NEW TETRAHYDROFURANOID STEROIDAL ALKALOIDS FROM THE LEAVES OF *BUXUS HILDEBRANDTII*

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Abstract– Four new tetrahydrofuranoid steroidal alkaloids, (+)-O^6-buxafuranamine (**1**), (+)-2-dehydroxy-O^2-buxafuranamine (**2**), (–)-O^{10}-buxafuranamine (**3**) and (–)- 6-dehydroxy-O^{10}-buxafuranamine (**4**) have been isolated from the leaves of *Buxus hildebrandtii*. These are the members of a new series of *Buxus* steroidal bases containing a tetrahydrofuran ring incorporated in their structures. The structures were established on the basis of extensive spectroscopic studies. Three new classes along with several more examples of the previously mentioned structural classes have been included in the rules which relates the structural features with the specific rotations.

The genus *Buxus* comprises evergreen shrubs which are spread out from Eurasia to South Africa, Malaysia, Indonesia and North and Central America. The genus *Buxus* is well known for the treatment of a wide variety of diseases in folk medicines.[1] We have reported over fifty new steroidal alkaloids from *B. papillosa, B. sempervirens* and *B. hildebrandtii*.[2-5] In this paper we report the isolation and structure elucidation of O^6-buxafuranamine (**1**), 2-dehydroxy-O^2-buxafuranamine (**2**), O^{10}-buxafuranamine (**3**) and 6-dehydroxy-O^{10}-buxafuranamine (**4**) all containing a tetrahydrofuran ring incorporated in their structures. Their structures were assigned on the basis of spectroscopic studies.

(+)-O^6-Buxafuranamine (**1**), $C_{35}H_{48}N_2O_5$ was obtained as a white amorphous solid from the ethanolic extract of *B. hildebrandtii* leaves (experimental). The uv spectrum showed absorption maxima at 228 nm

indicating the presence of a secondary benzamidic chromophore.[6] The ir spectrum displayed intense absorptions at 3400 (OH), 3350 (NH), 2900 (CH), 1710 (ester carbonyl), 1650 (α, ß-unsaturated amide carbonyl), 1590 (C=C) and 1100 (C-O) cm^{-1}.

The high - resolution electron impact mass spectrum of 1 showed the molecular ion peak at *m/z* 576.4589 corresponding to the molecular formula, $C_{35}H_{48}N_2O_5$, (calcd 576.4737) and indicating the presence of thirteen double bond equivalents in the molecule. The peak at *m/z* 561.3312 resulted by the loss of a methyl group from the molecular ion. A peak at *m/z* 225.2053 was due to the retro Diels-Alder cleavage of ring C, indicating the presence of a double bond in ring C. Compound (1) showed the base peak at *m/z* 72.0820 which arose by the cleavage of ring D nitrogen containing side chain. Another peak at *m/z* 105.0360 was due to fragmentation of the benzoyl ion from the molecular ion.

The ^{1}H-nmr spectrum featured three sharp singlets at δ 0.38, 0.83 and 0.85 corresponding to the protons of the three tertiary methyl groups. A three-proton doublet at δ 1.25 (J=6.4 Hz) was assigned to the C-21 secondary methyl protons. A three-proton sharp singlet at δ 2.01 was assigned to the acetyl methyl protons, while a broad singlet integrating for six protons appeared at δ 2.23 due to the *N*, *N*- dimethyl protons substituted at C-20 of the ring D side chain. A multiplet centered at δ 5.16 was assigned to the C-16 methine proton, geminal to the acetoxy group. The C-31 (C-4α)* methylene protons geminal to the oxygen appeared as AB doublets at δ 3.65 and 3.85 ($J_{31a,31b}$ = 8.7 Hz). An interesting multiplet at δ 3.86 was ascribed to C-6 methine proton, geminal to the oxygen forming an ether linkage between C-6 and C-31. The relatively upfield chemical shift of the C-6 proton as compared to the previously reported (+)- buxafuranamide (δ 4.51, C-6 H) may be attributed to the absence of the deshielding effect of the C-10 hydroxyl group found in (+)-buxafuranamide.[9] The C-19 methylene protons appeared as a sharp singlet at δ 2.97, while a broad singlet centered at δ 5.30 was assigned to the C-11 olefinic proton. A doublet centered at δ 5.92 ($J_{1,2\beta}$ =

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* X-Ray diffraction studies on *Buxus* alkaloids have shown that of the two methyl groups at C-4, it is C-31 (the C-4 α methyl) which undergoes preferential oxidation in comparison to the C-30 methyl (C-4β methyl).[7-8] The earlier reported structures in which the C-4 β methyl group had been shown to have undergone oxidation may need to be revised.

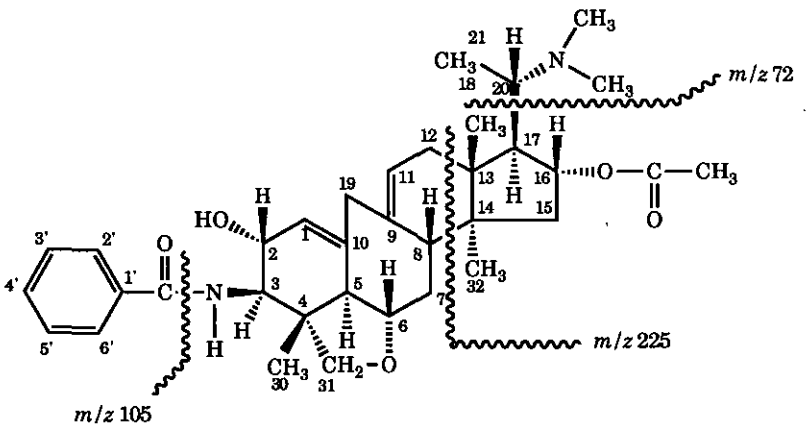
5.6 Hz) was due to the C-1 vinylic proton. The C-2 methine proton appeared as a double doublet at δ 4.24 ($J_{2\beta,3\alpha}$ = 4.7 Hz, $J_{2\beta,1}$ = 5.6 Hz) geminal to the hydroxyl group. A study of Dreiding models indicated that the ether bridge between C-6 and C-31 (C-4α) forces ring A into a twist-chair conformation in which the C-3/N bond is equatorial and the C-2/OH bond is axial. This was indicated by the coupling constant between C-2H (equatorial) and C-3H (axial) which was representative of equatorial-axial coupling. The C-3 methine proton appeared as a doublets of double doublet at δ 4.49 ($J_{3\alpha,NH}$ = 8.9 Hz, $J_{3\alpha,2\beta}$ = 4.7 Hz, $J_{3\alpha,5\alpha}$ = 0.8 Hz), while the amidic NH proton appeared as a doublet at δ 5.66 ($J_{NH,3\alpha}$ = 8.9 Hz). The aromatic protons resonated as two groups of three- and two-proton multiplets at δ 7.30 and 7.70 respectively.

The ^{1}H-nmr spectrum of **1** was also recorded in pyridine-d_5 to confirm the ethereal nature of the oxygen between C-6 and C-31. The C-31 methylenic protons showed a paramagnetic shift from δ 3.65 to 3.72 and 3.85 to 3.87 while the C-6 methine proton shifted from δ 3.86 to 4.00. It has been reported earlier that a pronounced shift of $\approx$ 0.2 ppm was observed in case of protons adjcent to hydroxy group when ^{1}H-nmr spectrum was recorded in pyridine-d_5.[10] The C-2 methine proton geminal to the hydroxyl group also showed a paramagnetic shift from δ 4.24 to 4.50.

Two-dimensional ^{1}H-nmr measurements were also performed.[11-12] The COSY-45° spectrum showed strong cross peaks between the C-1 olefinic proton (δ 5.92) and the C-2 methine proton (δ 4.24), while the C-2 methine proton also showed a cross-peak with the C-3 methine proton (δ 4.49). Strong COSY-45° interactions were also observed between the C-19 methylene protons (δ 2.97) and the C-11 vinylic proton (δ 5.30) representing the allylic coupling between the C-11 and C-19 protons. The NOESY spectrum showed the strong interactions of the C-19 methylene protons with the C-1 and C-11 vinylic protons. NOE interaction between C-6β proton and C-30 methyl was also observed in the NOESY spectrum of **1**. In the light of above spectroscopic studies, structure (**1**) was assigned to this new compound.

Our second compound (+)- 2-dehydroxy-O^2-buxafuranamine (**2**), $C_{35}H_{48}N_2O_4$, was also isolated as an amorphous solid. The uv and ir spectra of compound (**2**) closely resembled those of **1**.

The HREIMS spectrum of **2** include M^+ peaks at *m/z* 560.4899 in agreement with the molecular formula $C_{35}H_{48}N_2O_4$ (calcd 560.4788), indicating thirteen degrees of unsaturation in the molecules. The peak at *m/z*



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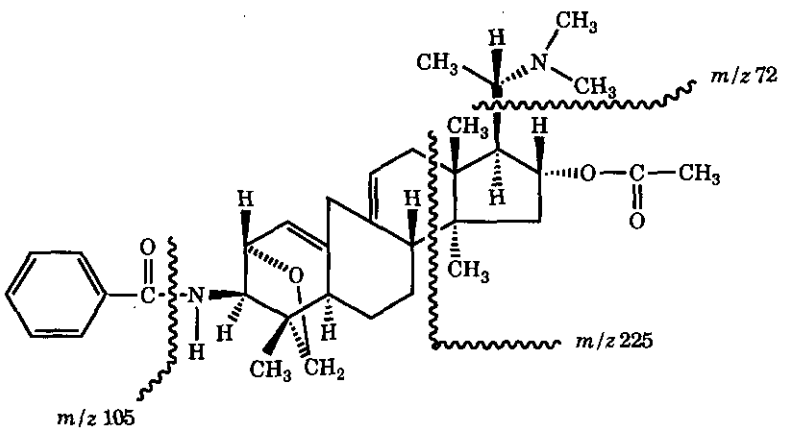
545. 4628 was due to the loss of a methyl group from the molecular ion, while the peak at *m/z* 105.0342 was due to the benzoyl cation. Compound (**2**) showed base peak at *m/z* 72.1311 indicating a trimethylimminium cation formed by the cleavage of ring D.

The ^{1}H-nmr spectrum of compound (**2**) was distinctly similar to **1** and featured three tertiary methyl signals at δ 0.24, 0.66 and 1.25. The C-21 secondary methyl protons appeared as a doublet at δ 1.26 ($J_{21,20}$ = 6.4 Hz), while a three-proton singlet at δ 2.01 was assigned to the acetyl methyl protons. A six-proton broad singlet centered at δ 2.37 was ascribed to the *N,N*-dimethyl protons. The C-16 methine proton appeared as a multiplet at δ 5.16. Two AB doublets resonating at δ 3.65 and 3.82 ($J_{31a,31b}$ = 8.4 Hz) were assigned to the C-31 methylene protons. An interesting double doublet centered at δ 4.25 ($J_{2\beta,3\alpha}$ = 4.8 Hz, $J_{2\beta,1}$ = 5.5 Hz) was ascribed to the C-2 methine proton, geminal to the oxygen linking C-2 with C-31 through an ether bridge. The α-stereochemistry of the ether bridge was apparent from the C-2H C-3H coupling constant ($J_{2\beta,3\alpha}$ = 4.8 Hz) while the α-orientation of the oxygenated C-4 methyl (C-31 methylene) was based on the above mentioned X-ray diffraction results. The Drieding model of compound (**2**) also showed that ring A exists in a twist chair conformation in which the C-3/ N and C-2/O bonds are both axially oriented. A doublet of double doublet at δ 4.49 ($J_{3\alpha,NH}$ = 8.8 Hz, $J_{3\alpha,2\beta}$ = 4.8 Hz, $J_{3\alpha,5\alpha}$ = 0.8 Hz) was assigned to the C-3 methine proton. A doublet centered at δ 5.65 ($J_{NH,3\alpha}$ = 8.8 Hz) was due to the amidic NH. The vinylic C-1 proton appeared as a doublet at δ 5.92 ($J_{1,2\beta}$ = 5.5 Hz), while a two-proton singlet at δ 2.97 was assigned

to the C-19 methylene protons. A one-proton broad singlet centered at δ 5.33 was assigned to the C-11 olefinic proton. The aromatic protons resonated as multiplets between δ 7.39 - 7.70.

The COSY-45° spectrum of **2** showed the strong interactions between C-3α H (δ 4.49) with the C-2 methine proton (δ 4.25) and the C-1 vinylic proton (δ 5.92). Strong COSY-45° interaction between the C-19 methylene proton (δ 2.97) with the C-11 olefinic proton (δ 5.33) was also observed in the spectrum. The ^{13}C-nmr spectrum ($CDCl_3$) of **2** indicated the presence of thirty five carbon atoms in the molecule. In order to establish the multiplicity of carbon signals, DEPT experiments were carried out with the last polarization pulse angle Θ = 45°, 90° and 135° which established the presencce of seven CH_3, six CH_2, fourteen CH and, by difference from the broad band spectrum, eight quaternary carbon atoms.[13] The C-21, C-30, C-18 and C-32 methyl carbons resonated at δ 11.42, 16.50, 17.78 and 19.92 respectively. The acetyl methyl carbon resonated at δ 21.25, while the C-31 methylene carbon and the C-2 methine carbon which were linked together through ether bridge appeared at δ 72.25 and 63.68 respectively. The olefinic C-1 resonated at δ 119.00, while the olefinic C-11 appeared at δ 127.10 whereas the allylic C-19 appeared at δ 33.10. The C^{13}-nmr chemical shift assignments to various carbon atoms are shown in Table **1**. These studies led to structure (**2**) for this new steroidal base.



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Our third compound (−)-O^{10}-buxafuranamine (**3**), $C_{35}H_{48}N_2O_5$, was isolated as a gummy material from the leaves of *B. hildebrandtii*. The uv and ir spectra of compound (**3**) were similar to those of **1** and **2**, indicating similar structural features.

The HREIMS spectrum of **3** showed the molecular ion peak at *m/z* 576.4691 corresponding to the molecular formula, $C_{35}H_{48}N_2O_5$. The compound showed the base peak at *m/z* 72.0810 again indicating the presence of a dimethylamino substituent at C-20 of the ring D side chain. A peak at *m/z* 105.0339 was due to the benzoyl cation. The fragment at *m/z* 225.1983 resulted by the retro Diels-Alder cleavage of ring C suggesting the presence of an unsaturation site in ring C.

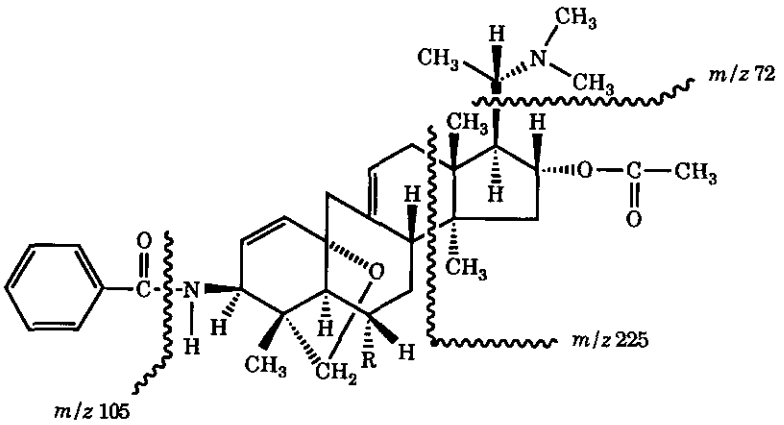
The ^{1}H-nmr spectrum of **3** displayed three sharp singlets at δ 0.72, 0.78 and 1.16 for the three tertiary methyl groups. A three-proton doublet resonating at δ 1.02 (J = 6.6 Hz) was due to the secondary C-21 methyl. Another three-proton singlet resonated at δ 1.91 was ascribed to the acetyl methyl protons, while a six-proton broad singlet at δ 2.36 was assigned to the *N,N*-dimethyl protons. The C-16 methine proton geminal to acetoxy group appeared as a multiplet at δ 4.96. Two AB doublets at δ 3.63 and 3.72 ($J_{31a,31b}$ = 8.6 Hz), were assigned to the C-31 methylene protons geminal to the ethereal oxygen connecting C-31 with C-10 by the ether linkage. A broad multiplet centered at δ 4.15 was due to the C-6 methine proton, geminal to the hydroxyl group. The C-19 allylic protons appeared as a broad singlet at δ 2.75. A broad singlet centered at δ 5.41 was assigned to the C-11 olefinic proton, while the C-1 and C-2 vinylic protons resonated as a sharp "singlet" at δ 5.86, which was splitted into two distinct signals when the ^{1}H-nmr spectrum was recorded in pyridine-d_5. A double doublet at δ 4.80 ($J_{3\alpha,NH}$ = 8.6 Hz, $J_{3\alpha,5\alpha}$ = 0.8 Hz) was assigned to the C-3 methine proton, while the amidic NH resonated as a doublet at δ 6.62 ($J_{NH,3\alpha}$ = 8.6 Hz). The aromatic protons appeared as two sets of multiplets at δ 7.43 and 7.85.

The ^{1}H-nmr spectrum of **3** was also recorded in pyridine-d_5. As compared to the spectrum $CDCl_3$, the C-31 methylene protons showed small paramagnatic shifts from δ 3.63 to 3.73 and 3.72 to 3.80, indicating the ethereal nature of the geminal oxygen. The two-proton "singlet" for the C-1 and C-2 in the $CDCl_3$ spectrum at δ 5.86 was splitted in the pyridine-d_5 spectrum into two double doublets at δ 6.07 ($J_{2,1}$ = 9.5 Hz, $J_{2,3\alpha}$ = 1.0 Hz), assigned to the C-2 vinylic proton and 6.10 ($J_{1,2}$ = 9.5 Hz) assigned to the C-1 olefinic proton. The C-3 methine proton was also seen to be further splitted from double doublets (in $CDCl_3$ spectrum) to doublets of double doublet (pyridine-d_5 spectrum) ($J_{3\alpha,NH}$ = 8.6 Hz, $J_{3\alpha,2}$ = 1.0 Hz, $J_{3\alpha,5\alpha}$ = 0.8 Hz) and shifted from δ 4.80 to 5.27. The C-6 methine proton geminal to the hydroxyl group also showed solvent induced paramagnetic shift from δ 4.15 ($CDCl_3$) to 4.42 (pyridine-d_5) which further confirmed that it was not involved in the ether linkage.

The COSY-45° spectrum (pyridine-d_5) revealed the strong cross peaks between the C-1 proton (δ 6.10) and C-2 olefinic proton (δ 6.07). The C-2 vinylic proton also showed cross peaks with the C-3 methine proton (δ 5.27). A strong COSY-45° interactions between allylic C-19 proton (δ 2.90) and C-11 olefinic protons (δ 5.49) was also observed.

The ^{13}C-nmr spectrum ($CDCl_3$) of **3** showed four signals at δ 14.05, 17.40, 19.41 and 19.91 which were assigned to the C-21, C-30, C-18 and C-32 methyl carbons respectively. The C-31 methylene and C-10 quaternary carbons connected by an ether linkage resonated at δ 64.75 and 63.49 respectively. The olefinic C-1 appeared at δ131.8, the olefinic C-2 resonated at δ 132.56 where as the olefinic C-11 resonated at δ 125.9. The rest of the ^{13}C-nmr chemical shift assignments are shown in Table **1**.

Acetylation of compound (**3**) with acetic anhydride/pyridine yielded the corresponding diacetate, $C_{37}H_{50}N_2O_6$. The ^{1}H-nmr spectrum of the acetate derivative showed downfield shift of the C-6 methine proton from δ 4.15 to 5.06. An additional three-proton singlet appeared at δ 1.95 was due to the C-6 acetyl methyl group. The rest of the ^{1}H-nmr spectrum was similar to that of the parent alcohol. It further proved the presence of a hydroxyl group at C-6. These studies led to the establishment of structure (**3**) for this compound.



3 R = OH
4 R = H

Table 1: ^{13}C-nmr Assignments of Compounds (2), (3) and (4) ($CDCl_3$)

Carbon No.	2 (δ)	3 (δ)	4 (δ)
1	119.00(CH)	131.80(CH)	131.80(CH)
2	63.68(CH)	132.56(CH)	132.56(CH)
3	55.30(CH)	56.90(CH)	56.90(CH)
4	44.32(-C-)	45.41(-C-)	45.43(-C-)
5	53.40(CH)	58.73(CH)	52.11(CH)
6	26.29(CH_2)	65.95(CH)	26.71(CH_2)
7	29.06(CH_2)	35.97(CH_2)	28.90(CH_2)
8	49.90(CH)	50.01(CH)	49.91((CH)
9	134.40(-C-)	135.67(-C-)	135.67(-C-)
10	131.60(CH)	63.49(-C-)	63.49(-C-)
11	127.10(CH)	125.90(CH)	125.90(CH)
12	32.95(CH_2)	35.13(CH_2)	35.13(CH_2)
13	44.59(-C-)	43.19(CH)	42.99(-C-)
14	48.33(-C-)	2.99(-C-)	45.09(-C-)
15	43.39(CH_2)	42.88(CH_2)	42.88(CH_2)
16	77.70(CH)	78.91(CH)	78.91(CH)
17	61.40(CH)	59.89(CH)	58.90(CH)
18	17.78(CH_3)	19.41(CH_3)	19.41(CH_3)
19	33.10(CH_2)	44.34(CH_2)	44.34(CH_2)
20	49.98(CH)	52.01(CH)	52.01(CH)
21	11.42(CH_3)	14.05(CH_3)	14.05(CH_3)
30	16.50(CH_3)	17.40(CH_3)	17.72(CH_3)
31	72.25(CH_2)	64.75(CH_2)	64.75(CH_2)
32	19.92(CH_3)	19.91(CH_3)	19.01(CH_3)
$OCOCH_3$	170.00(-C-)	171.09(-C-)	169.99(-C-)
$OCOCCH_3$	21.25(CH_3)	20.99(CH_3)	21.00(CH_3)
OCNH	165.99(-C-)	169.30(-C-)	169.30(-C-)
$N_b(CH_3)_2$	37.89(CH_3)	39.72(CH_3)	39.72(CH_3)
1'	134.48(-C-)	135.07(-C-)	134.99(-C-)
2'	127.21(CH)	127.32(CH)	127.32(CH)
3'	128.58(CH)	128.65(CH)	128.65(CH)
4'	131.09(CH)	129.89(CH)	129.89(CH)
5'	128.58(CH)	128.65(CH)	128.65(CH)
6'	127.21(CH)	127.32(CH)	127.3(CH)

Our fourth compound, (-) - 6-dehydroxy-O^{10}-buxafuranamine (**4**), $C_{35}H_{48}N_2O_4$, was separated as a gummy material. The uv and ir spectral data of compound (**4**) was very similar to those of the three new compounds discussed above. The ^{1}H-nmr spectrum of **4** resembled that of **3**, except that the downfield multiplet for the C-6 proton was absent. This indicated that **4** was a 6-deoxy derivative of **3**

The HREIMS spectrum of **4** include M^+ peak at *m/z* 560.4362 corresponding to the molecular formula $C_{35}H_{48}N_2O_4$ (calcd 560.4787). A peak at *m/z* 545.3091 was due to the loss of a methyl group from the molecular ion. The base peak at *m/z* 72.0815 corresponded to the loss of trimethylimminium cation by the cleavage of ring D. Another peak at *m/z* 105.0911 was due to the cleavage of the benzoyl cation.
The ^{13}C-nmr ($CDCl_3$) of **4** was very similar to that of **3** except that the C-6 methylene carbon resonated at δ 26.71. The ^{13}C-nmr chemical shift assigments are given in the Table **1**.On the basis of the spectral data structure (**4**) was assigned to this new compound.

We have reported previously the relationship between the structures and specfic rotations of steroidal alkaloids isolated from the genus *Buxus*. [14] Since than a number of new steroidal alkaloids has been isolated from *Buxus* species. With more data in hand three new classes have been included in the rules along with several examples of previously mentioned structural classes. Folowing are names along with the specific rotations of the new *Buxus* alkaloids.

1. 9(10→19) *abeo* Diene alkaloids of type **A** are dextrorotatory. Examples are homobuxaquamarine [+22°],[15] *N*-formylpapilicine [+36°],[16] *N*-formylharappamine[+40°],[16] 16α-acetoxybuxabenzamidienin [+60°],[14] sempervirine [+ 2°],[17] benzoylbuxidienine [+53°],*[17] *nor*-16α-acetoxybuxabenzamidienine [+17.5°],[18] *O*-acetyl-*N*-benzoylbuxidienine [+15°],*[18] N_a-demethylharappamine [+12.4°], 30-hydroxybuxamine A [+61°],[5] 30-*nor*-buxamine A [+19.7°],*[5] except 30(*O*)-benzoyl-16-deoxybuxidienine C [-75°],[5] 30-acetyl-N_a-benzoylbuxidienie [-4°],[19] and buxaminone [22°][20] are levorotatory.

2. 9β,19-Cyclo-11-oxo alkaloids of type **B** are also dextrorotatory, but magnitude of the specific rotation is usually larger than for *abeo* dienes of type A. One more example is avialable, *N*-benzoyl-16-acetylcyclobuxidine [+ 42°]. [21]

* Optical rotations are remeasured and found to be according to the rules.

3. 9β,19-Cyclo-16-oxo-$\Delta^{(17-20)}$ -alkaloids of type **C** are levorotatory, regardless of the geomatrical isomerism about the C-17 (20) double bond. Following are the examples of type C alkaloids. Sempervirone [-65°], [22] *Z*-buxenone [-67°],[23] *Z*-cyclobuxaphylamine [-30°],[24] *E*-cyclobuxaphylamine [-76°].[24] The latter two compounds also contian double bond at C-6/C-7.

4. 9β,19-Cyclo-20-oxo-$\Delta^{(16-17)}$ -alkaloids of type **D** are dextrorotatory as examplified by *nor*-cyclomicrobuxine [+ 34°],[15] *N*-formylcyclomicrobuxiene [+ 16°],[25] *N*-acetyl-*N*-demethylcyclomicrobuxine [+ 36°].[26]

5. 9β,19-Cyclo-$\Delta^{(6-7)}$ -alkaloids of type **E** are levorotatory for examples 31-acetylcyclomicrophylline A [- 40°]. [17]

6. Simple *Buxus* alkaliods with no unsaturation but with 9β,19-cyclo system of type **F** are dextrorotatory, examplified as semperviramidine [+ 33°],[27] deoxybuxandonine [+1°],[28] cyclomicrobuxinine [+162°],[23] and cyclomicrobuxamine [+140°].[30]

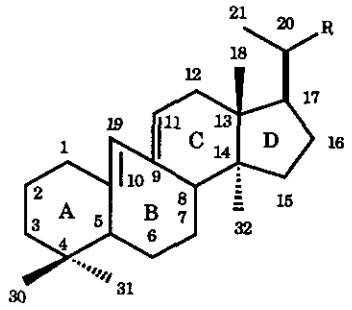
7. 9 (10→19) *abeo* Triene alkaliods of type **G** are dextrorotatry as indicated by following examples. Buxotrieneine[+13.5°],[14] buxitrienine-C [+57°],[29] papillotrienine[+ 60°] and *N*a-demethylpapillotrienine [+ 62°].

8. 9 (10→19) *abeo* $\Delta^{(1-10)\ (9-11)}$ -Alkaliods of type **H** are dextrorotatory. Examples include 31-acetylbuxanoldine [+50°],[30] buxupapine [+11°],[15] N_b-*nor*-buxupapine [+ 20°],[15] 16α,31-diacetylbuxanoldine [+ 22°], except buxanldine [-27°][14] and buxapapinolamine [- 16°][25] are levorotatory.

9. 9 (10→19) *abeo* $\Delta^{(1-10)}$-Alkaloids of type **I** are also dextrorotatory. Examples are buxanoldinine [+12°],[14] buxabenzacine [+48°],[9] and semperviraminol [+52°].

EXPERIMENTAL

General- Mass spectra were recorded on a Varian MAT 112 mass spectrometer connected to a DEC PDP 11/34 computer system. HREIMS were recorded on a Jeol-JMS HX110 mass spectrometer. ^{1}H-Nmr spectra were recorded in $CDCl_3$ or C_5D_5N, on Bruker AM 400 instrument at 400 MHz, while ^{13}C-nmr spectra were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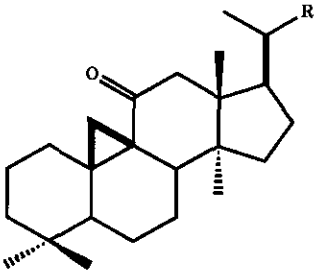
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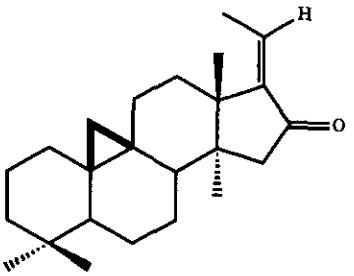
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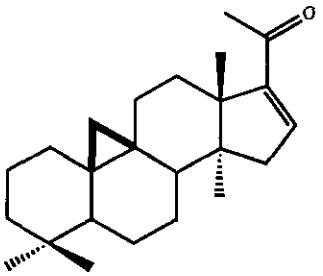
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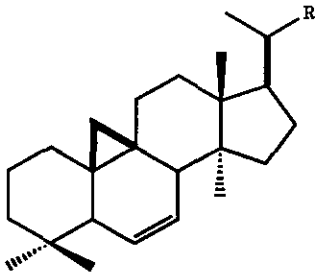
R

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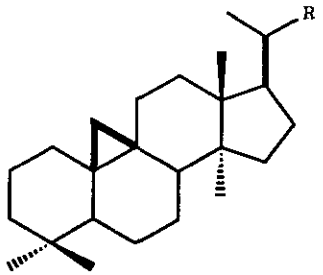
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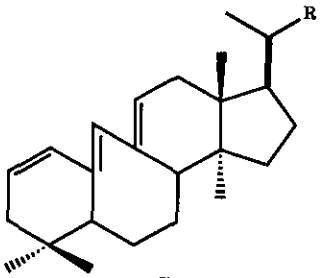
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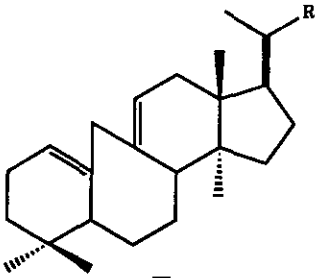
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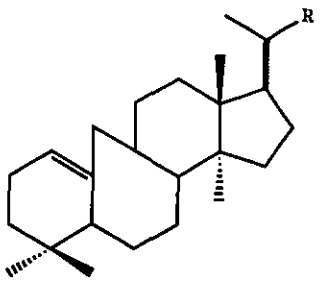
R

I

also recorded in $CDCl_3$ on the same instrument at 100 MHz. Ultraviolet and infrared spectra were recorded on Shimadzu UV 240 and Shimadzu IR 240 spectrophotometers, respectively. The optical rotations were measured on JASCO DIP - 360 digital polarimeter. The purity of the samples was checked by tlc (silica gel precoated plates).

Plant Material: Leaves of *B. hildebrandtii* were collected in April 1988 from the Bale province of Ethiopia near Sof Omar, altitude 136 m. The plant was identified by Dr. Sebsebe Demissew (National Herbarium Addis Ababa University) where a voucher specimen Sebsebe 2121 has been deposited.

Isolation: The EtOH extract of the leaves of *Buxus hildebrandtii* (80 g) was evaporated to a gum, defatted with pet. ether and loaded on a silica gel column (70-230 mesh, ASTM, Merck) (500 g). Eluation with $CHCl_3$ afforded a mixture of two alkaloids. This mixture was subjected to tlc (silica gel precoated plates, 0.2 mm) employing pet. ether - CH_3COCH_3 - $(C_2H_5)_2NH$ (9:1:0.1) as the eluting solvent to yield compounds **1** (7 mg) and **2** (6 mg) (R_f values, 0.75 and 0.49 respectively). Further elution with MeOH yielded a mixture of two more alkaloids which were purified by tlc (silica gel precoated plates, 0.2 mm) using pet. ether - $CHCl_3$ - $(C_2H_5)_2NH$ (8:2:0.5) as compounds **3** (10 mg) and **4** (7 mg) (Rf values, 0.69 and 0.54 respectively).

(+)-O^6-Buxafuranamine (1):White coloured amorphous solid (7 mg, $3.5x10^{-4}$ %) $[\alpha]^{20}_D$ $+20^o$ (c 0.7, $CHCl_3$); uv λ_{max} (MeOH) 228 nm; ir ($CHCl_3$) ν_{max} cm^{-1}: 3400 (OH), 3350 (NH), 2900 (CH), 1710 (ester carbonyl), 1650 (α,β-unsaturated amide carbonyl), 1590 (C=C), 1100 (C-O); ^{1}H-nmr ($CDCl_3$) δ: 0.38 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.85 (3H, s, CH_3), 1.25 (3H, d, $J_{21,20}$ = 6.4 Hz, 21-CH_3), 2.01 (3H, s, $OCOCH_3$), , 2.23 (6H, s, $N(CH_3)_2$), 2.97 (2H, s, H-19), 3.65 (1H, d, $J_{31a,31b}$ = 8.7 Hz, H-31a), 3.85 (1H, d, $J_{31b,31a}$ = 8.7 Hz, H-31b), 3.86 (1H, m, H-6), 4.24 (1H, dd, $J_{2\beta,3\alpha}$ = 4.7 Hz, $J_{2\beta,1}$ = 5.6 Hz, H-2ß), 4.49 (1H, ddd, $J_{3\alpha,2\beta}$ = 4.7 Hz, $J_{3\alpha,NH}$ = 8.9 Hz, $J_{3\alpha,5\alpha}$ = 0.8 Hz, H- 3α), 5.16 (1H, m, H-16ß), 5.30 (1H, br s, H-11), 5.66 (1H, d, $J_{NH,3\alpha}$ = 8.9 Hz, OCNH), 5.92 (1H, d, $J_{1,2\beta}$ = 5.6 Hz, H-1), 7.30-7.72 (5H, m, Ar H); ms *m/z* (rel. int. %): 576.4589 ($C_{35}H_{48}N_2O_5$, 3.5), 561.3312 ($C_{34}H_{45}N_2O_5$, 3), 225.2053 ($C_{13}H_{23}NO_2$, 2), 105.0360 (C_6H_5CO, 17), 72.0820 ($C_4H_{10}N$, 100).

(+)-2-Dehydroxy-O^2- buxafuranamine (2):White coloured amorphous solids, (6 mg, $3x10^{-4}$ %), $[\alpha]^{20}_D$ $+130^o$ (c 1.7, $CHCl_3$); uv λ_{max} (MeOH) 228 nm; ir ($CHCl_3$) ν_{max} cm^{-1}: 3400 (OH), 3310 (NH), 2900

(CH), 1700 (ester carbonyl), 1650 (α,β–unsaturated amide carbonyl), 1590 (C=C), 1090 (CO); ^{1}H-nmr ($CDCl_3$) δ: 0.24 (3H, s, CH_3), 0.66 (3H, s, CH_3), 1.25 (3H, s, CH_3), 1.26 (3H, d, $J_{21,20}$ = 6.4 Hz, 21-CH_3), 2.01 (3H, s, $OCOCH_3$), 2.37 (6H, s, $N(CH_3)_2$), 2.97 (2H, s, H-19), 3.65 (1H, d, $J_{31a,31b}$ = 8.4 Hz, H-31a), 3.82 (1H, d, $J_{31b,31a}$ = 8.4 Hz, H-31b), 4.25 (1H, dd, $J_{2\beta,3\alpha}$ = 4.8 Hz, $J_{2\beta,1}$ = 5.5 Hz, H-2β), 4.49 (1H, ddd, $J_{3\alpha,2\beta}$ = 4.8 Hz, $J_{3\alpha,NH}$ = 8.8 Hz, $J_{3\alpha,5\alpha}$ = 0.8 Hz, H-3α), 5.16 (1H, m, H-16), 5.33 (1H, br s, H-11), 5.65 (1H, d, $J_{NH,3\alpha}$ = 8.8 Hz, OCNH), 5.92 (1H, d, $J_{1,2\beta}$ = 5.5 Hz, H-1), 7.39-7.70 (5H, m, Ar H); ms *m/z* (rel. int. %): 560.4899 ($C_{35}H_{48}N_2O_4$, 0.6), 545.4628 ($C_{34}H_{45}N_2O_4$, 0.6), 105.0342 (C_7H_5O, 10), 72.1311 ($C_4H_{10}N$, 100).

(-)-O^{10}-Buxafuranamine (3): White coloured gummy material,(10 mg, 5x10^{-4} %) $[\alpha]^{20}_D$ -126.20° (c 1.3 $CHCl_3$); uv λ max (MeOH) 226 nm; ir ($CHCl_3$) ν_{max} cm^{-1}: 3410 (OH), 3330 (NH), 2910 (CH), 1710 (ester carbonyl), 1640 (α,β-unsaturated amide carbonyl), 1600 (C=C), 1110 (C-O); ^{1}H-nmr ($CDCl_3$) δ: 0.72 (3H, s, CH_3), 0.78 (3H, s, CH_3), 1.16 (3H, s, CH_3), 1.02 (3H, d, $J_{21,20}$ = 6.6 Hz, 21-CH_3), 1.91 (3H, s, $OCOCH_3$), 2.36 (6H, s, $N(CH_3)_2$), 2.75 (2H, s, H-19), 3.63 (1H, d, $J_{31a,31b}$ = 8.6 Hz, H-31a), 3.72 (1H, d, $J_{31b,31a}$ = 8.6 Hz, H-31b), 4.15 (1H, m, H-6β), 4.50 (1H, dd, $J_{3\alpha,NH}$ = 8.6 Hz, J3α,5α = 0.8 Hz, H-3α), 4.96 (1H, m, H-16ß), 5.41 (1H, br s, H-11), 5.86 (2H, s, H-1 and H-2), 6.62 (1H, d, $J_{NH,3\alpha}$ = 8.6 Hz, OCNH), 7.43-7.85 (5H, m, ArH); ms *m/z* (rel. int. %): 576.4691 ($C_{35}H_{48}N_2O_5$, 1.6), 561.3580 ($C_{34}H_{45}N_2O_5$, 1.4), 225.1983 ($C_{13}H_{23}NO_2$, 0.38), 105.0339 (C_7H_5O, 28), 72.0810 ($C_4H_{10}N$, 100).

Acetylation of (-)-O^{10}-buxafuranamine : Compound (**3**) (3 mg) was acetylated by stirring at room temperature using acetic anhydride (0.1 ml) in pyridine (0.3 ml). After stirring for 24 h the mixture was purified on tlc (silica gel) using pet. ether-$(C_2H_5)_2O$-$(C_2H_5)_2NH$ (9:1:0.5) to afford the corresponding diacetate $C_{37}H_{50}N_2O_6$ (2.5mg).

(-)-6-Dehydroxy-O^{10}-buxafuranamine (4): Colourless gummy material, (7 mg, 3.5 x 10^{-4} %), $[\alpha]^{20}_D$ -333° (c 1.5, $CHCl_3$); uv λ_{max} (MeOH) 226 nm; ir ($CHCl_3$) ν_{max}, cm^{-1}: 3410 (OH) 3300 (NH), 1700 (ester carbonyl), 1650 (α,β - unsaturated amide carbonyl), 1595 (C=C), 1100 (C-O); ^{1}H-nmr ($CDCl_3$) δ: 0.71 (3H, s, CH_3), 0.75 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.10 (3H, d, $J_{21,20}$=6.4 Hz, 21-CH_3), 1.91 (3H, s, $OCOCH_3$), 2.31 (6H, s, $N(CH_3)_2$), 2.77 (2H, s, H-19), 3.63 (1H, d, $J_{31a,31b}$ = 8.6 Hz, H-31a), 3.70 (1H, d, $J_{31b,31a}$ =

8.6 Hz, H-31b), 4.49 (1H, dd, $J_{3\alpha,NH}$ = 9.6 Hz, $J_{3\alpha,5\alpha}$ = 0.8 Hz, H-3α), 4.96 (1H, m, H-16ß), 5.47 (1H, br s, H-11), 5.88 (2H, s, H-1 and H-2), 6.62 (1H, d, $J_{NH,3\alpha}$ = 9.6 Hz, OCNH), 7.41-7.81 (5H, m, ArH); ms *m/z* (rel. int.%), 560.4362 ($C_{35}H_{48}N_2O_4$, 0.5), 545.3091 ($C_{34}H_{45}N_2O_4$, 1.5), 105.0911 (C_7H_5O, 42), 72.0815 ($C_4H_{10}N$, 100).

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