DCI mass spectrometry using isobutane enabled the determination of the $[M]^+$ to be 720 when $[M+H]^+$ was recorded at 721. Although the relative abundance values are lower, the rest of the fragmentation pattern was very much in accordance with that reported by Ollis *et al.* [8] on (−)-epicatechin 3,5-digallate nonamethyl ether, viz. MS (rel. int.): 508 (7.7), 313 (7.0) and 195 (26). NOE experiments have shown an association of 11% from 5-OMe to 6-H, 11% from 3′-OMe to 2′-H and 9% from 4′-OMe to 5′-H resp. CD comparison of the 3,7-digallate was identical with that of tetra-*O*-methyl-(+)-catechin thus confirming the (2*R*,3*S*)-configuration. IR showed the presence of an ester carbonyl at 1760 cm^{-1}.

EXPERIMENTAL

IR: $CHCl_3$. ^1H NMR: 300 MHz, $CDCl_3$. MS: Kratos MS80RF for DCI.

Plant material was obtained and extracted as indicated before [1].

Nona-O-methyl-(+)-catechin-3,7-digallate. Non-crystalline, 8 mg. ^1H NMR (300 MHz, $CDCl_3$): δ5.16 H(C)-2 (*d*), δ5.51 H(C)-3 (*m*), δ3.20 H(C)-4e (*dd*), δ2.85 H(C)-4a (*dd*), δ6.49 H(A)-8 (*d*), δ6.35 H(A)-6 (*d*), δ6.94 H(B)-2′ (*d*), δ6.81 H(B)-5′ (*d*), δ6.98 H(B)-6′ (*dd*), δ7.11 3-TMB (2H, *s*), δ7.43 7-TMB (2H, *s*), δ3.80–3.86 OMe (9H, *t*), δ3.84 TMB-OMe (9H, *s*), δ3.92 TMB-OMe (9H, *s*).

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LIGNANS FROM *JUNIPERUS SABINA*

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Key Word Index—*Juniperus sabina*; Cupressaceae; leaves; lignans; methoxypodophyllotoxins; podorhizol acetate.

Abstract—Four new natural products, β-peltatin-B methyl ether, podorhizol acetate, 2′-methoxyepipicropodophyllotoxin and 2′-methoxypicropodophyllotoxin, were isolated from the lignan fraction of a *n*-hexane extract from the leaves of *Juniperus sabina*, along with picropodophyllotoxone, epipodophyllotoxin, (+)-dihydrosesamin, podorhizol, anhydropodorhizol, epipicropodophyllotoxin and 2′-methoxypodophyllotoxin.

INTRODUCTION

Juniperus sabina L. (Section sabina, Cupressaceae) is a shrub growing in the higher parts of European mountains. Its essential oil from berries or leaves is very irritant, producing inflammation of skin and mucoses and is considered abortive although it lacks a specific action on the uterus [1]. Extracts from this plants have also shown cytotoxic and cytostatic activities [2, 3], but any folk use is not recommended due to their toxicity.

In a previous report [4] we described the isolation of some new acetylated lignans, as well as some others previously known, which were the major lignan components. In this paper we report the identification of the remaining lignans isolated from the same extract, including four new compounds.

RESULTS AND DISCUSSION

The compounds identified as minor components are (+)-dihydrosesamin, picropodophyllotoxone, anhydropodorhizol, podophyllotoxin and 2′-methoxypodophyllotoxin **5**, which have been identified by direct comparison with authentic samples or by their properties described in the literature [5–9]. The new natural compounds are β-peltatin B methyl ether (**1**) podorhizol acetate (**2**), 2′-methoxyepipicropodophyllotoxin (**3**) and 2′-methoxypicropodophyllotoxin (**4**).

Compound **1** was shown to be identical with the product obtained by alkaline epimerization at position C-8 of β-peltatin A methyl ether [10, 11]. Compound **2** was identical to synthetic podorhizol acetate [12].

Compound **3** showed a $[M]^+$ at *m/z* 444 correspond-

ing to the formula $C_{23}H_{24}O_9$. Its IR spectrum, with bands for γ-lactone, aromatic rings, ethers and hydroxyl groups, was similar to the 2′-methoxypodophyllotoxin (**5**). Its ^1H and ^{13}C NMR spectra were also very similar to those of **5**, except for differences in chemical shifts of signals corresponding to H-8, H-7′, C-7 and C-10, and in the coupling constants of these protons. In the ^1H NMR spectrum of **5** H-8 appeared at 2.37 ppm as a doublet of doublets with coupling constants of 14.8 and 4.3 Hz, which corresponded to the *trans* configuration of the fused lactone ring, whereas in the case of **3** the same proton appeared at δ3.13 with coupling constants of 9.9 and 5.9 Hz, which clearly corresponded to the *cis* configuration. As expected, the coupling constant of H-7′ was smaller for **3** than for **5**, accounting for a somewhat axial disposition of the hydroxyl group in compound **3**. Furthermore the chemical shifts observed for C-7′ (64.0) and C-9 (178.1) closely agreed for the *cis* configuration of the fused lactone [5].

Compound **4** showed IR and NMR spectra similar to those of **3** and **5**. The main differences observed were the chemical shift and coupling constant of H-7′ (Table 1) which served to establish the equatorial disposition of the hydroxyl group at C-7′. Thus, compound **4**, was identified as 2′-methoxypicropodophyllotoxin.

In comparison with lignans found in *J. thurifera* [12], other species of the same section, the main differences observed are the presence of a variety of acetylated lignans and methoxypodophyllotoxins, whereas savinin, first described as a component of *J. sabina* [2] has not been detected in this study.

EXPERIMENTAL

General. Mps: uncorr. Optical rotations: $CHCl_3$. UV EtOH, λ_{max}^{EtOH} nm. IR $\nu_{max}^{CHCl_3}$ cm^{-1}. ^1H NMR (200.13 MHz) and ^{13}C NMR (50.3 MHz); $CDCl_3$ with TMS as int. std. EIMS (70 eV): *m/z*, rel. abundance (%). Flash CC was run on silica gel (Merck No 9385).

Plant material. Collected in October 1986, at Cardaño de Abajo (Palencia, Spain). Voucher specimens are deposited in the Botany Department, Faculty of Pharmacy, Salamanca. (Register No SALAF 15979).

Extraction and isolation. Juniperus sabina leaves (7 kg) were extd with *n*-hexane in a Soxhlet over 9 hr. After cooling at 0° overnight, the extract afforded an insol. fr. (360 g, 28.1% of extract) which was successively defatted with MeOH and urea-satd MeOH and later extracted with aq. 4% NaOH soln to yield a neutral for (56 g, 15.5%). This fraction (39 g) was successively chromatographed over silica gel and alumina yielding deoxypodophyllotoxin (580 mg), deoxypicropodophyllotoxin (211 mg), (−)-deoxypodorhizon (1.4 g), β-peltatin A Me ether (115 mg), acetyl epipodophyllotoxin (190 mg), acetyl epipicropodophyllotoxin (40 mg), picropodophyllotoxin (100 mg), **1** (16 mg), **2** (18 mg), picropodophyllotoxone (30 mg), **5** (60 mg), **3** (9 mg), **4** (10 mg), epipodophyllotoxin (19 mg), (+)-dihydrosesamin (28 mg), podorhizol (70 mg), anhydropodorhizol (18 mg) and epipicropodophyllotoxin (135 mg).

β-Peltatin B methyl ether (**1**). Eluted with *n*-hexane–EtOAc (7:3), mp 184° (MeOH). $[\alpha]^{24}$ (λ): +3.3° (589), +3.5° (578), +4.6° (546), +12.1° (436) (*c* 0.45). UV_{max} (ε): 209 (47 900), 282 (1600). IR: 2870, 1770, 1630, 1600, 1505, 1465, 1230, 1130, 1050, 1005, 910. ^1H NMR (Table 1). ^{13}C NMR (Table 2).

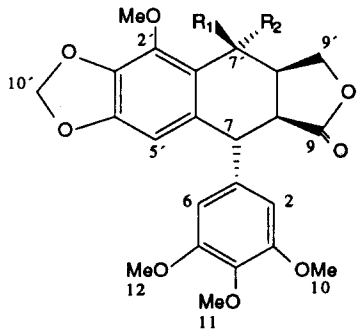
Podorhizol acetate (**2**). Eluted with CH_2Cl_2–EtOAc (9:1). $[\alpha]^{24}$ (λ): −26.9° (589), −28.9° (578), −33.8° (546), −69.3° (436)

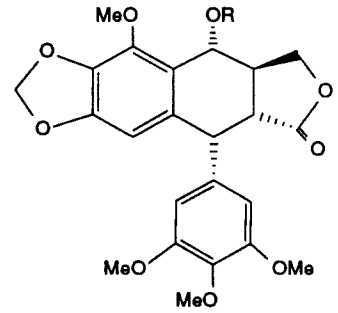
Table 1. ^1H NMR of lignans **1**, **2**, **3**, **3a**, **4**, **4a**, **5** and **5a** (δppm, *J* in Hz)

H	1	2	3	3a	4	4a	5	5a
2 and 6	6.34 *s*	6.39 *s*	6.47 *s*	6.51 *s*	6.49 *s*	6.43 *s*	6.44 *s*	6.47 *s*
7	4.34 *d* (3.0)	6.15 *d* (3.0)	4.32 *d* (5.8)	4.26 *d* (6.4)	4.38 *d* (2.2)	4.25 *d* (2.0)	4.52 *d* (4.1)	4.55 *d* (3.1)
8	3.29 *dd* (9.3, 3.0)	2.74 *dd* (6.1, 3.0)	3.14 *dd* (9.9, 5.8)	3.17 *dd* (9.9, 6.4)	3.06 *dd* (8.3, 2.0)	3.20 *dd* (8.7, 2.2)	2.73 *dd* (14.8, 4.3)	2.74–2.80 *m*
10 and 12	3.78 *s*	3.82 *s*	3.83 *s*	3.85 *s*	3.79 *s*	3.80 *s*	3.76 *s*	3.77 *s*
11	3.82 *s*	3.81 *s*	3.86 *s*	3.87 *s*	3.80 *s*	3.81 *s*	3.80 *s*	3.81 *s*
2′	—	6.25 *d* (1.7)	—	—	—	—	—	—
6′		6.33 *dd* (7.8, 1.7)		—			—	
5′	6.33 *s*	6.61 *d* (7.8)	6.07 *s*	6.01 *s*	6.33 *s*	6.41 *s*	6.29 *s*	6.32 *s*
7′a	2.78 *dd* (16.0, 6.3)	2.31 *dd* (13.9, 7.5)	5.38 *d* (3.5)	6.74 *d* (2.8)	5.00 *d* (4.1)	6.08 *d* (2.2)	5.03 *d* (8.5)	6.1 *d* (7.9)
7′b	2.65 *dd* (16.0, 5.8)	2.49 *dd* (13.9, 7.4)	—	—	—	—	—	—
8′	2.98 *m*	2.80 *m*	2.70 *m*	2.90 *m*	2.95 *m*	2.91 *m*	2.90 *m*	2.74–2.80 *m*
9′a	4.43 *dd* (9.2, 7.0)	3.98 *dd* (9.0, 5.3)	4.49 *dd* (9.3, 7.0)	4.42 *dd* (9.6, 6.9)	4.40 *dd* (9.1, 4.8)	4.40 *dd* (9.6, 6.0)	4.06 *dd* (9.7, 4.8)	4.17 *dd* (10.6, 9.0)
9′b	3.96 *dd* (9.2, 3.0)	4.31 *dd* (9.0, 7.4)	4.62 *dd* (9.3, 1.9)	4.49 *dd* (9.6, 2.0)	4.32 *dd* (9.1, 2.0)	4.48 *dd* (9.6, 2.9)	4.62 *dd* (8.7, 6.9)	4.42 *dd* (9.0, 6.5)
10	5.88 *d* (1.5)	5.90 *d* (1.6)	5.88 *d* (1.5)	5.87 *d* (1.2)	5.89 *d* (1.4)	5.93 *d* (1.4)	5.94 *s*	5.95 *d* (1.3)
	5.91 *d* (1.5)	5.94 *d* (1.6)	5.90 *d* (1.6)	5.91 *d* (1.3)	5.91 *d* (1.5)	5.94 *d* (1.4)		5.96 *d* (1.4)
MeO-2′	4.00 *s*	—	4.05 *s*	6.51 *s*	4.11 *s*	4.02 *s*	4.15 *s*	4.02 *s*
AcO	—	2.15 *s*	—	2.03 *s*	—	1.92 *s*	—	2.09 *s*

Table 2. ^{13}C NMR of lignans **1**, **2**, **3**, **3a**, **4**, **5** and **5a** (δ ppm)

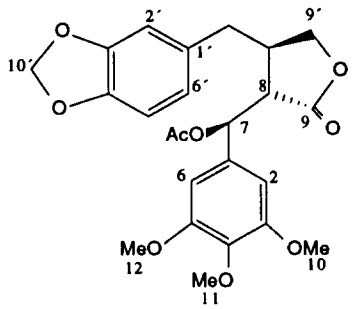
C	1	2	3	3a	4	5	5a
1	137.9	133.0	137.6	137.0	137.5	135.0	135.9
2	108.1	102.9	106.6	106.7	105.6	108.5	108.3
3	154.0	153.6	153.7	153.8	153.7	152.7	152.8
4	139.0	138.3	134.9	135.2	135.2	134.7	134.6
5	154.0	153.6	153.7	153.8	153.7	152.7	152.8
6	108.1	102.9	106.6	106.7	105.6	108.5	108.3
7	45.9	73.8	43.8	44.0	44.3	44.6	44.3
8	46.8	50.8	44.1	44.2	46.3	45.1	46.0
9	178.8	175.8	178.1	178.3	177.0	174.3	173.8
10	56.8	56.3	56.5	56.4	56.4	56.2	56.3
11	61.6	60.8	61.0	60.9	60.9	60.6	60.8
12	56.8	56.3	56.5	56.4	56.4	56.2	56.3
1′	120.7	131.1	122.5	119.5	122.2	125.1	120.8
2′	141.5	108.6	140.5	140.0	141.3	141.7	142.5
3′	136.0	148.0	139.6	139.3	139.7	137.5	137.5
4′	148.5	146.4	149.4	149.9	149.8	149.5	150.2
5′	104.8	108.1	104.1	103.6	104.2	104.3	104.1
6′	132.0	121.6	133.8	130.8	130.6	132.9	134.3
7′	24.8	39.3	64.0	65.6	67.1	70.5	70.3
8′	33.0	37.6	39.6	39.7	40.4	39.1	39.4
9′	73.6	72.1	69.4	69.2	73.0	71.8	71.9
10′	101.4	101.2	101.2	101.2	101.2	101.3	101.5
MeO-2′	60.2	—	60.2	60.1	60.0	59.8	59.6
$MeCOO^-$	—	169.0	—	170.4	—	—	170.9
$MeCOO^-$	—	20.8	—	20.0	—	—	20.9





R_1	R_2		
H	H	**1**	β-peltatin B methyl ether
OH	H	**3**	2′-methoxypipicropodophyllotoxin
OAc	H	**3a**	2′-methoxypipicropodophyllotoxin acetate
H	OH	**4**	2′-methoxypicropodophyllotoxin
H	OAc	**4a**	2′-methoxypicropodophyllotoxin acetate

R		
H	2′-methoxypodophyllotoxin	**5**
Ac	2′-methoxypodophyllotoxin acetate	**5a**



podorhizol acetate **2**

(*c* 0.69). IR: 3060, 2860, 1780, 1760, 1605, 1510, 1500, 1240, 1140, 1010, 950. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

2'-Methoxyepipicropodophyllotoxin (**3**). Eluted with CH_2Cl_2–EtOAc (9:1), mp 228° (hexane–CH_2Cl_2), $[\alpha]^{24}$ (λ): −2.8° (589), −3.4° (578), −4.0° (546) (*c* 0.5). UV λ_{max} (ε): 213 (58000), 279 (2900), 290 (2100). EIMS: 444 (100), 399 (36), 369 (28), 338 (12), 276 (28), 234 (22), 217 (23), 181 (36), 153 (20), 83 (82). IR: 3610, 2940, 2820, 1770, 1620, 1600, 1505, 1480, 1240, 1130, 1090, 1050, 1010, 915. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

Acetylation of **3** (Ac_2O–pyridine, room temp.) gave **3a**, $[\alpha]^{24}$ (λ): −20.3° (589), −22.6° (578), −25.0° (546), −47.4° (436) (*c* 0.34). UV λ_{max} (ε): 212 (55500), 275 (3800), 291 (2300). IR: 2915, 2840, 1780, 1740, 1620, 1600, 1500, 1480, 1235, 1130, 1050, 1015, 955. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

2'-Methoxypicropodophyllotoxin (**4**). Eluted with CH_2Cl_2–EtOAc (9:1). $[\alpha]^{24}$ (λ): −73.2° (589), −79.3° (578), −90.7° (546), −163.4° (436) (*c* 0.36). UV λ_{max} (ε): 221 (24800), 279 (2100), 291 (1700). IR: 3580, 2870, 2825, 1780, 1635, 1600, 1510, 1490, 1470, 1250, 1140, 1090, 1060, 1010, 970, 950. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

Acetylation of **4** (Ac_2O–pyridine, room temp.) gave **4a**, $[\alpha]^{24}$ (λ): −32.5° (589), −34.4° (578), −39.7° (546), −70.8° (436) (*c* 0.36). UV λ_{max} (ε): 209 (29700), 277 (2000), 290 (1600). IR ν 2940, 2860, 1780, 1745, 1635, 1600, 1510, 1490, 1470, 1250, 1140, 1100, 1060, 1030, 970, 920. ^{1}H NMR (Table 1).

2'-Methoxypodophyllotoxin (**5**). Eluted with $CHCl_3$–Me_2CO (19:1), mp 203° (hexane–CH_2Cl_2), $[\alpha]^{24}$ (λ): −124.2° (589), −130.9° (578), −150.0° (546) (*c* 0.86). UV λ_{max} (ε): 212 (43000), 279 (2300), 290 (2500). IR: 3560, 2940, 2900, 2840, 1780, 1620, 1595, 1505, 1480, 1250, 1130, 1100, 1045, 1000, 940, 845. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

Acetylation of **5** (Ac_2O–pyridine, room temp.) gave **5a**, $[\alpha]^{24}$ (λ): −137.9° (589), −144.7° (578), −165.7° (546), −293.9° (436) (*c* 1.27). UV λ_{max} (ε): 213 (53800), 278 (2300), 286 (1800). IR: 2940, 2900, 2840, 1780, 1740, 1620, 1595, 1505, 1480, 12545, 1130, 1000, 970, 850. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

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