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ACIDIC AND PHENOLIC LIGNANS FROM *JUNIPERUS SABINA*

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Key Word Index—*Juniperus sabina*; Cupressaceae; leaves; lignans; junaphtoic acid; (−)-3-*O*-demethylyatein.

Abstract—Along with some known compounds, two new lignans, a naphthalene derivative named junaphtoic acid and (−)-3-*O*-demethylyatein, were isolated from the acidic fraction of a *n*-hexane extract from the leaves of *Juniperus sabina*. Their structures were established by spectroscopic and chemical means.

INTRODUCTION

Juniperus sabina is a mountain shrub whose essential oil is an abortive [1] and whose extracts have shown cytotoxic and cytostatic activities [2, 3]. In previous reports we described the isolation and structural determination of neutral lignans from a *n*-hexane extract [4, 5] as well as those other compounds [6, 7]. We now report studies on the acidic fractions of the same extract and the structural assignment of junaphtoic acid (**1**) and demethylyatein (**2**), which are new natural products.

RESULTS AND DISCUSSION

Junaphtoic acid (**1**) was initially isolated as its acetate (**1a**) whose mass spectrum showed $[M]^+$ at *m/z* 454 which, together with data from DEPT and BB ^{13}C NMR spectra (showing the presence of 5 methyl groups, 1 methylene, 4 methines and 14 non-protonated carbon atoms) were in agreement with the molecular formula of $C_{24}H_{22}O_9$ for **1a**. The IR spectrum of **1a** showed absorptions at 3500–2500 (br.) and 1710 (CO_2H), 1760 and 1245 cm^{-1} (phenolic acetate), as well as those of aromatic rings. The naphthalene nature of the compound was indicated by the molecular formula and from the presence of 14 different signals assignable to aromatic carbons in the ^{13}C NMR spectrum, two of them showing *ca* double intensity as compared with the others. The free carboxyl group (δ172.6), the methyl absorbing at high field (δ13.5), the methylenedioxy moiety (δ101.5) and the three methoxy groups ($2\times\delta$56.1 and 60.8) arranged on positions 3, 4 and 5 of the phenyl group suggested structure **1a** for the compound, which was supported by ^1H NMR data (Table 1), UV absorption maxima at 212, 248 and 245 nm, and the fragmentation observed in the mass spectrum, with the base peak at *m/z* 412 resulting from the loss of ketene. Definitive proof of the structure was obtained through the synthesis of junaphtoic acid methyl ester (**1b**) from podophyllotoxin. This will be the subject of another report.

The spectral data (IR, ^1H and ^{13}C NMR, Tables 1 and 2) for **2** were closely related to those of yatein (dihydroanhydropodorhizol, deoxypodorhizon), **2b**. The main differences observed were readily explained on the basis of the existence of a hydroxyl group in **2**, which must be at position 3 because the signals for the remaining methoxy groups in the ^1H NMR spectrum absorbed separately from each other. Furthermore, the acetylated derivative **2a** displayed an acetate methyl signal at δ2.31 characteristic of a phenolic acetate. Finally, methylation of **2** with dimethyl sulphate gave the known yatein **2b** [8], thus confirming the 3-demethylated nature of **2**.

EXPERIMENTAL

Mps: uncorr. Optical rotations: $CHCl_3$. UV EtOH. ^1H NMR (200 MHz) and ^{13}C NMR (50 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).

Extraction and isolation. Plant material was 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). Leaves of *J. sabina* (7 kg) were extracted with *n*-hexane in a Soxhlet for 9 hr. After cooling at −20° overnight the ext. afforded an insol. lignan-enriched fr. (612 g), which was successively defatted with MeOH and urea-satd MeOH and later extracted with an aq. 4% NaOH soln to yield the acidic fr. (19.2 g, 1.3% of total ext.) Due to its complexity, this fr., in Et_2O soln, was refractionated by extraction with aq. satd $NaHCO_3$ and Na_2CO_3 (5%) solns, thus obtaining strong (5.1 g), medium (9.3 g) and weak (4.1 g) acidic frs. Isolation of the components of each fr. was achieved by flash CC, prep. TLC and crystallization. In many instances, providing that no acetate groups were present, the chromatographic frs were acetylated to facilitate the sepn and purification of products. Thus, the following lignans were isolated: 320 mg of junaphtoic acid (**1**) and 200 mg of dehydropodophyllotoxin (**3**) from the $NaHCO_3$ fr.; 108 mg of (+)-epipinoresinol (**4**) from the Na_2CO_3 fr. and 59 mg of 3-*O*-demethylyatein (**2**) and 58 mg of the epoxylignan **5** from the NaOH fr. Known compounds were identified by comparison of their physical constants with those described in the lit. [9–11]. As proof of their structures, **3** was prepd from podophyllotoxin and **4** was transformed into (+)-eudesmin (**4a**) by methylation with Me_2SO_4 [10].

Junaphtoic acid (**1**). Oil. UV λ_{max} nm (ε): 212 (24 000), 255 (21 200), 295 (4500). IR ν cm^{-1}: 3600, 3500–2500, 1705, 1590, 1500, 1465, 1420, 1240, 1130, 1050, 950, 910. ^1H NMR (Table 1)

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Table 1. ^{1}H NMR spectral data* of compounds **1**, **1a**, **1b**, **1c**, **2** and **2a** ($CDCl_3$–TMS, 200 MHz)

H†	1	1a	1b	1c	2	2a
2	6.62 *s*	6.61 *s*	6.55 *s*	6.57 *s*	6.38 *d* (2.0)	6.60 *d* (1.7)
6	6.62 *s*	6.61 *s*	6.55 *s*	6.57 *s*	6.28 *d* (2.0)	6.49 *s*
7a	—	—	—	—	2.79 *dd* (13.8, 6.7)	2.88 *m*
7b	—	—	—	—	2.95 *dd* (13.8, 4.6)	2.40–2.64 *m*
8	—	—	—	—	2.58–2.61 *m*	2.40–2.64 *m*
10	3.80 *s*	3.78 *s*	3.83 *s*	3.83 *s*	—	—
11	3.84 *s*	3.89 *s*	3.92 *s*	3.92 *s*	3.86 *s*	3.80 *s*
12	3.80 *s*	3.78 *s*	3.83 *s*	3.83 *s*	3.81 *s*	3.81 *s*
2′	7.50 s	7.03 s	7.50 s	7.05 s	6.45 *d* (1.7)	6.49 *s*
5′	6.75 *s*	6.91 *s*	6.90 *s*	6.95 *s*	6.68 *d* (8.3)	6.69 *d* (8.6)
6′	—	—	—	—	6.46 *dd* (6.7, 1.7)	6.43 *dd* (8.3, 1.5)
7′	—	—	—	—	2.48–2.61 *m*	2.40–2.64 *m*
8′	—	—	—	—	2.48–2.61 *m*	2.40–2.64 m
9′a	2.36 *s*	2.27 *s*	2.33 *s*	2.24 *s*	4.13 *dd* (9.1, 6.5)	4.06–4.19 *m*
9′b	—	—	—	—	3.86–3.90 *m*	3.77–3.88 *m*
10′	5.95 *s*	5.99 *s*	6.01 *s*	6.02 *s*	5.91 *s*	5.91 *s*
$MeCO_2$-3	—	—	—	—	—	2.31 *s*
$MeCO_2$-7′	—	2.48 *s*	—	2.50 s	—	—
CO_2H	—	8.29 *sa*	—	—	—	—
CO_2Me	—	—	3.58 *s*	3.57 *s*	—	—

*Values in parentheses are coupling constants (*J*) in Hz.
†Numbering is that of Agrawal and Thakur [12].

Table 2. ^{13}C NMR spectral data of compounds **1**, **1a**, **1b**, **1c**, **2** ($CDCl_3$) and **2a** (CD_3OD) (50 MHz)

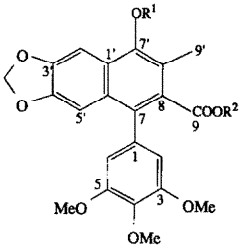
C	1	1a	1b	1c	2	2a
1	129.0	128.8	128.9	129.0	133.9	133.2
2	109.9	108.0	108.3	108.0	109.1	116.2
3	154.1	152.8	153.0	153.1	149.3	153.7
4	138.8	137.7	134.6	137.9	133.9	133.2
5	154.1	152.8	153.0	153.1	152.3	153.8
6	109.9	108.0	108.3	108.0	105.2	111.3
7	134.5	133.1	134.0	134.9	35.2	34.7
8	149.1*	143.8	137.2	144.0	46.2	46.5
9	173.8	172.6	170.1	169.4	178.5	178.3
10	56.7	56.1	56.3	54.4	—	—
11	61.2	60.8	61.0	61.0	60.9	60.7
12	56.7	56.1	56.3	56.4	55.9	56.2
1′	129.9	131.4	129.7	132.0	131.9	131.6
2′	99.4	97.5	98.3	97.7	108.9	109.0
3′	149.2*	148.2	148.0	148.3	147.9	148.0
4′	150.3	149.0	148.1	149.1	146.4	146.5
5′	103.4	103.5	103.2	103.7	108.3	108.4
6′	114.2	121.4	111.9	121.8	121.6	121.7
7′	123.9	124.6	121.8	124.8	38.3	38.4
8′	135.9	134.4	131.8	133.5	41.3	41.1
9′	13.4	13.5	12.8	13.6	71.1	71.2
10′	102.5	101.5	101.3	101.5	101.0	101.1
Acetate	—	20.4	—	20.6	—	20.7
		168.9		167.9		169.1
OMe	—	—	51.7	51.9	—	—

*Values may be interchanged.

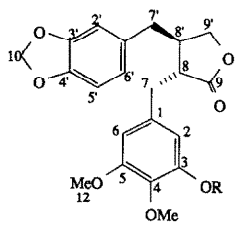
^{13}C NMR (Table 2). *Acetate* (**1a**). Eluted with HOAc. UV λ_{max} nm (ε): 212 (19000), 248 (20900), 285 (5700). EIMS: 454 ([M]$^+$, 30), 412 (100), 396 (28), 362 (6), 319 (5), 165 (5), 152 (6), 139 (6), 43 (55). IR ν cm^{-1}: 3500–2500, 1760, 1710, 1590, 1505, 1470, 1420, 1374, 1245, 1130, 1050, 1010, 950, 870, 850. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). Methyl ester (**1b**). UV λ_{max} nm (ε): 212 (26600), 256 (22700), 293 (6100). IR νcm^{-1}: 3600, 1725, 1590, 1500, 1465, 1360, 1200, 1130, 1040, 1000, 950, 860. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). Acetate methyl ester (**1c**). UV-λ_{max} nm: 206 (22300), 248 (22100), 285 (5200). IR ν cm^{-1}: 1770, 1730, 1600, 1510, 1470, 1440, 1370, 1240, 1135, 1050, 1015, 950. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2).

3-O-*Demethylyatein* (**2**). Eluted with hexane–EtOAc (1:1). $[\alpha]^{22}$ (λ nm): −10.3° (589), −11.4° (578), −13.0° (546), −26.3° (436) (*c* 0.9%). UV λ_{max} nm (ε): 210 (26600), 230 (10400), 285 (4200). IR ν cm^{-1}: 3530, 1770, 1600, 1510, 1495, 1450, 1360, 1245, 1200, 1170, 1130, 1110, 1040, 1020, 930, 910, 815. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). Acetate (**2a**). $[\alpha]^{22}$ (λ nm): −14.9° (589), −16.2° (578), −18.7° (546), −34.4° (436) (*c* 0.9%). UV λ_{max} nm (ε): 208 (39400), 226 (16000), 282 (6600). IR ν cm^{-1}: 2780, 1770, 1600, 1510, 1490, 1450, 1375, 1240, 1200, 1100, 1050, 1025, 950, 930, 900, 870. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). *Methylation of* **2**. Compound **2** (44 mg), 1 ml Me_2SO_4, 1 ml 10 m NaOH and 2 ml EtOH were refluxed for 5 hr. After usual work-up this afforded 40 mg of **2b**, an oil identical to yatein.

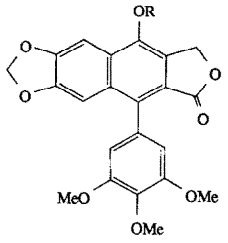
Synthesis of compound **3**. Podophyllotoxin (**6**) (1.2 g) in 30 ml of CH_2Cl_2 was treated with 1.6 g of pyridine dichromate, at room temp for 3 hr, worked-up as usual and chromatographed yielding 1 g of podophyllotoxone (**7**). **7** (80 mg) in diphenyl was heated at 260° for 2 hr in the presence of S (40 mg). After standing, 30 mg of dehydropodophyllotoxin, identical to the natural compound **3**, were sepd by filtration.



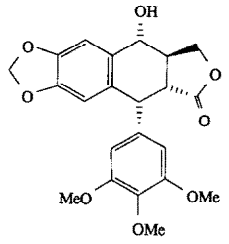
	R^1	R^2
1	H	H
1a	Ac	H
1b	H	Me
1c	Ac	Me



	R
2	H
2a	Ac
2b	Me



	R
3	H
3a	Ac



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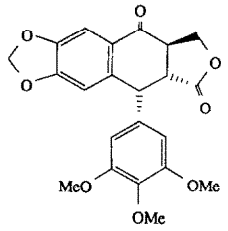

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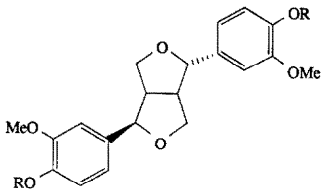
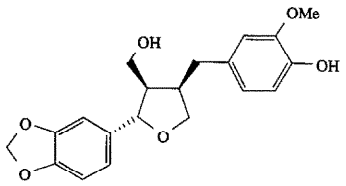


	R
4	H
4a	Me



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Methylation of compound **4**. Compound **4** (23 mg) was treated with Me_2SO_4 as described above for **2** to yield 24 mg **4a**.

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