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Spirofornabuxine, a novel type of *Buxus* alkaloid

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Abstract : From the leaves of *Buxus sempervirens* has been isolated, in addition to known alkaloids, a new alkaloid, spirofornabuxine (**1**), and its structure was determined by NMR spectroscopic analysis. This new alkaloid can be considered as the first member of a new group of steroidal derivatives, the spirobuxus alkaloids. Its implication in the biosynthesis of other *Buxus* alkaloids is discussed. © 1997 Published by Elsevier Science Ltd.

Buxus sempervirens (L.), Buxaceae, is a shrub widely distributed in Eurasia. Recent studies showed interesting properties of *Buxus sempervirens* extracts for treating human immunodeficiency virus infection[1]. Until now, most steroidal alkaloids isolated from *Buxus sempervirens* belonged to the 9,19-cyclobuxus or the 9,19-cyclo-9,10-secobuxus structural types[2,3].

In the present paper, we describe, in addition to known alkaloids such as cycloprotobuxin C, cyclobuxophyllin M or buxamin E, the isolation[4] and structure determination of spirofornabuxine (**1**), the first member of a new structural family of *Buxus* alkaloids. Its structure was established by HRMS and NMR spectroscopy.

The molecular formula $C_{25}H_{35}NO$ of **1** was deduced from HR-EIMS ($[M]^+$ at m/z 365.2754, calc. 365.2718). The ^1H NMR spectrum (Table 1) reveals the usual structural features of a steroidal alkaloid, with a methylamino group at δ 2.74, a cyclopropane methylene (δ 0.24 and δ 0.74), three adjacent olefinic methines and an isolated one.

The HMQC data and HMBC correlations (Table 2) of the four olefinic protons with quaternary carbons at 50.4, 143.4 and 150.3 revealed the presence of a cycloheptatriene ring (Figure 1). Further correlations of C-5 and C-19 with the inequivalent methylene protons at C-1, and of H-5 and H-19 with C-4, were indicative of an unusual spiro junction at C-10. Confirmation of this substitution by a cyclopentane ring was obtained by ^1H-^1H COSY (Table 1) and HMBC data. Correlations of the methylamino group with C-3 and of both methyl groups with C-3, C-4 and C-10, were indicative of the position of these substituents and brought the confirmation of the "spiro-cyclopentane" structure.

The tricyclic moiety (C,D,E rings) was built from the remaining quaternary carbons (C-8 and C-9) of the cycloheptatriene ring. ^3J correlations were observed between H-7 and C-14, and between H-19 and C-11 in the HMBC spectrum. H-11 was further correlated with the cyclopropane methylene at C-12 (COSY spectrum) and with a quaternary carbon at δ 32.0 in HMBC.

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The determination of the cyclohexanone D-ring structure was subsequently achieved by the observation of HMBC correlations of the three quaternary carbons. The carbonyl group at C-16 (δ 211.4) and the spiro carbon at C-13 (δ 32.0) were both ^{2}J or ^{3}J correlated with the inequivalent protons of the methylenes at C-15 and C-18 and with the methine proton at C-17, indicating a 1,4 position of the two carbons. Correlations observed from C-14 to H-15 and H-22, and from C-22 to H-15 established undoubtedly the position of the methyl group at C-14. Similar correlations between C-21 and H-17, and between H-20 and the three carbons at C-16, C-17 and C-18 clearly indicated the position of the ethyl group at C-17. The magnetic inequivalence of the C-20 methylene proton was explained by the proximity of the carbonyl group.

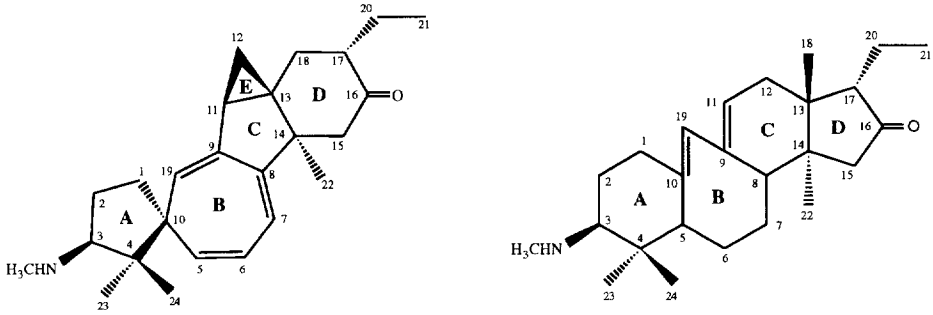

Figure 1 : Comparison between Spirofornabuxine **1** and secobuxus alkaloid type

The relative configuration of the two alicyclic moieties of spirofornabuxine was elucidated by a NOESY experiment (Table 1). The correlations observed between H-3, H-5, the C-23 methyl group and the C-3 methylamino group indicated a "syn" relationship between these protons. A NOE enhancement was also observed between H-19 and the C-24 methyl group.

The relative configuration of the C,D,E-rings moiety was established by correlations of the cyclopropane methylene protons (δ 0.24 and 0.74) with the two axial protons at C-15 (δ 2.52) and C-17 (δ 2.36), respectively, supporting an "anti" relationship between the cyclopropane ring and the C-17 ethyl group. The configuration at C-14 was then determined by the observation of NOE enhancements between the C-22 methyl group and both axial H-18 and equatorial H-15 methylene protons.

Until now, all the alkaloids isolated from Buxaceae have been described with an α configuration for the methyl group at C-14 and a β configuration for the amino group at C-3[2,3], so the absolute configuration of these carbon atoms can be likely proposed as 3*S* and 14*R*[2,3]. These two absolute configurations and the relative configurations established by NOESY suggest a 3*S*, 10*R*, 11*S*, 13*S*, 14*R*, 17*S* absolute configuration for **1** (Figure 1).

Table 1 : ^1H NMR data of spirofornabuxine (recorded at 400 MHz in $CDCl_3$, *J* Hz).

H	^1H NMR	COSY	NOESY
1α	1.55 *m*	H-1β, H-2α, H-2β	H-1β, H-2α
1β	1.20 *m*	H-1α, H-2α, H-2β	H-1α, H-24
2α	1.93 *m*	H-1α, H-1β, H-2β, H-3α	H-1α, H-2β, H-3α
2β	1.77 *m*	H-1α, H-1β, H-2α, H-3α	H-2α
3α	3.35 *t* (9)	H-2α, H-2β	H-2α, H-5, H-23, N-CH_3
5	5.35 *d* (10)	H-6	H-3α, H-7, H-23
6	6.02 *m*	H-5, H-7	H-7
7	6.07 *m*	H-6	H-5, H-6, H-15α, H-15β
11	2.26 *dd* (2, 7)	H-12α, H-12β	H-12α, H-18β, H-19
12α	0.74 *dd* (2, 7)	H-11, H-12β	H-11, H-12β, H-17β, H-18β
12β	0.24 *s br.*	H-11, H-12α	H-12α, H-15β
15α	2.61 *d* (13)	H-15β	H-7, H-15β, H-22
15β	2.52 *d* (13)	H-15α	H-7, H-12β, H-15α, H-17β
17β	2.36 *dddd* (6, 6, 6.5, 12.5)	H-18α, H-18β	H-12α, H-15β, H-18β, H-20_A, H-20_B, H-21
18α	2.14 *dd* (12.5, 13)	H-17, H-18β	H-11, H-18β, H-22
18β	1.60 *dd* (6.5, 13)	H-17, H-18α	H-12α, H-17β, H-18α
19	5.40 *s*		H-11, H-23, H-24
20_A	1.90 *m*	H-20_B, H-21	H-17β, H-20_B, H-21
20_B	1.35 *m*	H-20_A, H-21	H-17β, H-20_A
21	0.91 *t* (7)	H-20_A, H-20_B	H-17β, H-20_A
22	1.00 *s*		H-15α, H-18α
23	1.36 *s*		H-3α, H-5, H-19
24	1.32 *s*		H-19
N-CH_3	2.74 *s*		H-3α

Table 2 : ^{13}C NMR data and HMBC NMR of spirofornabuxine (recorded at 400 MHz in $CDCl_3$).

C	^{13}C NMR	HMBC
1	28.1	
2	23.6	H-3α
3	67.8	H-23, H-24, N-CH_3
4	45.5	H-3α, H-5, H-19, H-23, H-24
5	127.0	H-1β, H-7, H-19
6	124.5	
7	121.8	H-5
8	150.3	H-6, H-11, H-15β, H-19, H-22
9	143.4	H-7, H-11, H-12α
10	50.4	H-1β, H-5, H-6, H-19, H-23, H-24
11	27.2	H-19
12	22.6	H-18α, H-18β
13	32.0	H-11, H-15α, H-15β, H-17β, H-18α, H-18β, H-22
14	49.3	H-7, H-15α, H-15β, H-18β, H-22
15	49.8	
16	211.4	H-15α, H-15β, H-17β, H-18β, H-20_A, H-20_B
17	51.7	H-15α, H-18α, H-18β, H-20_A, H-20_B
18	32.0	H-11, H-17β, H-20_A, H-20_B
19	116.5	H-1β, H-5
20	21.8	H-17β, H-21
21	11.4	H-17β, H-20_A, H-20_B
22	25.9	H-15α, H-15β
23	20.6	H-3α, H-24
24	19.2	H-3α, H-23
N-CH_3	33.5	H-3α

Spirofornabuxine **1** constitutes a novel type of alkaloids by its original spiro junction at C-10 and the presence of a cycloheptatriene ring, the other *Buxus* alkaloids containing a seven numbered ring, the secobuxus alkaloids, present only one or two double bonds. Like the *Buxus* alkaloids and cycloartenol, spirofornabuxine is probably derived biogenetical from mevalonic acid[5]. While there is no experimental evidence available for this hypothesis, it seems reasonable to assume that cycloartenol, the universal biosynthetic precursor of sterols in plants, is also the precursor of the cyclobuxus, secobuxus and spirobuxus alkaloids, by degradations and aminations in all cases, by a further ring opening in the second, and by more complex reactions, opening and closing rings, in the last[6].

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4 - Leaves of *Buxus sempervirens* (4 kg) were extracted by MeOH-H_2O-CH_3COOH (10:10:1). Usual liq./liq. partitions followed by chromatographic separations over neutral alumina and silica gel afforded **1** (15 mg) as a cristalline powder, $[\alpha_D]$ +36 (c 0.31 in $CHCl_3$), IR (neat) 3400, 2920, 2852, 1712, 1463, 1031 cm^{-1}.

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