warranted.

Table 3. Anti-AChE Activity Data of Compounds **1**−**12**

compound	AChE (IC_{50}) (μM)[a]
1	3.0 ± 0.5
2	8.5 ± 1.5
3	22.9 ± 2.2
4	25.8 ± 1.8
5	29.8 ± 4.4
6	14.0 ± 1.6
7	30.2 ± 3.5
8	80.0 ± 2.0
9	58.2 ± 3.1
10	40.0 ± 4.0
11	NA
12	NA
huperzine A	1.7 ± 0.3
galanthamine	0.53 ± 0.5

[a] IC_{50} value represents the concentration of compounds required to inhibit the 50% activity of AChE. NA: Not active at the concentration of 50 μg/mL. "±" represents standard error of mean of these enzyme inhibition assays. Huperzine A and galanthamine were used as positive controls.

Experimental Section

General Experimental Procedures. The UV spectra were recorded in methanol on a Shimadzu UV-2501 PC spectrophotometer. Optical rotations were measured on an Autopol-1 V automatic polarimeter. IR spectra were acquired on a Varian 1000 FT-IR (Scimitar Series). Mass spectral studies were carried out on a Hewlett-Packard 5989B MS. The high-resolution mass spectra were recorded on a Kratos Analytical MS-50G mass spectrometer at the University of Alberta. ^1H NMR experiments including 1D and 2D were performed on a Bruker Avance-3 spectrometer at 400 MHz. The ^{13}C NMR spectra were recorded on the same instrument at 100 MHz. AChE inhibitory activity was measured spectrophotometrically using a Synergu HT multidetection BioTek microplate reader. Column chromatography was carried out on 60 Å silica gel (230−400 mesh).

Plant Material. *Buxus natalensis* was collected from uMhalanga Rocks nature reserve, Durban, South Africa, in February 2007 by one of the authors (R.M.G.). The plant was identified by Mkhipheni A Ngwenya, and a voucher specimen (132176) was deposited in the herbarium of South Africa National Biodiversity Institute, Durban, South Africa.

Extraction and Isolation. The bark (6.1 kg) was pulverized and extracted with MeOH (15 L × 3) at room temperature. The solvent was removed in vacuo to a gummy residue (1.5 kg), and this gum was dissolved in 70:30 (v/v) MeOH−H_2O. The aqueous methanolic extract was defatted with hexane. AChE inhibition assays were performed on hexane and defatted extracts. The defatted extract was active in this bioassay. The bioactive fraction (400 g) was fractionated using solvent−solvent partitioning with $CHCl_3$ at pH 3.5, 7.0, and 9.5, respectively. The $CHCl_3$ extracts obtained at pH 3.5, 7.0, and 9.5 exhibited anti-AChE inhibition activity with IC_{50} values of 70, 110, and 20 μg/mL, respectively. The $CHCl_3$ extract (pH 9.5, 26.0 g) was loaded onto a silica gel column (230−400 mesh, Merck), and the column was eluted with hexane−EtOAc (0−100%) and EtOAc−MeOH (0−100%). This afforded fractions that were pooled on the basis of the same R_f values on analytical TLC to afford fractions F_1−F_{20}. Fractions F_1−F_3 were active in our bioassay. Fraction F_1 was subjected to PTLC using hexane−acetone−diethylamine (7:3:0.01, v/v/v) as a mobile phase to separate compounds **1** (11.3 mg, R_f = 0.65), **6** (13.0 mg, R_f = 0.72), **7** (10.1 mg, R_f = 0.78), and **10** (2.3 mg, R_f = 0.16). PTLC of F_2 using hexane−acetone−diethylamine (80:20:0.5, v/v/v) afforded compound **2** (2.7 mg, R_f = 0.33). Compounds **4** (8.0 mg, R_f = 0.22) and **5** (14 mg, R_f = 0.27) were isolated from F_3 using hexane−EtOAc−diethylamine (90:5:5, v/v/v). The $CHCl_3$ extract (pH 3.5, 31.2 g) was loaded onto a silica gel column, and this column was eluted with hexane−EtOAc (0−100%) and EtOAc−MeOH (0−100%) to afford several fractions. These fractions were pooled on the basis of TLC to afford fractions FA_1−FA_{10}. Fractions FA_2−FA_4 were active in the AChE inhibition assay. Fraction (FA_3) was subjected to PTLC using $CHCl_3$−MeOH−acetic acid (85:15:0.1, v/v/v) to afford compound **12** (20 mg, R_f = 0.18). Compounds **3** (16 mg, R_f = 0.28) and **9** (10.6 mg, R_f 0.76) were obtained from fraction FA_2 after PTLC using hexane−EtOAc−diethylamine (90:5:5, v/v/v). Fraction FA_4 was also subjected to PTLC using hexane−acetone−diethylamine (75:25:0.1, v/v/v) to isolate **8** (12.0 mg, R_f = 0.62) and **11** (40 mg, R_f = 0.16). The $CHCl_3$ extract (pH 7.0) was not obtained in sufficient quantity to perform phytochemical studies.

***O*^{2}-Natafuranamine (1):** yellow gum (11.8 mg); $[\alpha]^{20}_D$ +5.3 (*c* 0.75, $CHCl_3$); UV (MeOH) λ_{max} 224 nm; IR ($CHCl_3$) ν_{max} 3418, 2936, 1729, 1662, and 1444 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* 592 $[M]^+$ (0.07), 195 (0.4), 105 (24), 72 (100), 59 (1.3); HRESIMS *m/z* 592.3517 (calcd for $C_{35}H_{48}N_2O_6$, 592.3513).

***O*^{10}-Natafuranamine (2):** yellow solid (2.7 mg); $[\alpha]^{20}_D$ +20.7 (*c* 0.048, $CHCl_3$); UV (MeOH) λ_{max} 221 nm; IR ($CHCl_3$) ν_{max} 3356, 2925, 1650, 1528, and 1459 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* 534 $[M]^+$ (0.2), 517 (0.02), 105 (60), 72 (100); HREIMS *m/z* 535.3523 $[M + H]^+$ (calcd for $C_{33}H_{47}N_2O_4$, 535.3530).

Cyclonataminol (3): yellow, amorphous powder (16 mg); $[\alpha]^{20}_D$ −70 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} 210 nm; IR ($CHCl_3$) ν_{max} 3435, 2935, and 1552 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 444 $[M]^+$ (0.5), 429 (0.2), 399 (0.3), 72 (100), 71 (2); HREIMS *m/z* 444.3708 $[M]^+$ (calcd for $C_{28}H_{48}N_2O_2$, 444.3716).

31-Demethylbuxaminol A (4): white, amorphous powder (8 mg); $[\alpha]^{20}_D$ +9.4 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} 254, 245, 238, and 231 nm; IR ($CHCl_3$) ν_{max} 3301, 2933, and 1551 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 414 $[M]^+$ (2), 399 (0.01), 369 (0.03), 72 (100), 71 (4); HREIMS *m/z* 414.3614 $[M]^+$ (calcd for $C_{27}H_{46}N_2O$, 414.3610).

Buxaminol A (5): white, amorphous powder (14 mg); UV (MeOH) λ_{max} 254, 246, 239, and 230 nm; IR ($CHCl_3$) ν_{max} 3298, 2940, 1550, and 1453 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.26 and 2.10 (1H, m, H-1), 1.70 and 1.48 (1H, m, H-2), 2.08 (1H, m, H-3), 1.93 (1H, m, H-5), 2.11 and 1.39 (1H, m, H-6), 1.52 (2H, m, H-7), 2.13 (1H, m, H-8), 5.47 (1H, s, H-11), 2.04 and 1.93 (1H, m, H-12), 2.01 and 1.49 (1H, m, H-15), 4.22 (1H, m, H-16), 1.81 (1H, m, H-17), 0.89 (3H, s, H_3-18), 5.89 (1H, s, H-19), 2.97 (1H, m, H-20), 0.88 (3H, d, J = 6.4 Hz, H_3-21), 0.99 (3H, s, H_3-30), 0.70 (3H, s, H_3-31), 0.64 (3H, s, H_3-32), 2.26 (3H, s, $N_a(CH_3)_2$), 2.23 (6H, s, $N_b(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 41.5 (CH_2, C-1), 23.2 (CH_2, C-2), 71.7 (CH, C-3), 43.2 (C, C-4), 52.2 (CH, C-5), 30.2 (CH_2, C-6), 25.8 (CH_2, C-7), 49.6 (CH, C-8), 138.8 (C, C-9), 137.0 (C, C-10), 128.5 (CH, C-11), 39.1 (CH_2, C-12), 43.7 (C, C-13), 46.3 (C, C-14), 43.4 (CH_2, C-15), 72.6 (CH, C-16), 53.0 (CH, C-17), 17.4 (CH_3, C-18), 128.6 (CH, C-19), 57.4 (CH, C-20), 9.5 (CH_3, C-21), 25.1 (CH_3, C-30), 15.3 (CH_3, C-31), 17.7 (CH_3, C-32), 44.7 (CH_3, $N_a(CH_3)_2$), 44.7 (CH_3, $N_b(CH_3)_2$); EIMS *m/z* 428 $[M]^+$ (0.4), 413 (0.01), 383 (0.3), 72 (100), 71 (0.27); HREIMS *m/z* 428.37668 $[M]^+$.

AChE Inhibition Assay. AChE inhibitory activities of **1**−**12** were determined using Ellman's method with slight modification.[39] The assay was carried out at room temperature in 100 mM sodium phosphate buffer at pH 7.8. In a typical assay, 126 μL of buffer, 50 μL of 0.01 M DTNB [5,5′-dithiobis(2-nitrobenzoic acid)], 2 μL of enzyme, and 2 μL of solutions containing test compounds (**1**−**12**) were mixed and incubated for 30 min. The reaction was then initiated by the addition of 20 μL of 0.075 M acetylthiocholine. Hydrolysis of acetylthiocholine was monitored by the formation of yellow-colored 5-thio-2-nitrobenzoate anion at a wavelength of 406 nm. All assays were carried out in triplicate using a 96-well microplate reader. The percentage inhibition was calculated using the formula $[(A_o - A_1/A_o] \times 100$, where A_o is the absorbance of the blank with no test compound and A_1 is the absorbance value of each concentration of the test compounds. The IC_{50} values were calculated by plotting a concentration−response curve.

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Supporting Information Available: ^{1}H NMR chemical shift assignments of **1** (recorded in acetone-d_6 and pyridine-d_5) and **2** (acquired in pyridine-d_5), copies of the ^{1}H, ^{13}C, COSY, HSQC, HMBC, and mass spectra of compounds **1**−**4**, plausible biosynthesis of compounds **1** and **2**, and structures of compounds **6**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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