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LIGNANS FROM POLAR EXTRACTS OF *JUNIPERUS THURIFERA*

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Key Word Index—*Juniperus thurifera*; Cupressaceae; methyl deoxypodophyllotoxinate; podophyllotoxinic acid; 7β-hydroxydihydrosesamin; lignans.

Abstract—Three new natural lignans, methyl deoxypodophyllotoxinate, podophyllotoxinic acid and 7β-hydroxydihydrosesamin, 11 known lignans and two cinnamyl alcohols were isolated and identified from a chloroform extract of *Juniperus thurifera* leaves. Assignment of the ^{13}C NMR spectrum of β-methyl peltatin B was also performed through direct and long-range heteronuclear 2D NMR analysis, amending previous assignments.

INTRODUCTION

In previous studies on the chemical composition of the leaves of *Juniperus thurifera* we described the isolation and characterization of a number of lignans from the *n*-hexane extract [1]. In the present work, we describe the lignans isolated from a chloroform extract obtained after depletion with *n*-hexane. The presence of lignans, substances with hitherto poorly understood biological activities in the different extracts of this plant led us to develop some activity assays with either extracts and fractions or purified substances. The hexane and chloroform extracts have both shown significant cytotoxic and cytostatic effects against KB cells together with inhibition of tubulin polymerization (San Feliciano *et al.* unpublished data).

RESULTS AND DISCUSSION

Dried and triturated leaves of *Juniperus thurifera* were extracted first with hexane and then with chloroform in a Soxhlet apparatus. From the neutral part, obtained after partitioning with basic aqueous solutions, by repeated chromatography 15 lignans were isolated and identified. Most of these were already known, viz. yatein, podorhizol, nemerosin, deoxypodophyllotoxin, deoxypicropodophyllotoxin, podophyllotoxin, picropodophyllotoxin, epipicropodophyllotoxin, β-methylpeltatin B, dihydrosesamin [1, 2] and (2*R*,3*R*)-2,3-bis-(3,4-dimethoxybenzyl)-1,4-butanodiol [3], which was isolated as its diacetate identical to ariensin. Two new lignans, methyl deoxypodophyllotoxinate (**1**) and 7′β-hydroxy-dihydrosesamin (**2**) were also isolated as their corresponding diacetates, together with 3′,4′-dimethoxy- and 3′,4′,5′-trimethoxycinnamyl alcohols. The only lignan found in the acidic part of the extract, mainly composed of diterpene acids, was podophyllotoxinic acid, which was isolated as its diacetate methyl ester **3**.

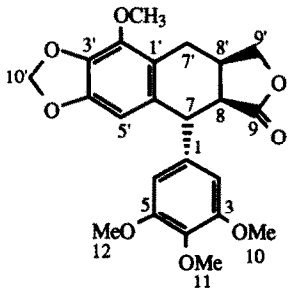
Compound **1** was an oil, with a $[M]^+$ at *m/z* 430 corresponding to the molecular formula $C_{23}H_{26}O_8$. Its ^{1}H and ^{13}C NMR (Table 1) data suggested a cyclolignan structure, with methylenedioxy and 3,4,5-trimethoxyphenyl groups presenting signals similar to those of deoxypodophyllotoxin although important differences were apparent. Thus, its IR showed a band at 1730 cm^{-1} instead of a γ-lactone band at 1780 cm^{-1}. Its ^{13}C NMR, also close to that of deoxypodophyllotoxin, showed an additional signal at δ51.38 corresponding to a methoxy group. These data, together with the signal in the ^{1}H NMR spectrum at δ3.60 assignable to the cited methoxy group, indicated the presence of a methyl ester in the molecule instead of the usual lactone group, thereby prompting us to propose structure **1** for this compound.

The *trans* configuration of substituents at C-8/C-8′ was deduced from the value of $J_{H_8, H_{8'}} = 11.4$ Hz [4]; indirect confirmation of the structure was achieved through synthesis of its 8-epimer **4**, which showed a value of $J_{H_8, H_{8'}} = 3.7$ Hz. Attempts to synthesize **1** from podophyllotoxin were unsuccessful due to the tendency of the latter to epimerize at C-8. Compound **4**, on standing, progressively lactonized to deoxypicropodophyllotoxin, whereas **1** was stable. Additionally, reduction of **1** with $LiAlH_4$ yielded the diol **5**, whose spectroscopic data (Tables 1 and 2) were consistent with those of the reduction product of podophyllotoxin, once again confirming the *trans* stereochemistry of the new compound at C-8/C-8′. Compound **1** cannot be an artefact, because it is well known that deoxypodophyllotoxin in methanol epimerizes to deoxypicropodophyllotoxin even in the absence of acid or base.

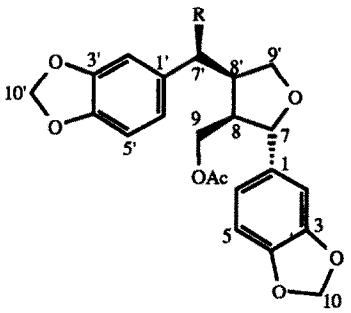
Compound **2** isolated after acetylation was also an oil with a $[M]^+$ at *m/z* 456 corresponding to the molecular formula $C_{24}H_{24}O_9$. Its IR spectrum showed bands for acetate (1740 cm^{-1}), aromatic rings (1620, 1505 cm^{-1}) and methylenedioxy groups (2865, 935 cm^{-1}). These data, together with the study of its NMR spectra (Tables 1 and 2), led us to propose a monoepoxylignan structure for this compound. Both spectra displayed signals very similar to those shown by dihydrosesamin acetate (**9**). However, **2** showed an additional secondary acetate, which was deduced by the presence in the ^{1}H spectrum of a methyl singlet at δ1.99 and a one proton doublet at δ5.67 as well as signals for two acetate groups in the ^{13}C NMR spectrum. The location of the second acetate group at the benzyl position 7′ became obvious and the configuration

Table 1. ^{1}H NMR spectral data for compounds **1**–**7** (200 MHz)

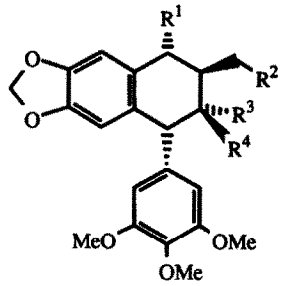
H	1	1a	2	3	4	5	6	7
2	6.13 *s*	6.09 *s*	6.74–6.87 *m*	6.34 *s*	6.26 *s*	6.24 *s*	6.36 *s*	6.34 *s*
5	—	—		—	—	—	—	
6	6.13 *s*	6.09 *s*		6.34 *s*	6.26 *s*	6.24 *s*	6.36 *s*	6.34 *s*
7	4.38 *d* (5.7)	4.33 *d* (5.8)	4.52 *d* (8.0)	4.37 *d* (5.1)	4.36 *d* (6.4)	4.09 *d* (5.1)	4.67 *d* (5.1)	4.34 *d* (3.0)
8	3.00 *dd* (11.4, 5.7)	2.96 *dd* (5.8, 10.9)	2.34 *m*	3.19 *dd* (13.2, 5.2)	3.07 *dd* (6.4, 3.7)	2.11 *m*	2.80 *m*	3.29 *dd* (9.3, 3.0)
9a	—	—	4.26 *dd* (11.2, 4.6)	—	—	3.49 *m*	3.48 *m*	—
9b	—	—	4.10 *dd* (11.2, 6.7)	—	—	—	—	—
10	3.75 *s*	3.76 *s*	5.96 *s*	3.77 *s*	3.77 *s*	3.75 *s*	3.79 *s*	3.78 *s*
11	3.80 *s*	3.78 *s*	—	3.80 *s*	3.82 *s*	3.80 *s*	3.84 *s*	3.82 *s*
12	3.75 *s*	3.76 *s*	—	3.77 *s*	3.77 *s*	3.75 *s*	3.79 *s*	3.78 *s*
2′	6.63 *s*	6.58 *s*	6.74–6.87 *m*	6.73 *s*	6.58 *s*	6.61 *s*	6.63 *s*	—
5′	6.40 *s*	6.36 *s*		6.43 *s*	6.36 *s*	6.36 *s*	6.56 *s*	6.33 *s*
6′	—	—		—	—	—	—	—
7′a	3.08 *d* (5.8)	2.66 *dd* (10.6, 16.0)	5.67 *d* (9.3)	6.27 *s*	2.74 *dd* (5.4, 16.7)	2.80 *m*	4.38 *d* (3.4)	2.78 *dd* (16.0, 6.3)
7′b	—	2.98 *dd* (5.8, 16.0)	—	—	2.94 *dd* (7.6, 16.7)	—	—	2.65 *dd* (16.0, 5.8)
8′	2.44 *m*	2.55 *m*	2.60 *m*	2.67 *m*	2.41 *m*	2.11 *m*	2.65 *m*	2.98 *m*
9′a	3.68 *dd* (6.4, 4.5)	4.09 *dd* (3.0, 10.8)	3.78 *dd* (9.7, 5.3)	4.14 *dd* (11.6, 2.3)	3.70 *m*	3.64 *m*	3.70 *m*	3.96 *dd* (9.2, 3.0)
9′b	—	4.18 *dd* (5.4, 10.8)	3.70 *dd* (16.8, 7.2)	4.26 *dd* (11.6, 3.6)	—	—	—	4.43 *dd* (9.2, 7.0)
10′a	5.82 *s*	5.85 *s*	5.95 *s*	5.92 *s*	5.85 *d* (1.4)	5.86 *s*	5.92 *d* (1.4)	5.88 *d* (1.5)
10′b	—	—	—	—	5.88 *d* (1.4)	—	5.90 *d* (1.4)	5.91 *d* (1.5)
2′-OMe	—	—	—	—	—	—	—	4.00 *s*
9-OAc	—	—	2.05 *s*	—	—	—	—	—
7′-OAc	—	—	1.99 *s*	2.03 *s*	—	—	—	—
9′-OAc	—	—	—	2.16 *s*	—	—	—	—
CO_2Me	3.60 *s*	3.51 *s*	—	3.60 *s*	3.63 *s*	—	—	—



7



2 R=OAc
9 R=H



1 R^1=H, R^2=OH, R^3=COOMe, R^4=H
1a R^1=H, R^2=OAc, R^3=COOMe, R^4=H
3 R^1=OAc, R^2=OAc, R^3=COOMe, R^4=H
4 R^1=H, R^2=OH, R^3=H, R^4=COOMe
5 R^1=H, R^2=OH, R^3=CH_2OH, R^4=H
6 R^1=OH, R^2=OH, R^3=CH_2OH, R^4=H

of the new chiral centre was deduced from the value of the coupling constant $J_{H_{7'},H_{8'}}$ = 9.3 Hz. This implied a predominantly *anti* conformation for these two protons. After detailed study of Dreiding models for both epimers at 7′, it was found that for the 7′*R* epimer the most stable conformation corresponded to values for the coupling constant of $J_{H_{7'},H_{8'}}$ = 8–10 Hz and for the 7′*S* the conformation corresponds to values of $J_{H_{7'},H_{8'}}$ = 4–6 Hz; this led us to assign structure **2** for this compound.

From the acidic part extracted with aqueous sodium bicarbonate, after acetylation and methylation with diazomethane, only lignan **3** was isolated, whose spectroscopic data indicated a cyclolignan structure. Its EI mass spectrum with $[M]^+$ at *m/z* 530 corresponded to the molecular formula $C_{27}H_{30}O_{11}$. Its IR, ^{1}H NMR and ^{13}C NMR (Tables 1 and 2) spectra were very similar to those of methyl deoxypodophyllotoxinate acetate, isolated from the neutral fraction. Compared to **1a**, compound **3** showed more intense acetate bands in its IR spectrum and the mass spectrum displayed the loss of two acetic acid molecules, indicating the presence of two acetate groups. This was confirmed by the presence in the ^{1}H NMR spectrum of two singlets at *ca* 2.1 ppm. The location of the second acetate group at 7′ was deduced from the presence of a methine signal at *ca* δ71 in the ^{13}C NMR spectrum and the absence of the signal corresponding to the methylene carbon in the ^{13}C NMR spectrum (δ32.2), as well as those of its protons in the ^{1}H NMR spectrum (δ2.16 and 2.03). The configuration of C-7′ was established considering there was no coupling in the signal corresponding to H-7′; this observation can only be explained if this proton is *trans* to H-8′. The structure of methyl diacetylpodophyllotoxinate was thus established for compound **3** and confirmed by hydride reduction of **3** and podophyllotoxin, both of which gave the same triol **6** (Tables 1 and 2). Podophyllotoxinic acid, the corresponding natural product, has not been described before as a component of plants, but its natural character is certain because it cannot be obtained under acidic or basic conditions from podophyllotoxin.

Complementary to the isolation of the above products, the unambiguous assignment of the ^{13}C NMR spectra of some compounds was made by direct H–C and long-range H/C 2D NMR correlations. These particular experiments allowed us to assign spectra (Tables 1 and 2) for β-methylpeltatin B (**7**), amending the previously published [5] ^{13}C NMR data. In addition, we were able to ascertain that in the *cis*-lactone series, the chemical shifts for carbon atoms at positions 1 and 4 are, respectively, δ139.0 and 137.9, contrary to the assignments performed by us for compounds of the *trans*-lactone series.

EXPERIMENTAL

General. Mps are uncorr. Optical rotations were measured in $CHCl_3$. UV spectra were recorded in EtOH, IR spectra in $CHCl_3$. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were measured in $CDCl_3$ with TMS as int. std; δ values are expressed in ppm. EIMS were obtained at 70 eV. Flash CC was run on silica gel.

Plant material, extraction and isolation. Juniperus thurifera was collected in November at Prádena (Segovia, Spain). Voucher specimens are deposited at the Botany Department, Faculty of Pharmacy, Salamanca (register number SALAF No. 15980).

Leaves (4.6 kg) were extracted in a Soxhlet first with hexane and then with $CHCl_3$. The $CHCl_3$ extract was partitioned between a 4% aq. soln of NaOH obtaining an acidic part (76.8 g) and a neutral part (46.1 g), which were successively defatted with MeOH and a satd soln of urea in MeOH. Once defatted (15.3 g, 12.4%), the neutral part was chromatographed over silica gel with hexane–EtOAc mixts of increasing polarity yielding: yatein (0.6 g), podorhizol (0.2 g), nemerosin (0.1 g), deoxypodophyllotoxin (0.2 g), deoxypicropodophyllotoxin (1.1 g), picropodophyllotoxin (3.0 g), epipicropodophyllotoxin (0.2 g), β-methylpeltatin B (20 mg), dihydrosesamin (0.2 g) and (2*R*,3*R*)-2,3-bis-(3,4-

Table 2. ^{13}C NMR spectral data for compounds 1–7

C	1	1a	2	3	4	5	6	7
1	137.88	137.63	130.30	135.91	137.23	137.20	136.87	139.0
2	107.41	107.20	106.93	107.12	106.78	107.59	107.04	108.1
3	152.96	152.91	148.18	153.03	153.25	152.88	153.22	154.0
4	137.88	148 31	148.18	137.66	140.93	138.60	138.87	137.9
5	152.96	152.91	108.19	153.03	153.25	152.88	153.22	154.0
6	107.41	107.20	120.13	107.12	106.78	107.69	107.04	108.1
7	48.64	47.67	85.33	46.43	49.68	43.55	47.49	45.9
8	47.91	47.67	50.41	47.84	46.07	48.66	48.17	46.8
9	173.72	172.52	64.49	171.40	174.44	65.36	68.92	178.8
10	56.34	56.28	101.32	56.20	56.37	56.33	56.36	56.8
11	60.84	60.80	—	60.84	60.82	60.85	60.83	61.6
12	56.34	56.28	—	56.20	56.37	56.33	56.36	56.8
1′	128.58	128.01	133.33	127.91	128.28	129.01	129.96	120.7
2′	108.00	107.87	108.33	107.34	108.36	109.32	109.93	141 5
3′	146.80	146.37	155.69	147.98	146.30	146.10	147.67	136.0
4′	146.37	146.81	145.69	147.56	146.48	146.41	146.25	148.5
5′	109.92	109.27	107.42	108.66	109.77	107.97	108.00	104.8
6′	130.27	129.97	121.20	131.67	129.82	131.85	132.26	132.0
7′	32.23	31.87	77.05	71.02	30.97	33.00	77.03	24.8
8′	33.19	30.18	47.93	36.04	35.97	34.98	43.25	33.0
9′	65.98	66.69	69.58	63.06	63.90	64.94	60.21	73.6
10′	100.86	100 86	101.32	101.34	100.80	100.70	100.99	101.4
2′-OMe	—	—	—	—	—	—	—	60.2
9-CO-Me	—	—	181.31	—	—	—	—	—
9-CO-Me	—	—	20.24	—	—	—	—	—
7′-CO-Me	—	—	170.77	171.02	—	—	—	—
7′-CO-Me	—	—	20.72	21.29	—	—	—	—
9′-CO-Me	—	170.98	—	170.61	—	—	—	—
9′-CO-Me	—	20.81	—	20.75	—	—	—	—
CO_2Me	51.38	51.33		51.55	51.77	—	—	—

dimethoxybenzyl)-1,4-butanodiol diacetate (2 mg), 3′,4′-dimethoxycinamyl alcohol (60 mg), 3′,4′,5′-trimethoxycinamyl alcohol (70 mg), **1** (90 mg) and **2** (5 mg).

Methyl podophyllotoxinate (**1**). Oil. eluted with CH_2Cl_2–EtOAc. $[\alpha]^{23}(\lambda)$ −72.5° (589); −75.0° (578); −90.0° (546); −106.25° (436) (EtOH; *c* 0.1%). EIMS *m/z* (rel. int.): 430 (87), 429 (51), 414 (19), 398 (16), 397 (11), 353 (16), 352 (13), 340 (13), 339 (59), 338 (49), 324 (11), 283 (33), 282 (39). UV λ_{max} (ε): 215 (20215), 289 (3750). IR ν cm^{-1}: 3620, 3460, 1730, 1600, 1510, 1490, 1240, 1130, 1050, 1010, 945. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2). Acetate (**1a**). Oil. IR: 1780, 1740, 1600, 1505, 1485, 1240, 1130, 1045, 1005, 945 cm^{-1}. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2).

Compound **9** (30 mg) in 5 ml of 0.25% KOH in MeOH was stirred for 10 min at room temp. After neutralization and extraction with EtOAc the reaction mixt. was methylated to afford 25 mg of Me deoxypicropodophyllotoxinate (**4**). Oil. IR ν cm^{-1}: 3320, 1730, 1600, 1510, 1240, 1135, 1050, 1010, 950. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2).

$LiAlH_4$ *reduction of compound* **1**. To an Et_2O soln of **1** (25 mg), $LiAlH_4$ (30 mg) was added and the mixt. stirred for 4 hr. Usual work-up of the reaction mixt. afforded 24 mg of **5**, identical to the reduction product obtained from deoxypodophyllotoxin. IR νcm^{-1}: 3600, 1600, 1510, 1470, 1240, 1130, 1040, 1010, 940. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2).

7β-Hydroxydihydrosesamin diacetate (**2**). Oil, eluted with CH_2Cl_2–EtOAc. UV λ_{max} (ε): 284 (15300), 235 (23800), 205 (83650). EIMS *m/z* (rel. int.): 456 (11), 336 (5), 202 (10), 187 (11), 161 (31), 151 (42), 150 (20), 149 (84), 134 (30). IR ν cm^{-1}: 2865, 1740, 1620, 1505, 1455, 1390, 1260, 1060, 935. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2).

From the acidic part, after acetylation and methylation, by standard methods with Ac_2O–pyridine and CH_2N_2–Et_2O, respectively, repeated CC yielded 25 mg of **3**.

Methyl podophyllotoxinate diacetate (**3**). Oil eluted with *n*-hexane–EtOAc. $[\alpha]^{23}(\lambda)$, −164,0° (589); −156,0° (578); −133,0° (546); −131° (436) (*c* 0,8%). UV λ_{max} (ε): 288 (3 000), 207 (34 600). EIMS *m/z* (rel. int.): 530 (6), 470 (1), 410 (4), 364 (6), 351 (5), 350 (18). IR ν cm^{-1}: 1735, 1590, 1505, 1240, 1130, 1045, 935 cm^{-1}. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2).

$LiAlH_4$ *reduction of compound* **3**. To an Et_2O soln of **3** (25 mg), $LiAlH_4$ (30 mg) was added and the mixt. stirred for 3 hr. Usual work-up afforded 24 mg of **6**, identical to the reduction product of podophyllotoxin. IR: 3620, 1600, 1510, 1240, 1130, 1045, 1010, 995 cm^{-1}. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2).

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REFERENCES

1. San Feliciano, A., Medarde, M., Lopez, J. L., Puebla, P., Miguel del Corral, J. M. and Barrero, A. F. (1989) *Phytochemistry* **28**, 2863.
2. San Feliciano, A., Miguel del Corral, J. M., Lopez, J. L. and Pascual-Teresa, B de. (1990) *An. Quim.* **86**, 561.
3. Hernández, J. D., Román, L. U., Espiñeira, J. and Joseph-Nathan, J. (1983) *Planta Med.* **47**, 215.
4. Hartwell, J. L. and Schrecker, A. W. (1953) *J. Am. Chem. Soc.* **75**, 5916.
5. San Feliciano, A., Miguel del Corral, J. M., Gordaliza, M. and Castro, M. (1990) *Phytochemistry* **29**, 1335.