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LIGNANS FROM *JUNIPERUS THURIFERA*

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Key Word Index—*Juniperus thurifera*, Cupressaceae, deoxypicropodophyllotoxin; (-)*epi*-podorhizol; lignans.

Abstract—The isolation and identification of two new natural lignans, (-)*epi*-podorhizol and deoxypicropodophyllotoxin, and 12 known lignans from a hexane extract of *Juniperus thurifera* is described.

INTRODUCTION

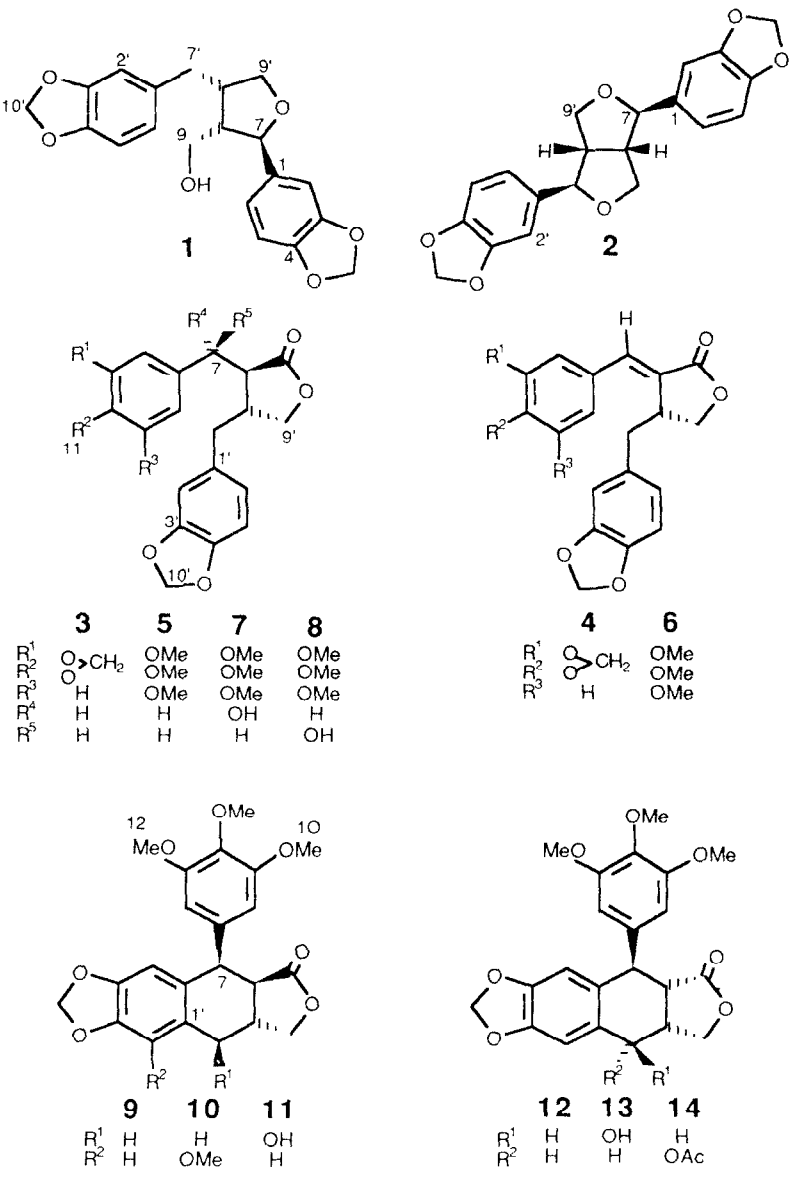
Juniperus thurifera is a tree of variable size native to mountainous zones of the Mediterranean area with cold winters. It is sporadically distributed throughout the western Mediterranean and appears in three varieties: *africana* Maire (in Morocco and Algeria), *hispanica* Mill (Spain) and *gallica* Coiny (in France and Corsica). It belongs to the section *sabina* and in Spain it is known as 'Cedro de España', 'Sabina albar', 'Sabina turifera', and 'Sabina española'.

Crude extracts from leaves have been tested on neoplastic KB celules, showing an appreciable cytostatic activity. They also show a remarkable inhibition on tubulin polymerization (unpublished observations), which is in agreement with other data in the literature reporting these types of activities for some lignans [1, 2].

In the present work, we report the identification of lignans in the hexane extract from *Juniperus thurifera* var. *hispanica* Mill. In previous papers, we have described other components of the plant: phenylpropane derivatives [3], coumarins [4], monoterpenoids [5], sesquiterpenoids and diterpenoids [6], and some two-dimensional NMR studies carried out some lignans [7, 8] and coumarins [4].

RESULTS

Chromatographic separation of the cold insoluble part of the hexane extract afforded 14 lignans with varied structures: (−)-dihydrosesamin(**1**) [9], (−)sesamin (**2**) [10, 11], (−)-hinokinin (**3**) [12, 13], (−)-balactone (**4**) [14, 15], (−)-deoxypodorhizon (**5**) [16–18], nemerosin (**6**) [19, 20], podorhizol (**7**) [19, 20], (−)-*epi*-podorhizol (**8**), deoxypodophyllotoxin (**9**) [21, 22], β-peltatin-A-methylether (**10**) [23], podophyllotoxin (**11**) [21, 22], deoxypicropodophyllotoxin (**12**), picropodophyllotoxin



(**13**) [21, 22] and acetyl-epipicropodophyllotoxin (**14**) [24] Compounds **8** and **12** are new natural products The identification of these substances was performed by spectroscopic techniques, and certain chemical transformations. The identity of the substances was confirmed by direct comparison with authentic samples or through their properties described in the literature Some ^{13}C NMR data previously undescribed [25] are included in this paper The assignment of ^{1}H and ^{13}C signals is based on previous results [7, 8] obtained for some two dimensional NMR correlations performed on compounds **4, 5** and **12**.

Compound **8** is an isomer of **7** Its mass spectrum shows the molecular ion at *m/z* 416 corresponding to the molecular formula $C_{22}H_{24}O_8$ Its IR spectrum, with bands of γ-lactone, aromatic rings, ethers and a hydroxyl group, is practically identical to that of podorhizol Its ^{1}H NMR and ^{13}C NMR spectra are also very similar to those of podorhizol, except for differences in the chemical shifts of the signals corresponding to H-7 and C-7 (δ 4 80 and 74.74 respectively) and their environment, which suggests that the substance is an epimer of podorhizol at C-7 This is confirmed by the splitting pattern of the signal corresponding to the proton geminal to the hydroxyl, by the value of its coupling constant (*d*, J = 7 7 Hz), and by the deshielding ($\Delta\delta$2 64) of the carbon atom supporting it Its physical and spectroscopic properties match those reported for synthetic **8** [26]

Compound **12** shows $[M]^+$ at *m/z* 398. Its spectroscopic properties suggest a lactonic cyclolignan, with trimethoxyphenyl and methylendioxyphenyl groupings, very similar to that of deoxypodophyllotoxin (**9**) The main differences between the ^{1}H and ^{13}C NMR spectra of **9** and **12** are restricted to the γ-lactone signals, this fact denotes a change towards the *cis* junction of the lactonic ring Compound **12** was thus identified as deoxypicropodophyllotoxin, and was obtained as the single product from basic treatment of **9** Compound **12** previously

obtained by epimerization of **9** [27] or described as an artefact [28], is in this case present in the extract, as deoxypodophyllotoxin subjected to the same procedure as the crude extract under stronger conditions and over longer periods of time was recovered unchanged, whereas treatment under epimerization conditions (KOH–MeOH 10%) quantitatively produced **12**.

EXPERIMENTAL

General Mps uncorr. Optical rotations were measured in $CHCl_3$. ^1H NMR (200 13 MHz) and ^{13}C NMR (50 3 MHz) spectra were measured in $CDCl_3$ with TMS as int standard EIMS were obtained at 70 eV. Flash chromatography was performed on silica gel (Merck No 9385)

Plant material, extraction and isolation The plant material was collected in September, at Prádena (Segovia, Spain) Voucher specimens are deposited in the Botany Department, Faculty of Biology, Salamanca (register number SALA No 7193)

J thurifera leaves (15 kg) were extracted with hexane, and the resulting extract cooled at 0° overnight to give 615 g (34 0 % over whole extract) of insoluble fraction, which was successively defatted with MeOH and a saturated solution of urea in MeOH and fractionated with aq. 4% NaOH. The neutral part (130 g 7 1%) was chromatographed over silica gel with hexane–EtOAc mixtures of increasing polarity, yielding **1** (1 9 g), **2** (0 5 g), **3** (0 7 g), **4** (0.5 g), **5** (23.0 g), **6** (0.4 g), **7** (0 9 g), **8** (0 3 g), **9** (0 05 g), **10** (0.04 g), **11** (0.02 g), **12** (14 8 g), **13** (2 5 g), **14** (0 4 g)

Acetylation of **7** (50 mg) (Ac_2O–pyridine, room temp) gave **7a**, $[\alpha]^{23}(\lambda) = -33\,1°$(589), $-35\,2°$ (578), $-41\,1°$ (546) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3020, 2780, 1780, 1760, 1595, 1510, 1490, 1230, 1120, 925, ^1H NMR δ 2 15 (3H, *s*, OAc), 2.74 (1H, *dd*, $J = 3\,0, 3\,0$, H_8), 2 80 (1H, *m*, H_8'), 2 31 (1H, *dd*, $J = 13\,9, 7\,5$, $H_{7a'}$) 2 49 (1H, *dd*, $J = 13\,9, 7\,4$, $H_{7b'}$), 3 81 (3H, *s*, H_{11}), 3 82 (6H, *s*, H_{10}, H_{12}), 3 98 (1H, *dd*, $J = 9\,0, 5\,3$, $H_{9a'}$), 4 31 (1H, *dd*, $J = 9\,0, 7\,4$, $H_{9b'}$), 5 90 (1H, *d*, $J = 1\,6$, $H_{10a'}$), 5 94 (1H, *d*, $J = 1\,6$, $H_{10b'}$), 6 25 (1H, *d*, $J = 1\,7$, $H_{2'}$), 6 33 (1H, *dd*, $J = 7\,8, 1\,7$, $H_{6'}$), 6 39 (2H, *s*, H_2, H_6), 6 61 (1H, *d*, $J = 7\,8$, $H_{5'}$); ^{13}C NMR Table 1 Treatment of **7a** (50 mg) with 5% KOH–MeOH (0.5 ml) gave 45 mg of reaction mixture, which on flash chromatography afforded **6** (35 mg)

epi-*Podorhizol* (**8**). Colourless crystals, mp 129–130° (CH_2Cl_2–hexane) $[\alpha]^{23}$ (λ) = $-20\,8°$ (589), $-21\,9°$ (578), $-26\,3°$ (546), $-29\,5°$ (436), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3500, 3010, 2780, 1760, 1600, 1510, 1490, 1230, 1120, 925, EIMS *m/z* (rel int) 416 (46), 397 (24), 367 (3), 263 (2), 237 (2), 236 (2), 221 (12), 197 (100), 194 (32), 169 (37), 135 (76), 77 (37), ^1H NMR Table 2; ^{13}C NMR Table 1.

Deoxypicropophyllotoxin (**12**) Colourless crystals, mp 172–173° (MeOH) $[\alpha]^{23}(\lambda) = +27\,3°$ (589), $+28\,5$ (578), $+33\,3°$ (546), $+167\,5°$ (436), $+140\,0°$ (365), UV λ_{max}^{MeOH} nm (ε) 217 (10 700), 294 (2 200), EIMS *m/z* (rel. int) 398 (98), 367 (43), 353 (20), 329 (37), 283 (54), 282 (36), 252 (29), 251 (13), 250 (13), 249 (25), 229 (91), 185 (100), 181 (67), 168 (80), 153 (84); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3010, 2780, 1780, 1600, 1510, 1490, 1240, 1050, 940, 875, 840, ^1H NMR: Table 2, ^{13}C NMR, Table 1 Treatment of deoxypodophyllotoxin (9) (105 mg) with 10% KOH/MeOH (0 5 ml) at room temp followed by acid with 2M HCl, neutralization and extraction gave 102 mg of **12**

Table 1. ^{13}C NMR (50.3 MHz) spectra of lignans **6**, **7**, **7a**, **8**, **10** and **12**

C*	6	7	7a	8	10	12
1	129.50	136 79	132 92	135.78	136 29	138 29
2	107 78	102.61	102.66	104 11	108 89	105 60
3	153.71	153 42	153 53	153 77	152 69	153 52
4	140 28	137 60	138 06	138 68	137 63	137 37
5	153 51	153.42	153 53	153 77	152 69	153 52
6	107 78	102 61	102 66	104 11	108 89	105 60
7	137 65	72 10	73 66	74 74	43 99	45 41
8	127 25	52 83	50 75	51 69	47 43	46 27
9	172.21	178 37	175 80	178 66	175 04	178 23
10	56 41	56.20	56 17	56 04	56 49	56 38
11	60 94	60 84	60 73	60 97	60 77	60 79
12	56 41	56 20	56 17	56.04	56 49	56 38
1′	131 36	131.55	131 05	131 59	121 10	128 41
2′	109.07	108.62	108 49	108 75	140 91	108 84
3′	148 07	147 88	147 90	148 11	134.63	146.98
4′	146 70	146 33	146 41	146 56	148 42	146.86
5′	108 48	107.95	107 97	108 42	104 56	109 84
6′	121 91	121 54	121 53	121 49	131 82	130 67
7′	37 89	39 42	39 15	38 35	27 58	32 07
8′	39 56	36.40	37 52	39 86	32 55	33 05
9′	69 75	72 85	72 03	71 97	72 40	72 75
10′	101 11	101 14	101 14	101.14	101.05	101 00
11′					59 41	
CO-Me			168 96			
CO-Me			20 29			

$CDCl_3$, TMS as int standard
*Numbering is that used in ref. [29].

Table 2. ^{1}H NMR (200 0 MHz) spectra of lignans **8** and **12**

H	8	12
2,6	6 65 *s*	6 35 *s*
7	4 80 *d* (7 7)	4 36 *d* (3 1)
8	2 61 *dd* (9 0, 7.7)	3 36 *dd* (9 5, 3 1)
10,12	3 88 *s*	3 78 *s*
11	3 83 *s*	3 82 *s*
2′	6 34 *d* (1 7)	6 66 *s*
5′	6 65 *d* (8 3)	6 58 *s*
6′	6.34 *dd* (8 3, 1.7)	—
7′	2 17 *dd* (13.8, 3.0)	2 47 *dd* (15 3, 5 4)
	2 26 *dd* (13 8, 5 0)	2 85 *dd* (15 3, 6 3)
8′	2.48 *m*	3 02 *m*
9′	3 81 *dd* (9 2, 5 5)	3 96 *dd* (9 2, 3 1)
	4.15 *dd* (9 2, 7 6)	4 42 *dd* (9 2, 7 3)
10′	5 91 *s*	5 89 *d* (1 4)
	5 94 *d* (1 6)	5 93 *d* (1 4)

$CDCl_3$, TMS as int. standard

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