Pergamon

TETRAHEDRON

Tetrahedron 56 (2000) 3133–3138

Novel Bioactive Steroidal Alkaloids from *Pachysandra procumbens*

Leng Chee Chang, Krishna P. L. Bhat, Harry H. S. Fong, John M. Pezzuto and A. Douglas Kinghorn*

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA

Received 29 January 2000; accepted 16 March 2000

Abstract—(+)-(20*S*)-20-(Dimethylamino)-3α-(methylbenzoylamino)-11-methylene-5α-pregnane (**1**), a steroidal alkaloid with a new substitution pattern, was isolated from the entire plant of *Pachysandra procumbens*, together with four other new steroidal alkaloids, (+)-(20*S*)-3-(benzoylamino)-20-(dimethylamino)-5α-pregn-2-en-4β-ol (**2**), (+)-(20*S*)-20-(dimethylamino)-16α-hydroxy-3β-(3′α-isopropyl)-lactam-5α-pregn-4-one (**3**), (+)-(20*S*)-20-(dimethylamino)-3α-(methylbenzoylamino)-5α-pregn-12β-yl acetate (**4**), and (+)-(20*S*)-20-(dimethylamino)-3α-(methylsenecioylamino)-5α-pregn-12β-ol (**5**), as well as two known compounds, (+)-pachysamine H (**6**) and (+)-pachysandrine B (**7**). The structures of the new compounds were determined by spectroscopic methods. Compounds **1–7** were evaluated for their potential cancer chemopreventive properties using an in vitro estrone sulfatase assay. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Cancer chemoprevention involves the prevention, delay, or reversal of cancer by the ingestion of dietary or pharmaceutical agents.[1–3] As part of our current work in this area, the entire plant of *Pachysandra procumbens* Michx. (Buxaceae) was chosen for detailed investigation, since its petroleum ether- and ethyl acetate-soluble extracts showed significant activity in an antiestrogen-binding site assay, as measured by inhibition of ^{3}H-tamoxifen binding.[4] We earlier reported the first phytochemical and biological investigation on this plant, with the isolation of several new and known steroidal alkaloids as antiestrogen-binding site inhibitors being described.[4] Continued investigation of this species has resulted in the isolation of five additional new compounds, **1–5**, and two known compounds (**6** and **7**). The structural determination of **1–5** and the biological evaluation of **1–7** are reported herein.

One approach worth considering for the treatment of breast cancer involves inhibition of the enzyme estrone sulfatase.[5] This enzyme hydrolyzes estrone sulfate forming estrone, and estrone sulfate thereby serves as a reservoir for the generation of estrogens. As demonstrated with clinical breast tumor specimens,[6] relative to aromatase, another enzyme that controls estrogen levels, sulfatase levels may be 10^6-fold greater. Sulfatase can also be amplified in human mammary carcinoma cell lines.[7] Hence, sulfatase inhibitors have considerable therapeutic potential for the treatment or control of estrogen-dependent breast cancers. Accordingly, isolates **1–7** were evaluated for their antiestrogenic properties in an estrone sulfatase inhibition assay.[8]

Results and Discussion

A molecular formula of $C_{32}H_{48}N_2O$ was determined from the HREIMS of compound **1**. Comparison of its IR, ^{1}H NMR, and ^{13}C NMR data (Tables 1 and 2) with values for pachysamine H (**6**)[14] and several other recently isolated analogs from plants in the family Buxaceae indicated that **1** was a steroidal alkaloid.[10–13] The presence of a methylbenzoyl moiety in **1** [δ_C 172.7 (C=O), δ_C 137.0 (C-1′), δ_C 128.4 (C-2′ and C-6′), 126.5 (C-3′ and C-5′), and 129.0 (C-4′)] was consistent with literature values for this functionality.[14] Compounds **1** and **6** are very closely related steroidal alkaloids structurally, with the only difference being the presence of an exomethylene group (δ_C 109.4, 151.0; δ_H 4.82, 4.66) in **1**. The location of the exomethylene group was assigned by analysis of the HMQC and HMBC (Table 1) spectral data, which ruled out the possibility of this functional group occurring at either C-1, C-2, C-4, C-6, or C-12. When compared with pachysamine H (**6**),[14] the signal for C-11, found at δ_C 20.8 (t), was shifted to δ_C 151.0 (s) indicating its involvement in an olefinic bond. In addition, a downfield shift of C-8 from δ_C 35.4 to 44.0, and upfield shifts of C-9 from δ_C 54.9 to 45.4, C-12 from δ_C 39.9 to 36.8, and C-13 from δ_C 41.7 to 36.5, respectively, were

Keywords: steroidal alkaloids; *Pachysandra procumbens*; estrone sulfatase inhibition.

* Corresponding author. Tel.: +1-312-996-0914; fax: +1-312-996-7107; e-mail: kinghorn@uic.edu

0040–4020/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0040-4020(00)00232-5

Table 1. ^{1}H NMR data and HMBC correlations for **1** and **2** (300 MHz, $CDCl_3$ ppm)

^{1}H #	**1**		**2**	
	δ_H (mult.)	Carbon signal correlated	δ_H (mult.)	Carbon signal correlated
1			2.25 dd (6.0, 18.2)	C-2, C-3, C-5, C-10, C-19
2			6.00 dd (2.2, 6.0)	C-3, C-4, C-10
4			4.21 d (4.3)	C-3, C-2, C-5, C-10
18	0.73 s		0.68 s	C-12, C-13, C-14, C-17
19	1.20 s		1.06 s	C-1, C-5, C-9, C-10
20	2.67 m		2.46 m	N–Me_2, C-17
21	0.89 d (6.5)	C-17, C-20	0.89 d (6.4)	C-17, C-20
N–Me_2	2.20 s	C-20, N–Me_2	2.19 s	C-20, N–Me_2
N–Me	3.11 s	N–C=O		
N–H			7.60 s	C-2, C-4, C=O
2′	7.38 m	N–C=O, C-4′	7.80 d (7.3)	C-4′, C-3′, C=O
3′	7.38 m	C-1′, C-5′	7.48 dd (7.3, 7.3)	C-1′, C-2′, C-5′
4′	7.38 m	C-2′, C-6′	7.49 dd (7.3, 7.3)	
5′	7.38 m	C-1′, C-3′	7.48 dd (7.3, 7.3)	C-1′, C-3′, C-6′
6′	7.38 m	N–C=O, C-4′	7.80 d (7.3)	C-4′, C-5′, C=O
=CH_2	4.82 s, 4.66 s	C-9, C-11		

observed in **1**. In an HMBC experiment (Table 1), correlations were observed for the resonances at δ_H 4.82 and 4.66 (H-22) with the signals of δ_C 45.4 (C-9) and δ_C 151.0 (C-11). The stereochemistry of the C-3 substituent was assigned as α in compound **1** by comparison with pachysamine H (**6**), from the observation of signals resonating at δ_C 36.0 (C-1), 26.0 (C-2), 39.8 (C-3), 32.5 (C-4), and 42.2 (C-5), respectively.[14] Several 11-methylene steroids have been synthesized[15] and the structure of 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol has been determined by single-crystal X-ray crystallography.[16] Thus, the structure of **1** (Fig. 1) was assigned as (+)-(20*S*)-20-(dimethylamino)-3α-(methylbenzoylamino)-11-methylene-5α-pregnane. Compound **1** exhibits a new substitution pattern among steroidal alkaloids reported to date from the family Buxaceae.

Table 2. ^{13}C NMR spectral data for **1**–**5** (75.0 MHz, $CDCl_3$ ppm)

C #	1	2	3	4	5
1	36.0 t	39.8 t	36.6 t	35.8 t	35.7 t
2	26.0 t	114.5 d	27.4 t	24.9 t	25.1 t
3	39.8 d	135.3 s	58.5 d	45.8 d	39.8 d
4	32.5 t	68.9 d	207.8 s	32.8 t	32.8 t
5	42.2 d	47.3 d	56.8 d	42.0 d	42.1 d
6	29.7 t	24.6 t	20.6 t	29.0 t	29.2 t
7	31.7 t	32.3 t	30.6 t	31.7 t	31.7 t
8	44.0 d	35.8 d	34.6 d	35.3 d	34.0 d
9	45.4 d	55.1 d	53.4 d	55.2 d	54.1 d
10	34.7 s	34.4 s	42.8 s	36.1 s	35.5 s
11	151.0 s	21.1 t	21.8 t	27.1 t	25.7 t
12	36.8 t	40.0 t	40.5 t	81.0 d	78.1 d
13	36.5 s	42.0 s	42.0 s	46.1 s	49.3 s
14	57.7 d	56.9 d	54.6 d	53.0 d	51.1 d
15	25.0 t	24.5 t	35.0 t	23.7 t	23.9 t
16	27.7 t	28.0 t	72.8 d	27.9 t	28.5 t
17	47.1 d	54.7 d	59.3 d	55.9 d	54.9 d
18	12.3 q	12.6 q	14.6 q	10.5 q	9.6 q
19	12.7 q	14.7 q	14.1 q	12.7 q	12.6 q
20	61.1 d	61.6 d	57.9 d	60.0 d	62.9 d
21	11.1 q	10.3 q	10.2 q	8.8 q	15.0 q
N–Me_2	40.1 q	40.3 q	39.9 q	40.3 q	42.4 q
N–C=O	172.7 s	166.5 s	171.0 s	172.0 s	169.1 s
N–CH_3	35.4 q			34.2 q	33.0 q
Me-5′			20.5 q, 20.6 q		
Me-3′					26.8 q, 20.5 q
=CH_2	109.4 t				
1′	137.0 s	135.3 s		137.0 s	
2′/6′	128.4 d	129.1 d		128.3 d	120.2 d
3′	126.5 d	127.3 d	57.1 d	126.5 d	144.4 s
4′	129.0 d	132.1 d	42.2 t	128.9 d	
5′	126.5 d	127.3 d	28.6 d	126.5 d	
OAc				170.5 s, 21.7 q	

Compound **2** was shown to possess a molecular formula of $C_{30}H_{44}N_2O_2$ by HREIMS. An amide carbonyl (IR, ν_{max} 1660 cm^{-1}; δ_C 166.5) as well as a hydroxyl group (ν_{max} 3440 cm^{-1}(br); δ_C 68.9) functionality were present. Analysis of its ^{1}H and ^{13}C NMR data suggested that **2** was a steroidal alkaloid closely related in structure to epipachysandrine A.[13] The presence of a benzoyl moiety in **2** was indicated by the observed ^{1}H and ^{13}C NMR data (Tables 1 and 2), consistent with literature values.[11,13] The linkage of the benzoyl group to C-3 (ring A) in the molecule of **2** was suggested by spectral data comparison with axillaridine A.[11] In the ^{1}H NMR spectrum of **2**, the H-2 signal was observed at δ_H 6.00 (J=2.2, 6.0 Hz) indicating that the hydroxyl group was located at C-4 and not at C-1. This inference was supported by a HMBC experiment, which was used to provide complete assignments of the ^{1}H and ^{13}C NMR spectra of **2** (Table 2). The stereochemistry of H-4 was assigned as α based on the coupling constant at δ_H 4.21 ($J_{4\alpha,5}$=4.3 Hz), consistent with published data for the H-4α substituent ($J_{4\alpha,5}$=4.5 Hz) of vaganine B.[12] In addition, H-4α (δ_H 4.21) showed a cross-peak with H-5α [δ_H 1.55 (determined from a HMQC experiment)], but not with Me-19 (δ_H 1.06) in a NOESY experiment. On the basis of the above evidence, the structure of compound **2** (Fig. 1) was assigned as (+)-(20*S*)-3-(benzoylamino)-20-(dimethylamino)-5α-pregn-2-en-4β-ol.

Compound **3** was assigned a molecular formula of $C_{29}H_{48}N_2O_3$ by HREIMS. Its IR and ^{13}C NMR spectral data showed an amide carbonyl (IR, ν_{max} 1647 cm^{-1}; δ_C 171.0), an unconjugated ketone (ν_{max} 1750 cm^{-1}; δ_C 207.8), a four-membered non-fused β-lactam ring (ν_{max} 1720 cm^{-1}; δ_C 171.0, 57.1; δ_H 2.94, δ_C 42.2; δ_H 3.26),[4] and a *gem*-dimethyl functionality (ν_{max} 1367–1328 cm^{-1}; δ_C 20.5, 20.6; δ_H 1.01, 1.07). The ^{1}H and ^{13}C NMR spectra

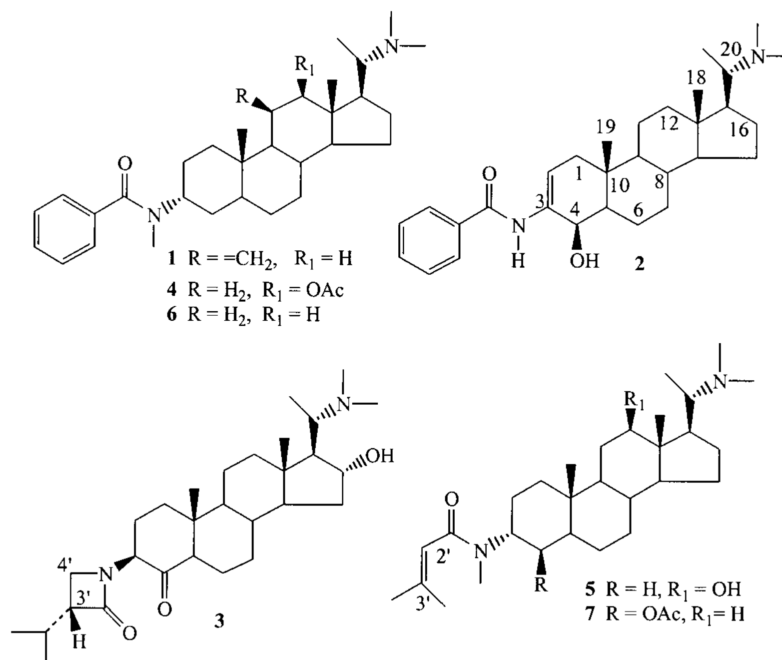

Figure 1. Structures of compounds **1**–**7**.

of **3** (Tables 2 and 3) were closely comparable to those of a model compound, (+)-(20*S*)-20-(dimethylamino)-16α-hydroxy-3-(3′α-isopropyl)-lactam-5α-pregn-2-en-one,[4] with the only difference being the absence of an olefinic proton at C-2 in **3**. The location of the OH proton at C-16 of **3** was confirmed by an HMBC experiment, in which correlations were observed for the resonance at δ_H 1.20 (H-17) with the signals at δ_C 72.8 (C-16), 42.0 (C-13), 35.0 (C-15), 57.9 (C-20), and 10.2 (Me-21). The stereochemistry of the C-16 hydroxyl group in **3** was investigated by a 1D NOE experiment. Thus, irradiation at H-16 (δ_H 4.30) gave no enhancement with H-17α, suggesting that H-16 is β. In addition, the coupling constant for H-16 (J=1.6, 6.0, 7.5 Hz) supported the presence of a 16α-OH substituent.[4] The same type of lactam ring and C-3 stereochemistry as proposed for **3** was confirmed for the model compound pachystermine A by chemical degradation and partial synthesis.[9] In addition, a cross-peak was observed between δ_H 4.05 (H-3α) and δ_H 2.05 [H-5α, from a HMQC experiment], but not with Me-19 (δ_H 0.74) in a NOESY experiment, strongly supporting the stereochemistry proposed. Thus, the structure of **3** (Fig. 1) was determined as (+)-(20*S*)-20-(dimethylamino)-16α-hydroxy-3β-(3′α-isopropyl)-lactam-5α-pregn-4-one.

The molecular formula of compound **4** was determined as $C_{33}H_{50}N_2O_3$ by HREIMS. Comparison of its ^{1}H and ^{13}C NMR data (Tables 2 and 3) with the previously mentioned

Table 3. ^{1}H NMR data for **3**–**5** and HMBC correlations for **3** and **4** (300 MHz, $CDCl_3$ ppm)

^{1}H #	**3**		**4**		**5**
	δ_H (mult.)	Carbon signal correlated	δ_H (mult.)	Carbon signal correlated	δ_H (mult.)
3	4.05 m	C-2, C-4, C-4′, N–C=O			
12			4.59 dd (4.6, 10.9)	C-11, C-17, Me-18, C-13, 170.5	3.23 dd (4.4, 10.9)
16	4.30 ddd (1.6, 6.0, 7.5)	C-17, C-13			
17	1.20 m	C-13, C-15, C-16, C-20, Me-21			
18	0.85 s	C-13, C-14, C-17	0.78 s	C-12, C-17	0.71 s
19	0.74 s	C-1, C-10, C-9	1.20 s	C-1, C-5, C-9	0.80 s
20	2.89 m	Me-21, 39.9, C-17	2.50 m	N–Me$_2$, C-17	2.21 m
21	0.91 d (6.1)	C-17, C-20	0.86 d (6.5)	C-17, C-20	1.12 d (6.5)
N–Me$_2$	2.20 s	C-20, 39.9	2.13 s	C-20, N–Me$_2$	2.33 s
N–Me			3.06 s	N–C=O	3.04 s
2′/6′			7.37 m	C-4′, C=O, C-1′	5.76 s
3′	2.94 m	N–C=O, C-4′, C-5′, 20.5	7.37 m	C-1′, C-2′, C-5′	
4′	3.26 d (3.5)	N–C=O, C-5′, C-3	7.37 m	C-3′, C-5′	
5′	2.05 m		7.37 m	C-1′, C-3′, C-6′	
Me-3′					1.80 s, 1.83 s
OAc			2.03 s	170.5	
Me-5′	1.01 d, 1.07 d (6.70)	20.5, 20.6, C-3′, C-5′			

known compound, pachysamine H (**6**)[14] indicated they were closely related steroidal alkaloids, with the only difference being the presence of an additional acetate group (IR ν_{max} 1733 cm^{-1}; δ_C 170.5, 21.7; δ_H 2.03) in **4**. The relative location of the OAc group was assigned at C-12, as opposed to C-1, C-4, or C-16, based on a HMBC experiment, in which correlations were observed for the resonance at δ_H 4.59 (H-12) with signals of δ_C 10.5 (Me-18), 27.1 (C-11), 55.9 (C-17), and 46.1 (C-13). In turn, the resonance at δ_H 0.78 (Me-18) correlated with signals at δ_C 81.0 (C-12) and 55.9 (C-17). The downfield shifts of the ^{13}C NMR signals due to C-11 (+6.3 ppm) and C-13 (+4.0 ppm) when compared with pachysamine H (**6**),[14] again indicated that the acetate group was affixed to C-12. The stereochemistry of this functionality in **4** was assigned as β based on the coupling constant for H-12 (J=10.9, 4.6 Hz) and by comparison with the model compound paxillarine B.[14,18] Thus, compound **4** (Fig. 1) was assigned as (+)-(20*S*)-20-(dimethylamino)-3α-(methylbenzoylamino)-5α-pregn-12β-yl acetate.

Compound **5** exhibited a molecular formula of $C_{29}H_{50}O_2N_2$, as determined by HREIMS. The ^1H and ^{13}C NMR spectra of **5** (Tables 2 and 3) were closely comparable to analogous data for pachysandrine B (**7**),[14] also isolated in this study, with the exception of additional signals occurring for a hydroxyl group (IR, ν_{max} 3425 cm^{-1}), with signals for an acetate group being absent. The presence of an *N*-methyl-senecioyl moiety was indicated by characteristic ^{13}C NMR resonances [δ_H 3.04 ($N-CH_3$), 1.80 and 1.83 (Me-3′), 5.76 (H-2′); δ_C 169.1 (C=O), 120.2 (C-2′), 144.4 (C-3′), 26.8 and 20.5 (Me-3′)].[14,18] The relative location of the OH group of compound **5** was determined as being at C-12 rather than at C-1, C-4, or C-16, based on analysis of the ^1H and ^{13}C NMR data.[14] This inference was confirmed by a HMBC experiment, in which correlations were observed for the resonance at δ_H 0.71 s (Me-18) with signals of δ_C 78.1 (C-12), 51.1 (C-14), and 49.3 (C-13). Also, the resonance at δ_H 3.23 (H-12) correlated with signals at δ_C 9.6 (Me-18), 25.7 (C-11), and 49.3 (C-13). The stereochemistry of H-12 was assigned as α based on the observed coupling constant value (δ_H 3.23, J=10.9, 4.4 Hz), and by comparison with the model compound, paxillarine B.[18] When compared with pachysandrine B (**7**),[14,17] the *N*-methylsenecioyl substituent of **5** was assigned as 3α, based on the signals resonating at δ_C 35.7 (C-1), 25.1 (C-5), 39.8 (C-3), 32.8 (C-4) and 42.1 (C-5).[14] Consequently, structure **5** (Fig. 1) was assigned as (+)-(20*S*)-20-(dimethylamino)-3α-(methylsenecioylamino)-5α-pregn-12β-ol.

Two known compounds, (+)-pachysamine H (**6**)[14] and (+)-pachysandrine B (**7**)[17] (Fig. 1), were also isolated, and identified by comparison with literature data.

Compounds **1**, **3**, **6**, and **7** were inactive (IC_{50} values>40 μM) as inhibitors of estrone sulfatase. On the other hand, compounds **2** and **5** (IC_{50} values of 0.11 and 0.41 μM, respectively) were more potent than the positive control danazol (IC_{50} 3.8 μM), while compound **4** (IC_{50} 5.7 μM) had comparable activity. Thus, on the basis of the in vitro data obtained, the therapeutic potential of these steroidal alkaloids is considered worthy of further investigation.

Experimental

General procedures

Mps: uncorr.; UV: MeOH; IR: film; ^1H and ^{13}C NMR, $^1H-^{13}C$ HMBC and HMQC spectra were recorded on a Bruker Avance DPX-300 and DRX-500 MHz spectrometers with TMS as internal standard; low- and high-resolution mass spectra were obtained on a Finnigan MAT-90 instrument.

Plant material

P. procumbens was obtained as seedlings from Greenwood Propagation, Hebron, IL, in April 1997, and plants were grown to maturity in a greenhouse. A voucher specimen (number PC-0398) has been deposited at the University of Illinois Pharmacognosy Field Station.

Extraction and isolation

The milled, dried plant material (5.9 kg) was extracted by maceration with MeOH–H_2O (9:1; 3×12 L). After filtration and evaporation of the solvent, the resultant extract was diluted with H_2O to afford an aqueous MeOH solution (80%), and then partitioned with petroleum ether and EtOAc, respectively, to afford dried petroleum ether- (19.5 g) and EtOAc-soluble (132.0 g) residues. The petroleum ether extract was subjected to column chromatography (Si gel) using petroleum ether–EtOAc–triethylamine mixtures in a gradient with a final wash using 100% MeOH, to afford 12 pooled fractions. Fractions 7 to 10 were combined, and eluted with gradient mixtures of petroleum ether–EtOAc–triethylamine. Fraction 17 from the initial column, eluted with petroleum ether–EtOAc–triethylamine (98:1:1), was purified further by passage over Sephadex LH-20 eluted with MeOH, resulting in the crystallization of compound **5** (8.0 mg, 0.00013%) from the mother liquor. Fraction 38 eluted with petroleum ether–EtOAc–triethylamine (95:4:1) was subjected to additional column chromatography (Si gel), using the isocratic solvent system petroleum ether–EtOAc–triethylamine (97:2:1), to afford compound **2** (10.0 mg, 0.00016%). Fraction 76 from the same column afforded compound **3** (25.0 mg, 0.00042%), by elution with petroleum ether–EtOAc–triethylamine (85:13:2). Subfraction 10, eluted with the same solvent system, was subjected to passage over Sephadex LH-20 (elution with MeOH), resulting in the crystallization of compound **4** (12.0 mg, 0.0002%).

The EtOAc-soluble fraction (132.0 g) was purified using silica gel as stationary phase and eluted with $CHCl_3$–MeOH–24% NH_4OH mixtures of increasing polarity, to afford ten pooled fractions. Fraction 4 from this first column afforded semi-pure compound **1**, by eluting with $CHCl_3$–MeOH–24% NH_4OH (98:1:1), which, by passage over Sephadex LH-20 and elution with MeOH, resulted in the crystallization of compound **1** (9.0 mg, 0.00015%) from the mother liquor. Fractions 6–8, eluted with $CHCl_3$–MeOH–NH_4OH (24%) (95:4:1), were combined and chromatographed on a silica gel column developed with $CHCl_3$–MeOH–triethylamine (93:6:1) as solvent system, to afford,

in turn, (+)-pachysamine H (6, 80.0 mg, 0.00135%) and (+)-pachysandrine B (**7**, 35.0 mg, 0.00059%).

(+)-(20*S*)-20-(Dimethylamino)-3α-(methylbenzoylamino)-11-methylene-5α-pregnane (1). Colorless needles; mp 185°C; $[\alpha]_D^{20}=+60.2°$ (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (3.86) nm; IR ν_{max} (film) 2926, 2852, 1622, 1450, 1404, 1364, 1056 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel. int. %) $[M]^+$ 476 (3), 461 (5), 446 (15), 445 (8), 433 (6), 391 (7); HREIMS *m/z* $[M]^+$ 476.3766 (calcd. for $C_{32}H_{48}ON_2$, 476.3755).

(+)-(20*S*)-3-(Benzoylamino)-20-(dimethylamino)-5α-pregn-2-en-4β-ol (2). Colorless needles; mp 189°C; $[\alpha]_D^{20}=+40.0°$ (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (3.66), 269 (3.43) nm; IR ν_{max} (film) 3440, 2927, 2841, 1712, 1660, 1558, 1445, 1059 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel. int.%) $[M]^+$ 464 (18), 449 (46), 377 (16), 314 (19), 288 (18), 272 (100); HREIMS *m/z* $[M]^+$ 464.3404 (calcd for $C_{30}H_{44}O_2N_2$, 464.3392).

(+)-(20*S*)-20-(Dimethylamino)-16α-hydroxy-3β-(3′α-isopropyl)-lactam-5α-pregn-4-one (3). Colorless needles; mp 205°C; $[\alpha]_D^{20}=+67.4°$ (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 242 (3.39) nm; IR ν_{max} (film) 3442 (br), 2934, 2875, 2783, 1750, 1720, 1647, 1450, 1367, 1328, 1259, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* (rel. int.%) $[M]^+$ 472 (7), 457 (42), 428 (41), 344 (100), 105 (56.5); HREIMS *m/z* $[M]^+$ 472.3661 (calcd. for $C_{29}H_{48}O_3N_2$, 472.3653).

(+)-(20*S*)-20-(Dimethylamino)-3α-(methylbenzoylamino)-5α-pregn-12β-yl acetate (4). Colorless needles; mp 190°C; $[\alpha]_D^{20}=+35.0°$ (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (4.26), 242 (3.76) nm; IR ν_{max} (film) 2929, 2868, 2771, 1733, 1712, 1623, 1457, 1383, 1238, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* (rel. int.%) $[M]^+$ 522 (13), 507 (12), 450 (32), 148 (100), 105 (60); HREIMS *m/z* 522.3811 (calcd. for $C_{33}H_{50}O_3N_2$, 522.3809).

(+)-(20*S*)-20-(Dimethylamino)-3α-(methylsenecioylamino)-5α-pregn-12β-ol (5). Colorless needles; mp 179°C; $[\alpha]_D^{20}=+61.4°$ (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 214 (4.35) nm; IR ν_{max} (film) 3425 (br), 2915, 2868, 1644, 1537, 1386, 1367, 1040 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* (rel. int. %) $[M]^+$ 458 (5), 443 (20), 154 (24), 136 (11), 114 (100), 105 (60); HREIMS *m/z* 458.3858 (calcd. for $C_{29}H_{50}O_2N_2$, 458.3860).

(+)-Pachysamine H (6). White powder; $[\alpha]_D^{20}=+60.4°$ (*c* 0.05, $CHCl_3$) [lit.[14] $[\alpha]_D^{25}=+66°$ ($CHCl_3$)]; IR, ^1H and ^{13}C NMR, and EIMS data consistent with literature values.[14]

(+)-Pachysandrine B (7). Colorless needles; mp 186°C; [lit.[17] 187–189°C]; $[\alpha]_D^{20}=+91.6°$ (*c* 0.06, $CHCl_3$) [lit.[17] $[\alpha]_D^{20}=+93°$ ($CHCl_3$)]; ^1H and ^{13}C NMR, and EIMS data consistent with literature values.[14]

Bioassay evaluation

All compounds were tested for their potential to inhibit estrone sulfatase by the procedure of MacIndoe et al.,[8] with slight modifications. Briefly, livers derived from female Sprague–Dawley rats were homogenized in 0.1 M Tris–acetate buffer, pH 6.5, and centrifuged at 100,000×g for 30 min at 4°C. The supernatant (cytosolic fraction) was used as the enzyme source. Duplicate reaction mixtures were prepared in 0.02 M Tris–citrate buffer, pH 6.5. Aliquots of tissue supernatants (20 μL; approximately 0.7 mg/mL protein) were mixed with [6,7-^3H] estrone sulfate (4×10^5 dpm, 3 pmol; NEN-Dupont, Boston, MA; adjusted to a final concentration of 20 μM with unlabeled estrone sulfate), with or without test agents, in a final reaction volume of 500 μL. The reaction mixture was incubated for a 2 h at 37°C. At the end of the incubation period, 400 μL aliquots were transferred to scintillation vials containing 2 mL of highly non-polar scintillation fluid (prepared by combining 666 mL dioxane, 330 mL xylene, 80 g naphthalene, and 5 g 2,5-diphenyloxazole) and 2 mL of water. This immiscible system separated the substance from the product and permitted detection of the latter. All compounds were tested initially at a concentration of 40 μM, and those showing greater than 50% inhibition were evaluated in dose-response studies. Danazol (Sigma Chemical Co., St. Louis, MO) was used as positive control.

Acknowledgements

We are grateful to Dr D. D. Soejarto and Mr R. Dvorak, College of Pharmacy, University of Illinois at Chicago, for taxonomic assistance and for the mass spectral data, respectively. Also, we thank Mr S. Totura, University of Illinois Pharmacognosy Field Station, Downers Grove, IL, for the plant cultivation, and Dr R. M. Moriarty, Department of Chemistry, University of Illinois at Chicago, for information on synthetic 11-methylene steroids. This work was supported by program project P01 CA48112, funded by the National Cancer Institute, NIH, Bethesda, Maryland.

References

1. Wattenberg, L. W. *Cancer Res.* **1985**, *45*, 1–8.
2. Sporn, M. B. *Lancet* **1993**, *342*, 1211–1213.
3. Pezzuto, J. M. *Recent Advances in Phytochemistry*; In *Phytochemistry of Medicinal Plants*, Arnason, J. T., Mata, R., Romeo, J. T., Eds.; Plenum: New York, 1995; Vol. 29, pp 19–44.
4. Chang, L. C.; Bhat, K. P. L.; Pisha, E.; Kennelly, E. J.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1998**, *61*, 1257–1262.
5. Reed, M. J.; Purohit, A.; Howarth, N. M.; Potter, B. V. L. *Drugs Future* **1994**, *19*, 673–680.
6. Santen, R. J.; Leszcynski, D.; Tilson-Mallet, N.; Feil, P. D.; Wright, C.; Manni, A.; Santner, S. J. *Ann. N. Y. Acad. Sci.* **1986**, *464*, 126–137.
7. Pasqualini, J. R.; Gelly, C.; Lecerf, F. *Breast Cancer Res. Treat.* **1986**, *8*, 233–240.
8. MacIndoe, J. H.; Woods, G.; Jeffries, L.; Hinkhose, M. *Endocrinology* **1988**, *23*, 1281–1285.
9. Kikuchi, T.; Uyeo, S. *Chem. Pharm. Bull.* **1967**, *15*, 549–570.
10. Chiu, M. H.; Nie, R.-L.; Li, Z.-R.; Zhou, J. *Phytochemistry* **1990**, *29*, 3927–3930.

11. Chiu, M. H.; Nie, R.-L.; Zhou, J. *Phytochemistry* **1992**, *31*, 2571–2572.
12. Qiu, M. H.; Li, Z.-R.; Cao, D.-Y.; Nie, R.-L. *Acta Bot. Sin.* **1993**, *35*, 885–890.
13. Atta-ur-Rahman; Khan, M. R.; Choudhary, M. I.; Iqbal, M. Z. *Phytochemistry* **1997**, *45*, 861–864.
14. Qiu, M. H.; Wang, D.-Z.; Nie, R.-L. *Chin. J. Magn. Reson.* **1995**, *12*, 155–165.
15. van den Broek, A. J.; van Bokhoven, C.; Hobbelen, P. M. J.; Leemhuis, J. *J. R. Neth. Chem. Soc.* **1975**, *94*, 35–40.
16. van Soest, T. C.; van Dijck, L. A.; Zeelen, F. J. *J. R. Neth. Chem. Soc.* **1980**, *99*, 323–325.
17. Kikuchi, T.; Uyeo, S. *Chem. Pharm. Bull.* **1967**, *15*, 207–213.
18. Qiu, M. H.; Nie, R.-L.; Li, Z.-R.; Zhou, J. *J. Acta Bot. Yunnan.* **1992**, *14*, 215–223.