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STEROIDAL ALKALOIDS FROM *BUXUS SEMPERVIRENS*

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Key Word Index—*Buxus sempervirens*, Buxaceae; steroidal alkaloids; (−)-buxadienine; (+)-buxaquamarine; (−)-31-acetoxy-N_a-benzoylbuxidienine

Abstract—*Buxus sempervirens* has yielded a new steroidal alkaloid, (−)-buxadienine along with two other bases (+)-buxaquamarine and (−)-31-acetoxy-N_a-benzoylbuxidienine.

INTRODUCTION

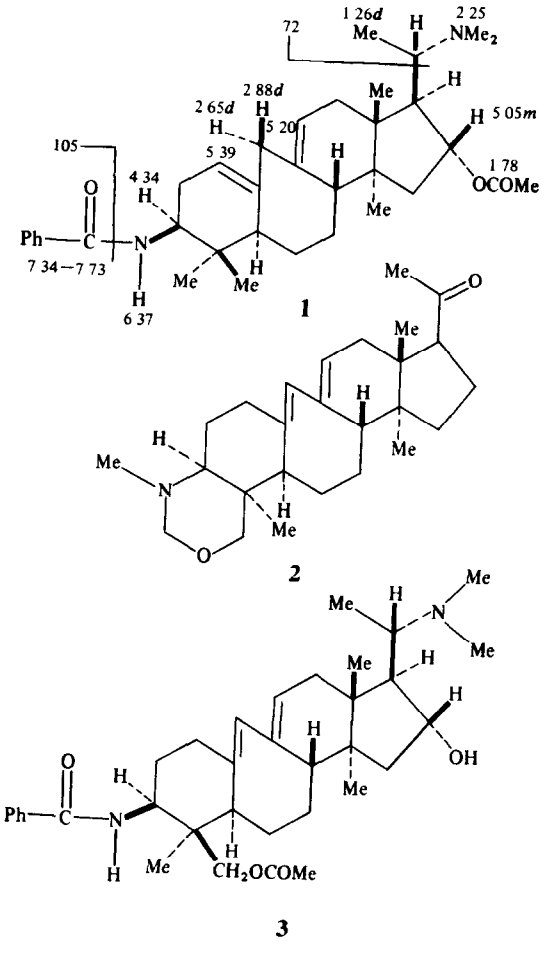
Buxus sempervirens L. (Buxaceae) is a shrub widely distributed in Eurasia Water extracts of this plant have been used in the indigenous system of medicine for a variety of purposes [1]. Continuing our investigations on the leaves of *B. sempervirens*, we report here the isolation and structure elucidation of a new steroidal base, (−)-buxadienine (**1**). Its structure has been determined through extensive spectroscopic studies. In addition to this, two other steroidal alkaloids isolated for the first time from this plant, have been identified as (+)-buxaquamarine (**2**) [2] and (−)-31-acetoxyl-N_a-benzoylbuxidienine (**3**) [3].

RESULTS AND DISCUSSION

Buxadienine (**1**) was isolated from the crude alkaloidal fraction by extracting into $CHCl_3$ at pH 9 Compounds **2** and **3** were isolated from the fractions obtained at pH 3.

(−)-Buxadienine (**1**), $C_{35}H_{50}N_2O_3$, showed UV absorption at 226 nm, characteristic of a secondary benzamidic chromophore [4, 5] The IR spectrum displayed absorptions at 1725 (ester carbonyl), 1655 (amide carbonyl) and 1600 (C=C) cm^{-1} [5]. The ^1H NMR spectrum of **1** ($CDCl_3$, 400 MHz) showed four 3H singlets at δ0 78, 0.88, 0.92 and 0.94 due to the four tertiary methyl groups. A doublet resonating at δ1.26 ($J_{21,20}$=6.4 Hz) was assigned to the 20-methyl group. A 3H singlet at δ1.78 was due to the acetate methyl group while the NMe_2 group appeared as a 6H singlet at δ2.25. A doublet at δ2 65 (J_{gem}=14.4 Hz) was due to C-19βH, while the doublet at δ2.88 (J_{gem}=14.4 Hz) was attributed to C-19αH A multiplet at δ4.34 was due to H-3. The 16β proton, geminal to the acetoxy group, appeared as a multiplet at δ5.05. Two broad singlets at δ5.20 and 5.39, each integrating for one proton, were assigned to the C-11 and C-1 olefinic protons, respectively [6]. The amidic NH proton appeared as a clean doublet at δ6.37 ($J_{3,NH}$ =8.4 Hz) Two multiplets integrating for 3H and 2H appeared centred at δ7.34 and 7 73 due to 3′/4′/5′/ and ′2/′6/ aromatic protons, respectively [4].

The high resolution mass spectrum of **1** showed the $[M]^+$ ion at *m/z* 546.3729, corresponding to the molecular formula $C_{35}H_{50}N_2O_3$ An intense peak at *m/z* 503.3601 was due to the loss of an acetyl group from the $[M]^+$. The base peak at *m/z* 72.0812 arose by the cleavage of the nitrogen-containing side chain of ring D [7]. In the light of these data structure **1** was assigned to the new alkaloid.



The second alkaloid was identified as (+)-buxaquamarine (**2**), reported by us previously from *B. papilosa*, by comparison of its spectral data and chromatographic behaviour with that of (+)-buxaquamarine [2]. The third alkaloid was identified as (−)-31-acetoxy-N_a-benzoylbuxidienine (**3**) by spectroscopic and chromatographic comparison with an authentic sample [3].

EXPERIMENTAL

^{1}H NMR 400 MHz. TLC was performed on silica gel (GF 254) precoated plates (Merck). Plant material was collected from Beynam Forest, Ankara, Turkey, in Sept. 1986. The EtOH extract of air-dried leaves was evapd. to a gum. The crude alkaloids (50 g) were obtained by extraction into 10% HOAc and partially sepd. into several fractions at different pH values.

(−)-*Buxadienine* (**1**). The fraction obtained at pH 9 was subjected to repeated prep. TLC in Me_2CO–hexane–$NHEt_2$ (5:2.5:1) to yield amorphous **1** (8 mg). $[\alpha]_D^{20} = -7$ ($CHCl_3$); UV λ_{max}^{MeOH} 226 nm; IR ν_{max} ($CHCl_3$) 1655 (amide carbonyl), 1725 (ester carbonyl), 1600 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.78 (3H, s, Me-18), 0.88 (3H, s, Me-32), 0.92 (3H, s, Me-30), 0.94 (3H, s, Me-31), 1.26 (3H, *d*, $J_{21,22}$ = 6.4 Hz, Me-21), 1.78 (3H, s, COMe), 2.25 (6H, s, NMe_2), 2.65 (1H, *d*, J_{gem} = 14.4 Hz, H-19β), 2.88 (1H, *d*, J_{gem} = 14.4 Hz, H-19α), 4.34 (1H, *m*, H-3), 5.05 (1H, *m*, H-16β), 5.20 (1H, *br* s, H-11), 5.39 (1H, *br s*, H-1), 6.37 (1H, *d*, $J_{3\ NH}$ = 8.4 Hz, NH), 7.34–7.73 (5H, *m*, ArH); MS *m/z* (rel. int.) 546.3729 ($C_{35}H_{50}N_2O_3$, calcd. 546.3720, 50), 503.3601 ($C_{33}H_{47}N_2O_2$ calcd. 503.3637, 90), 105.0339 (C_7H_5O, calcd. 105.0340, 100), 72.0812 ($C_4H_{10}N$, calcd. 72.0813, 95).

(+)-*Buxaquamarine* (**2**). The fraction obtained at pH 3 was loaded onto a silica gel column. Elution was carried out with $CHCl_3$–MeOH. Two major frs. were obtained, A and B. Fraction A afforded compound **2** (10 mg, amorphous), after prep. TLC (silica gel) in Me_2CO–hexane–$NHEt_2$ (5:2.0:1) $[\alpha]_D^{20} = +24$ ($CHCl_3$); UV λ_{max}^{MeOH} 238, 245, 205sh, 254 nm sh; IR ν_{max} ($CHCl_3$) 1685 (ketonic carbonyl), 1645 cm^{-1} (C=C); ^{1}H 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, MeCO), 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), 6.00 (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, 3), 127 (32), 85 (56), 71 (23), 58 (100), 57 (72).

(−)-31-*Acetoxy*-N_a-*benzoylbuxidienine* (**3**). Fr. B from the above CC was subjected to prep. TLC (silica gel) with Me_2CO–hexane–$NHEt_2$ (5:2.5:1) as eluent to obtain **3** as a white amorphous material (6 mg). $[\alpha]_D^{20} = -40$ ($CHCl_3$); UV λ_{max}^{MeOH} 238, 245, 253, 268, 279, and 290 nm; IR $\nu_{max}^{(CHCl_3)}$ 3400 (NH), 3350 (OH), 1716 (C=O ester), 1662 (C=O, amide), and 1610 cm^{-1} (C=C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.76 (3H, s, Me), 0.77 (3H, s, Me), 0.93 (3H, *d*, $J_{21\ 22}$ = 6.4 Hz, Me-21), 6.94 (3H, s, Me), 2.12 (3H, s, COMe), 2.61 (6H, s, NMe_2), 3.82 (1H, *d*, $J_{31\alpha\ 31\beta}$ = 10.9 Hz, H-31α), 4.02 (1H, *d*, $J_{31\beta\ 31\alpha}$ = 11.0 Hz, H-31β), 3.94 (1H, *m*, H-16), 5.52 (1H, *m*, H-11), 6.07 (1H, *s* H-19) and 7.43–7.71 (5H, *m*, ArH); MS *m/z* (rel. int.) 562.3801 ([M]$^+$, $C_{35}H_{50}N_2O_4$ calcd. 562.3770, 23), 547 (5), 503 (15), 85 (62), 72 (92), 71 (40), 58 (100).

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