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ALKALOIDS FROM *BUXUS* SPECIES

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Key Word Index—*Buxus sempervirens*; *B. longifolia*; Buxaceae; roots; leaves; steroidal alkaloids; (−)-16α-hydroxybuxaminone; (+)-cyclobuxomicreinine.

Abstract—Two new steroidal alkaloids, (−)-16α-hydroxybuxaminone (3β-dimethylamino-16α-hydroxy-9,10-seco-buxa-9(11),10(19)-dien-20-one) and (+)-cyclobuxomicreinine (3β-methylaminobuxa-5,16-dien-20-one) have been isolated from the roots of *Buxus sempervirens* and the leaves of *B. longifolia*, respectively.

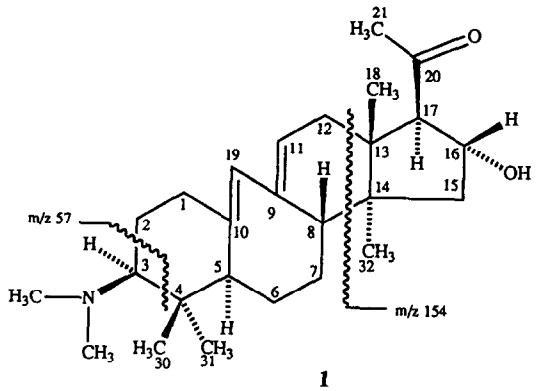
INTRODUCTION

The family Buxaceae is known to contain several species which occur in temperate regions of both hemispheres but only at higher elevations in the tropics. Extracts of shrubs of the genus *Buxus* are well known in folk medicine [1, 2]. The previous phytochemical studies on *B. sempervirens, B. papillosa* and *B. hildebrandtii* have resulted in the isolation of over 150 new steroidal alkaloids [3, 4]. We now describe the isolation and structure elucidation of two new steroidal alkaloids, (−)-16α-hydroxybuxaminone [3β-dimethylamino-16α-hydroxy-9,10-*seco*-buxa-9(11),10(19)-dien-20-one] (**1**) and (+)-cyclobuxomicreinine (3β-methylaminobuxa-5,16-dien-20-one) (**2**), isolated from the roots of *B. sempervirens* and leaves of *B. longifolia*, respectively. In addition to this, two other steroidal bases, isolated for the first time from *B. longifolia*, have been identified as (+)-buxaquamarine (**3**) [5] and (+)-*nor*-16α-acetoxybuxabenzamidienine (**4**) [6].

RESULTS AND DISCUSSION

Our first compound, (−)-16α-hydroxybuxaminone (**1**), $C_{26}H_{41}NO_2$, was isolated from ethanolic extracts of the roots of *B. sempervirens* by the method described in the Experimental. The compound showed UV absorptions at 246 and 242 nm indicating the 9(10→19)*abeo*-diene system [7]. The IR spectrum displayed strong absorptions at 3400 (OH), 2900 (C–H), 1695 (C=O) and 1590 cm^{-1} (C=O). The high-resolution electron-impact mass spectrum (HREIMS) of the alkaloid showed the $[M]^+$ at *m/z* 399.3931, corresponding to the molecular formula $C_{26}H_{41}NO_2$ (calcd 399.4069), indicating the degree of unsaturation as seven double bond equivalents in the molecule. The peaks at *m/z* 384.3163 and 356.2878 resulted from the respective losses of CH_3 and CH_3CO groups from the $[M]^+$. The ion peak at *m/z* 154.1295 resulted from retro Diels–Alder cleavage of ring C, indicating the presence of an unsaturation site in ring C. The compound showed a base peak at *m/z* 57.0679 due to the loss of the $(CH_3)_2N^+{=}CH$ cation by cleavage of ring A. The overall mass fragmentation pattern of compound **1** was very similar to that of *Buxus* alkaloids bearing a *N*-dimethyl amino group at C-3 [8].

The ^1H NMR spectrum of **1** ($CDCl_3$, 400 MHz) featured four tertiary methyl groups as sharp singlets at δ0.67, 0.71, 0.96 and 1.06. A three-proton singlet at δ2.15 was assigned to the proton of the methyl group adjacent to the carbonyl group. Another broad singlet integrating



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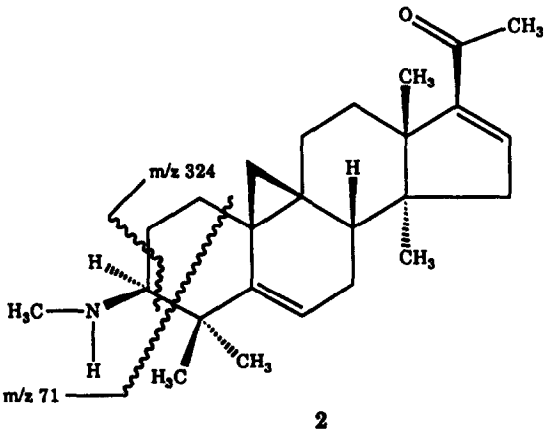

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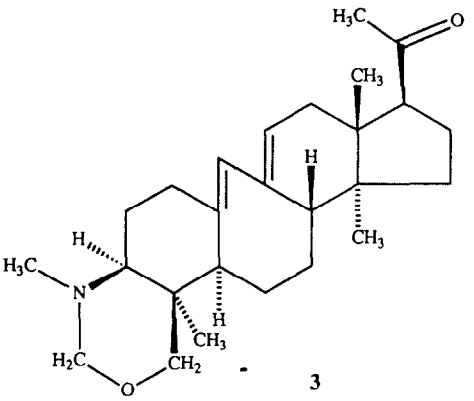

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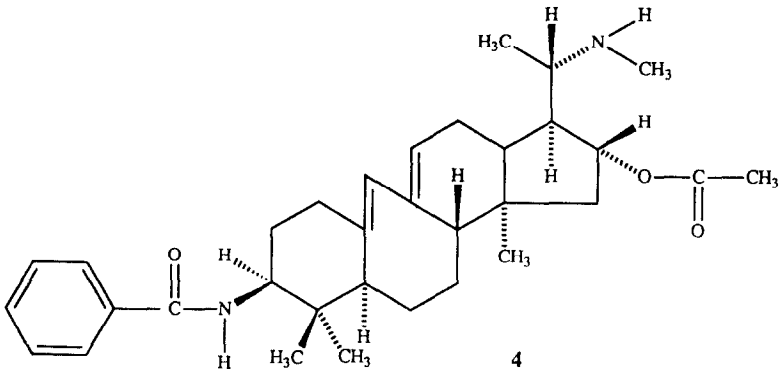

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for six-protons centred at δ2.31 was ascribed to the N_a-dimethyl protons. The C-16 methine proton appeared as a multiplet at δ4.88, being geminal to the hydroxy group. A broad singlet centred at δ5.62 was assigned to the C-11 olefinic proton, while the C-19 vinylic proton appeared as a singlet at δ5.91.

Acetylation of compound **1** with acetic anhydride/pyridine yielded the corresponding acetate, $C_{28}H_{43}NO_3$ [3]. Its ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) showed a downfield shift of the C-16 methine proton from δ4.88 to 5.16. An additional three-proton singlet centred at δ2.09 was due to the acetyl methyl protons. The rest of the ^{1}H NMR spectrum was similar to that of the parent alcohol. On the basis of the above mentioned spectroscopic studies structure **1** was assigned to this new steroidal base.

Our second compound, (+)-cyclobuxomicreinine (**2**), $C_{25}H_{37}NO$, was isolated from an ethanolic extract of the leaves of *B. longifolia*. The compound showed a UV absorption maximum at 240 nm indicating the presence of an α,β-unsaturated carbonyl group [5]. The IR spectrum displayed intense absorptions at 3080 (C–H), 1650 (α,β-unsaturated carbonyl), 1588 (C=C) and 1350 cm^{-1} (CH_2). The HREIMS of **2** showed the $[M]^+$ at m/z 367.2893, affording the molecular formula $C_{25}H_{37}NO$, indicating the degree of unsaturation as eight double bond equivalents in the molecule. The substance showed a peak at m/z 352. 264 resulting from the loss of a methyl group from the $[M]^+$. The base peak at m/z 71 corresponding to the composition C_4H_9N resulted from the cleavage of ring A along with the nitrogen containing side chain, as is usual in *Buxus* compounds [7].

The ^{1}H NMR spectrum of **2** (400 MHz, $CDCl_3$) showed four three-proton singlets at δ0.88, 0.89, 0.98 and 1.19, corresponding to the four tertiary methyl groups. Another three-proton singlet at δ2.10 was assigned to the methyl group adjacent to the carbonyl function [5]. The C-19 protons appeared as upfield AB doublets at δ0.40 and 0.34 ($J_{19\alpha,19\beta}$=4.6 Hz) [8], respectively, characteristic of cyclopropyl protons. The C-16 olefinic proton resonated as a doublet of doublets at δ6.65 (J_1=2.0, J_2=3.4 Hz) [7]. The C-6 proton resonated as multiplet at δ5.37 [8]. A three proton singlet at δ2.30 was assigned to the *N*-methyl group. From this evidence structure **2** was assigned to this new compound.

The two known steroidal bases, buxaquamarine (**3**) [5] and (+)-*nor*-16α-acetoxybuxabenzamidienine (**4**) [6], were also isolated for the first time from the leaves of *B. longifolia*.

EXPERIMENTAL

General. MS were recorded on a double-focusing spectrometer connected to a computer system. ^{1}H NMR were recorded in $CDCl_3$ at 400 MHz. The UV spectra were recorded on a Shimadzu UV-240 instrument. Purity of samples was checked on TLC (silica gel G-254).

Plant material. Roots of *B. sempervirens* L. were collected from Bolu-Yedigollar, 400 m Turkey, in Sept. 1989, leaves of *B. longifolia* Boiss. from Hatay-Antakya near St. Peter's Church, 220 m, Turkey, in March 1990. Species were identified by Dr Bilge Sener, Department of Pharmacognosy, voucher specimens are kept in the Herbarium of Gazi University, Faculty of Pharmacy, Ankara, Turkey.

Extraction and isolation of (−)-16α-*hydroxybuxaminone* (**1**). Air-dried and powdered roots of *B. sempervirens* (10 kg) were extd by cold percolation with EtOH. Removal of solvent gave 600 g of crude alcoholic exts. Partial sepn of alkaloids was carried out by extn with $CHCl_3$ at different pH values. The fr. obtained at pH 9.5 was evapd and concd to a gum. The gum (45 g) was loaded onto a silica gel column (600 g) and eluted with petrol (40–60°) and petrol–$CHCl_3$ mixs with increasing amounts of $CHCl_3$. On elution with petrol–$CHCl_3$ (9:1) an impure new steroidal base was obtained which was further purified by TLC using petrol–Et_2O–Et_2NH (8:2:0:5) to afford a white-coloured gummy material, $[\alpha]_D^{20}-54°$ ($CHCl_3$; *c* 1.56). UV λ_{max} (MeOH) 242, 246 nm. IR ($CHCl_3$) ν_{max} cm^{-1} 3400 (OH), 2900 (C–H), 1695 (C=O), 1590 (C=C). ^1H NMR ($CDCl_3$, 400 MHz) δ0.67 (3H, *s*, Me), 0.71 (3H, *s*, Me), 0.96 (3H, *s*, Me), 1.06 (3H, *s*, Me), 2.15 (3H, *s*, MeCO), 2.31 (6H, *s*, N $(Me)_2$), 4.88 (1H, *m*, H-16), 5.62 (1H, *br s*, H-11), 5.91 (1H, *s*, H-19). MS *m/z* (rel. int., %) 399.3931 ($C_{16}H_{41}NO_2$, 18), 384.3163 ($C_{25}H_{38}NO_2$, 4), 71 (C_4H_9N, 100), 57.0679 (C_3H_7N, 32).

Acetylation of (−)-16α-*hydroxybuxaminone* (**1**). Compound **1** (2 mg) was acetylated with Ac_2O (0.1 ml) in pyridine (0.3 ml) at room temp. After stirring for 24 hr, the mixt. was purified on TLC to afford the corresponding acetate. ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.67 (3H, *s*, Me), 0.71 (3H, *s*, Me), 0.96 (3H, *s*, Me), 1.06 (3H, *s*, Me), 2.09 (3H, *s*, MeCO), 2.15 (3H, *s*, OAcO), 2.31 [6H, *s*, $N(Me)_2$], 5.16 (1H, *m*, H-16), 5.62 (1H, *bs*, H-11), 5.91 (1H, *s*, H-19). MS *m/z* (rel. int., %): 441 ($C_{28}H_{43}NO_3$, 10), 426 ($C_{27}H_{40}NO_3$, 20), 71 (C_4H_9N, 100), 57 (C_3H_7N, 80).

Isolation of (+)-*cyclobuxomicreinine* (**2**), (+)-*buxaquamarine* (**3**) *and* (+)-nor-16α-*acetoxybuxabenzamidienine* (**4**). EtOH exts of air-dried and powdered leaves of *B. longifolia* were evapd to a gum and partial sepn of alkaloids was carried out by extn into $CHCl_3$ at different pH values. The fr. obtained at pH 3 (44.4 g) was loaded onto a silica gel column (70–230 mesh, 300 g). The column was eluted with petrol–$CHCl_3$ mixts of increasing polarity. Fr. A obtained by elution with petrol–$CHCl_3$ (9:1) contained a major compound which was further purified by prep. TLC in EtOAc–hexane–Et_2NH (8:1.8:0.2) to afford **2** as a white amorphous solid (4 mg). $[\alpha]_D^{20}+40°$ ($CHCl_3$). UV λ_{max} (MeOH) 240 nm. IR ($CHCl_3$) ν_{max} cm^{-1}; 3080 (C–H), 1650 (C=O), 1588 (C=C), 1350 (CH_2). ^1H NMR ($CDCl_3$, 400 MHz) δ0.34 (1H, *d*, $J_{19\alpha,19\beta}=4.6$ Hz, H-19α), 0.40 (1H, *d*, $J_{19\beta,19\alpha}=4.6$ Hz, H-19β), 0.88 (3H, *s*, 30-Me), 0.89 (3H, *d*, 31-Me), 0.98 (3H, *d*, 32-Me), 1.19 (3H, *s*, 18-Me), 2.10 (3H, *s*, 21-Me), 2.30 (3H, *s*, N-Me), 5.37 (1H, *m*, H-6), 6.65 (1H, *dd*, $J_1=2.0$ Hz, $J_2=3.4$ Hz, H-16). MS *m/z* (rel. int., %), 367 ($C_{25}H_{37}NO$, 34), 352 ($C_{24}H_{34}NO$, 22), 324 ($C_{23}H_{32}O$, 16), 71 (C_4H_9N, 100).

Fr. B obtained by elution with petrol–$CHCl_3$ (7:3) was further chromatographed by prep. TLC using petrol–EtOAC–Et_2NH (8:1.8:0.2) to afford two known steroidal bases, (+)-buxaquamarine (**3**) and (+)-*nor*-16α-acetoxybuxabenzamidienine (**4**). *Buxaquamarine* (**3**). Amorphous powder. $[\alpha]_D^{20}+24°$ ($CHCl_3$). UV λ_{max} (MeOH): 253, 238, 205 nm. IR ν_{max} cm^{-1} ($CHCl_3$): 2840 (CH), 1685 (ketonic carbonyl), 1645 (C=C). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.68 (3H, *s*, Me), 0.75 (3H, *s*, Me), 1.06 (3H, *s*, Me), 2.11 (3H, *s*, OAc), 2.17 (3H, *s*, N-Me), 3.27 (1H, *d*, $J_{31\alpha,31\beta}=10.7$ Hz, H-31α), 3.61 (1H, *d*, $J_{33\alpha,33\beta}=7.6$ Hz, H-33α), 3.84 (1H, *d*, $J_{31\beta,31\alpha}=10.7$ Hz, H-31β), 4.45 (1H, *d*, $J_{33\beta,33\alpha}=7.6$ Hz, H-33β), 5.60 (1H, *br m*, H-11), 6.0 (1H, *s*, H-19). MS *m/z* (rel. int., %): 397.2980 ($[M]^+$, $C_{26}H_{39}NO_2$, calcd 397.2981, 26), 382.7441 ($C_{25}H_{36}NO_2$ calcd 382.2745, 48), 127 ($C_7H_{13}NO$, 60), 85 ($C_5H_{11}N$, 19), 58 (C_3H_8N, 100), 71 (C_4H_9N, 28).

(+)-nor-16α-*Acetoxybuxabenzamidienine* (**4**). White amorphous solid. $[\alpha]_D^{20}+17.5°$ ($CHCl_3$). UV λ_{max} (MeOH): 230, 245, 254 nm. IR ν_{max} cm^{-1} ($CHCl_3$): 3350 (NH), 1725 (ester (C=O), 1650 (amide (C=O), 1605 (C=C). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.67 (3H, *s*, Me-32), 0.75 (3H, *s*, Me-31), 0.86 (3H, *s*, Me-38), 0.99 (3H, *s*, Me-30), 1.26 (3H, *d*, $J_{21,20}=6.5$ Hz, Me-21), 1.76 (3H, *s*, OAc), 2.43 (3H, *s*, $N(Me)_2$), 4.36 (1H, *m*, H-3), 5.08 (1H, *m*, H-16β), 5.57 (1H, *m*, H-11), 5.93 (1H, *s*, H-19), 6.34 (1H, *d*, $J_{3\alpha,NH}=8.5$ Hz, NH), 7.40–7.70 (5H, *m*, ArH). MS *m/z* (rel. int., %): 532.3665 ($[M]^+$, $C_{34}H_{48}N_2O_3$, calcd 532.3664, 28), 501.3234 ($C_{33}H_{43}N_3O$, 16), 58 (C_3H_8N, 40), 72 ($C_4H_{10}N$, 100).

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