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FOUR NEW STEROIDAL ALKALOIDS FROM *PACHYSANDRA AXILLARIS*

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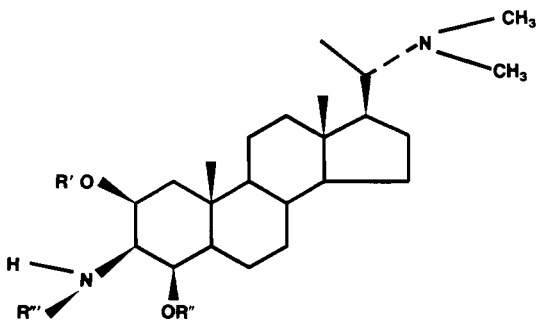
ABSTRACT.—The chemical structures of four new steroidal alkaloids, axillarines C [**1**], D [**2**], E [**3**], and F [**4**], from *Pachysandra axillaris* were elucidated as 20α-dimethylamino-3β-benzoylamino-2β-hydroxy-5α-pregnan-4β-yl acetate [**1**], 20α-dimethylamino-3β-benzoylamino-5α-pregnane-2β,4β-diol diacetate [**2**], 20α-dimethylamino-3β-benzoylamino-5α-pregnane-2β,4β-diol [**3**], and 20α-dimethylamino-3β-tigloylamino-2β-hydroxy-5α-pregnan-4β-yl acetate [**4**].

Alkaloids from *Pachysandra terminalis* Sieb. et Zucc. (Buxaceae) have been studied by Kikuchi *et al.* (7). With the exception of terminaline, all these alkaloids are derivatives of 3,20α-diamino-5α-pregnane and 3,20α-diamino-5α-pregnane with oxygen at C-4 and were called pachysandra-type alkaloids (1). Recently, we have studied the pachysandra-type alkaloids isolated from *Pachysandra axillaris* Franch. (Buxaceae) collected in Yunnan, China (2), and reported structural elucidation of pachyaximines A and B (3), isospropachysine (4), axillaridine A (5), and pachyaxiosides A and B (6). The present paper reports the chemical structures of four new alkaloids named axillarines C [**1**], D [**2**], E [**3**], and F [**4**].

The mixture of alkaloids was isolated from the concentrated 95% EtOH extracts of *P. axillaris* by partition at different pH values. The fraction obtained at pH 3 was repeatedly chromatographed on Si gel or alumina to afford axillarines C–F in 0.0029, 0.00018, 0.00067, and 0.00011 (%) yields, respectively.

Axillarine C [**1**] had molecular formula $C_{32}H_{48}O_4N_2$ as determined from its mass spectrum ($[M]^+$ 524). Its ir spectrum displayed absorptions at 3380 (NH, OH), 1732, 1226 (OAc), 1635 (benzamide C=O), 1600, 1520, 1455 (aromatic C=C), 715 cm^{-1}. The ms of **1** showed a base peak at *m/z* 72, resulting from cleavage of the nitrogen-containing side chain on ring D, a characteristic fragment in related alkaloids (7). Other significant ions were observed at *m/z* 453 $[M-71]^+$ and 105 $[C_6H_5CO]^+$. The ^{1}H-nmr spectrum exhibited two tertiary methyl groups at 0.65 and 1.26 ppm, while a



1 R′=H, R″=Ac, R‴=Bz
2 R′=R″=Ac, R‴=Bz
3 R′=R″=H, R‴=Bz
4 R′=H, R″=Ac, R‴=Tig

Bz = benzoyl Tig = tigloyl = Me-CH=CMe-CO

secondary methyl group resonated as a doublet at δ 0.87 ppm ($J = 6.2$ Hz), corresponding to Me-18, Me-19, and Me-21. A 6H singlet at δ 2.16 ppm was assigned to the protons of two methyl groups attached to a nitrogen. The NH proton of a secondary benzamide group resonated as a doublet at δ 6.98 ppm ($J = 8.1$ Hz). Signals at 5.44 (m) and 2.08 (s) were assigned to a CH-OAc group. Aromatic protons appeared as three groups of multiplets centered at δ 7.73 ppm (2H, brdd, $J = 7.4$, 7.4 Hz), and 7.39 ppm (2H, brdd, $J = 7.4$, 7.4 Hz), corresponding to H-2′,6′, H-4′,3′, and H-5′, respectively. The ^{13}C-nmr spectrum of **1** exhibited signals at δ 12.39, 16.58, and 9.91 ppm, which were assigned to the Me-18, Me-19, and Me-21 carbons, respectively. The ^{13}C data of the side chain and the C and D rings were similar to those of pachyaximine A and B, iso-spiropachysine, axillaridine A, and pachyaxiosides A and B (3–6). The signals of oxygen-substituted carbons appeared at δ 74.95, 69.79 ppm, and the signals of the benzoyl carbons were easily assigned. Assignments of the various carbons were confirmed by DEPT (Table 1). The ^{13}C-nmr data of **1** indicate that all substituents are on ring A. The secondary benzamide group is at the C-3 position, and hydroxyl and

TABLE 1. ^{13}C-nmr and DEPT Data of Axillarines C [**1**], D [**2**], E [**3**] diacetate, and F [**4**].

Carbon	Compound				
	1	**2**	**3** Diacetate	**4**	DEPT
C-1	44.59	40.96	40.97	44.59	CH_2
C-2	69.79	71.70	71.72	69.96	CH
C-3	52.13	50.91	50.91	51.60	CH
C-4	74.95	74.35	74.37	75.09	CH
C-5	48.66	48.88	48.89	48.59	CH
C-6	25.33	25.20	25.21	25.33	CH_2
C-7	31.85	31.80	31.82	31.85	CH_2
C-8	34.70	34.82	34.83	34.71	CH
C-9	56.23	55.81	55.82	56.23	CH
C-10	34.88	35.01	35.02	34.82	C
C-11	20.65	20.68	20.70	20.65	CH_2
C-12	39.64	39.58	39.62	39.64	CH_2
C-13	41.69	41.88	41.93	41.69	C
C-14	56.44	56.39	56.39	56.44	CH
C-15	23.98	24.04	24.07	23.98	CH_2
C-16	27.62	27.57	27.44	27.62	CH_2
C-17	54.95	54.70	54.67	54.95	CH
C-18	12.39	12.35	12.35	12.39	Me
C-19	16.58	15.39	15.41	16.59	Me
C-20	61.02	61.50	61.39	61.02	CH
C-21	9.91	10.25	10.34	9.91	Me
NMe_2	39.89	39.84	39.74	39.89	Me
C=O	167.06	166.92	166.93	168.82[a]	C
C-1′	134.56	134.52	134.54		C
C-2′	128.53	128.67	128.67	130.89[a]	CH
C-3′	127.06	126.95	126.96	131.76[a]	CH
C-4′	131.43	131.57	131.58	13.89[a]	CH
C-5′	127.06	126.95	126.96	12.86[a]	CH
C-6′	128.53	128.67	128.67		CH
2-OAc		170.76	170.76		C
		21.30	21.30		Me
4-OAc	170.30	170.25	170.25	170.11	C
	21.01	20.93	20.94	21.01	Me

[a]DEPT of tigloyl: C-1′–C-5′, C, C, CH, Me, Me.

acetoxyl carbons are at the C-2 and C-4 positions, respectively. This is shown in the diacetate **2** by the carbon signal of C-1 being shifted upfield from δ 44.59 to 40.96. Thus the chemical structure of axillarine C [**1**] may be assigned as 20α-dimethylamino-3β-benzoylamino-2β-hydroxy-5α-pregnan-4β-yl acetate.

Axillarine D (2) showed ir absorptions at 1740, 1735, 1233, 1245 cm^{-1} (2 × OAc). In the ^{1}H-nmr spectrum there were signals of two acetylmethyls at 2.12 and 2.08 ppm (s, 3H each), and the signal of the proton geminal to the C-2 hydroxy group shifted downfield from 4.14 (m) to 5.26 (m) ppm. In the ^{13}C-nmr spectrum new signals for acetyl carbons appeared at δ 170.76, 21.30 ppm, and the signals of the C-2 carbon shifted downfield from δ 69.79 in **1** to 71.70 ppm in **2**. These data indicate that **2** is an acetate of **1**. The ir, ms, ^{1}H-nmr and ^{13}C-nmr spectra of axillarine C acetate and axillarine D [**2**] were identical.

Axillarine E [**3**] was insoluble in common organic solvents. Its ir spectrum (Nujol) showed absorption at 3440 (NH), 3420, 3280, (OH), 1635 (benzamide C=O), 1596, 1515, 1457 (aromatic C=C) cm^{-1}. Axillarine E [**3**] was acetylated to afford axillarine E diacetate which proved to be **2** by comparison of ir, ms, ^{1}H-nmr, and ^{13}C-nmr spectra. Thus **3** is 2,4-deacetyl axillarine D, or 20α-dimethylamino-3β-benzoylamino-5α-pregnane-2β,4α-diol.

Axillarine F [**4**] showed ir absorptions at 3380 (NH, OH), 1733, 1226 (OAc), and 1655 cm^{-1} (C=C). The ms showed a molecular ion peak at *m/z* 502, corresponding to molecular formula $C_{30}H_{50}O_4N_2$, and a characteristic base peak at *m/z* 72 was observed. The ^{1}H-nmr spectrum showed signals for two tertiary methyl groups at δ 0.64, 1.22 (each 3H, s), one secondary methyl at δ 0.87 (d, J = 6.2 Hz), one *N*-dimethyl group at δ 2.16 (6H, s), and an aceto-methyl at δ 2.07 (3H, s) ppm. This was similar to the spectrum of axillarine C; the lack of benzoyl signals and the presence of an olefinic proton at δ 6.38 ppm (1H, brq, J = 6.6 Hz), as well as the two methyl protons at 1.79 (3H, brs) and 1.72 (3H, brd, J = 6.6 Hz) suggested that a tigloyl moiety was present instead. Signals at δ 4.1 (m) and 5.34 (m) ppm were assigned to the two protons geminal to hydroxyl and acetoxyl groups, respectively. The ^{13}C-nmr chemical shifts of the steroidal skeleton were essentially identical to those of axillarine C. Axillarine F has a tigloylamino group at the C-3 position. The signals of this group were assigned as 168.82 (C=O), 130.89 (C), 131.76 (CH), 13.89 (Me), 12.86 (Me) ppm. Therefore its structure is 20α-dimethylamino-3β-tigloylamino-2β-hydroxy-5α-pregnan-4β-yl acetate.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were uncorrected. The ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ on a Bruker AM-400 nmr spectrometer. Chemical shifts (δ) are given in ppm with TMS as the internal standard. The ir spectra (Nujol or KBr) were recorded on a Perkin-Elmer 577 spectrophotometer. The ms spectra (ei 70 eV or 20 eV) were recorded on a Finnigan-4510 spectrometer. Optical rotations were recorded on a JASCO-20C polarimeter.

EXTRACTION AND ISOLATION.—The 95% EtOH extracts of *P. axillaris* (dry wt 45 kg) collected from Yunnan, China in October 1985 were evaporated to a gum. The crude alkaloids were obtained by extraction into 5% HOAc. Partial separation of the alkaloids was achieved by extraction into $CHCl_3$ at different pH values. The fraction obtained with pH 3.0 buffer solution was evaporated to a gum. The gum was repeatly chromatographed on Si gel or alumina to afford four steroidal alkaloids: axillarines C [**1**] (1.3 g), D [**2**] (80 mg), E [**3**] (300 mg), and F [**4**] (50 mg) in 0.0029%, 0.00018%, 0.00067%, and 0.00011% yields, respectively.

Axillarine C [**1**].—Colorless crystals: mp 272–274°; $[\alpha]^{22}_D$ +22.4° (c = 0.981, $CHCl_3$); ir ν max (Nujol) 3380, 2950, 2920, 2850, 1732, 1635, 1520, 1455, 1372, 1360, 1226 cm^{-1}; ms *m/z* (%) $[M]^+$ 524 (0.2), $[M-Me]^+$ 509 (0.3), 453, 298, 272 (8), 256 (8), 122 (64), 105 (43), 72 (100); ^{1}H nmr (ppm) 7.72 (2H, brd, J = 7.4 Hz, H-2′, -6′), 7.47 (1H, brdd, J = 7.4, 7.4 Hz, H-4′), 7.39 (2H, brdd, J = 7.4, 7.4 Hz, H-3′, -5′), 6.98 (1H, d, J = 8.1 Hz, NH), 5.43 (1H, m, H-4), 4.25 (1H, ddd, J = 8.1, 3.9, 3.9

Hz, H-3), 4.14 (1H, m, H-2), 2.16 (6H, s, NMe_2), 2.08 (3H, s, OAc), 1.26 (3H, s, Me-19), 0.87 (3H, d, J = 6.2 Hz, Me-21), 0.65 (3H, s, Me-18).

Axillarine C acetate.—Mp 218–220°; ir ν max (KBr) 3440, 2920, 2860, 1742, 1735, 1665, 1632, 1600, 1580, 1510, 1482, 1445, 1390, 1240, 1235, 1055, 1020, 710 cm^{-1}; ms *m/z* (%) $[M]^+$ 566, $[M-Me]^+$ 551, 272, 256, 105 (12), 72 (100); ^1H nmr δ (ppm) 7.66 (2H, brd, J = 7.2 Hz, H-2′, -6′), 7.51 (1H, brd, J = 7.2 Hz, H-4′), 7.43 (2H, brdd, J = 7.2, 7.2 Hz, H-3′, -5′), 6.55 (1H, d, J = 8.4 Hz, NH), 5.35 (1H, m, H-1), 5.25 (1H, m, H-2), 4.46 (1H, ddd, J = 8.4, 3.9, 3.9 Hz, H-3), 2.25 (6H, s, NMe_2), 2.11 (3H, s, 2-OAc), 2.09 (3H, s, 4-OAc), 1.17 (3H, s, Me-19), 0.93 (3H, d, J = 6.2 Hz, Me-21), 0.65 (3H, s, Me-18).

Axillarine D [**2**].—Colorless crystals; mp 223–225°, $[\alpha]^{22}D$ +11.6° (c = 0.433, $CHCl_3$); ir ν max (KBr) 2920, 2860, 2760, 1740, 1735, 1665, 1630, 1600, 1580, 1510, 1480, 1445, 1390, 1370, 1245, 1233, 1050, 1020, 714 cm^{-1}; ms *m/z* (%) $[M]^+$ 566, $[M-Me]^+$ 551, 272, 256, 72 (100); ^1H nmr (ppm) 7.66 (2H, brd, J = 7.3 Hz, H-2′, -6′), 7.50 (1H, brdd, J = 7.3, 7.3 Hz, H-4′), 7.42 (2H, brdd, 7.3, 7.3 Hz, H-3′, -5′), 6.53 (1H, d, J = 8.4 Hz, NH), 5.34 (1H, m, H-4), 5.26 (1H, m, H-2), 4.45 (1H, ddd, J = 8.4, 3.9, 3.9 Hz, H-3), 2.23 (6H, s, NMe_2), 2.12 (3H, s, 4-OAc), 2.08 (3H, s, 2-OAc), 1.17 (3H, s, Me-19), 0.92 (3H, d, J = 6.3 Hz, Me-21), 0.65 (3H, s, Me-18). *Anal.* calcd for $C_{34}H_{50}O_5N_2$: C 72.08, H 8.83, N 4.93; found C 72.00, H 8.80, N 4.88%.

Axillarine E [**3**].—Colorless crystals; mp 285–290° insoluble in $CHCl_3$, MeOH, pyridine, and Me_2CO; ir ν max (KBr) 3440, 3420, 3280, 2930, 2860, 2840, 2760, 1635, 1615, 1596, 1573, 1515, 1480, 1440, 1150, 705 cm^{-1}; ms *m/z* (%) $[M]^+$ 482, $[M-Me]^+$ 467, 248, 122, 105, 72 (100). *Anal.* calcd for $C_{30}H_{46}O_3N_2$: C 74.69, H 9.54, N 5.81; found C 74.90, H 9.35, N 5.75%.

Axillarine E diacetate.—Mp 221–224°; ir ν max (KBr) 3340, 2930, 2860, 2768, 1736, 1370, 1650, 1626, 1598, 1575, 1515, 1480, 1440, 1385, 1365, 1240, 1230, 1055, 1020, 710 cm^{-1}; ms *m/z* (%) $[M]^+$ 556, $[M-Me]^+$ 551, 495, 272, 256, 105 (10), 72 (100); ^1H nmr δ (ppm) 7.68 (2H, brd, J = 7.3 Hz, H-2′, -6′), 7.50 (1H, brdd, 7.3, 7.3 Hz, H-4′), 7.43 (2H, brdd, J = 7.3, 7.3 Hz, H-3′, -5′), 6.54 (1H, d, J = 8.4 Hz, NH), 5.35 (1H, m, H-4), 5.26 (1H, m, H-2), 4.46 (1H, ddd, J = 8.4, 3.9, 3.9 Hz, H-3), 2.25 (6H, s, NMe_2), 2.12 (3H, s, 4-OAc), 1.17 (3H, s, Me-19), 0.93 (3H, d, J = 6.4 Hz, Me-21), 0.65 (3H, s, Me-18).

Axillarine F [**4**].—Colorless crystals; mp 241–244°; $[\alpha]^{22}D$ +29.5 (c = 0.398, $CHCl_3$); ir ν max (Nujol) 3380, 3950, 2865, 1733, 1655, 1630, 1226 cm^{-1}; ms *m/z* (%) $[M]^+$ 502 (0.1), $[M-Me]^+$ 487 (0.2), 431, 416, 272, 256, 100 (48), 72 (100); ^1H nmr δ (ppm) 6.47 (1H, d, J = 8.1 Hz, NH), 6.38 (1H, brq, J = 6.6 Hz, H-3′), 5.34 (1H, m, H-4), 4.10 (1H, ddd, J = 8.1, 3.9, 3.9 Hz, H-3), 4.04 (1H, m, H-2), 2.22 (6H, s, NMe_2), 2.08 (3H, s, 2-OAc), 1.79 (3H, br s, 2′-Me), 1.72 (3H, brd, J = 6.6 Hz, 3′-Me), 1.22 (3H, s, Me-19), 0.86 (3H, d, J = 6.3 Hz, Me-21), 0.64 (3H, s, Me-18). *Anal.* calcd for $C_{30}H_{50}O_4N_2$: C 71.71, H 9.96, N 5.57; found C 71.60, H 9.79, N 5.53%. All ^{13}C nmr and DEPT see Table 1.

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