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Oxygenated diterpenes and other constituents from Moroccan *Juniperus phoenicea* and *Juniperus thurifera* var. *africana*

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Abstract

Six new diterpenic acids isolated as their methyl ester derivatives, i.e., methyl 12-oxo-8α,15-dihydroxyabiet-13-en-19-oate, methyl 12-oxo-8α-hydroxyabiet-13-en-19-oate, methyl 15-hydroperoxy-8α,12α-epidioxiabiet-13-en-19-oate, methyl 15-hydroxy-8α,12α-epidioxiabiet-13-en-19-oate, methyl 15-hydroperoxy-8α,14α,12α,13α-diepoxiabietan-13-en-19-oate, and methyl 7α,12β-dihydroxy-sandaracopimarate, together with two new isovalerate derivatives of *p*-methoxycinnamyl alcohol and linalool, were isolated from the leaves of *Juniperus thurifera* var. *africana* and *Juniperus phoenicea*, grown in Morocco. The structures of these compounds were established by using spectroscopic techniques, including 2D NMR spectra. The cytotoxicity of the abietane diterpenoids was tested against five cell lines.

Keywords: *Juniperus thurifera*; *Juniperus phoenicea*; Cupressaceae; Diterpenoids; Cytotoxic activity

1. Introduction

Continuing our studies on the chemical composition of Spanish and Northern Moroccan plants with the aim of finding both new natural compounds with interesting biological activities and also investigating the occurrence of natural terpenoids which could be used as intermediates for the synthesis of added-value compounds, we present herein the study of two *Juniperus* species growing in Morocco. On one hand, *Juniperus thurifera* L. var. *africana* Maire (Cupressaceae), a tree endemic to North Africa, and on the other hand, *Juniperus phoenicea* L. (Cupressaceae). Since species of *J. phoenicea* have already been studied, this re-evaluation is directed to a comparison of how the geographical situation affects the production of secondary metabolites. The mixture of the leaves and cones of *J. phoenicea* is used as an oral hypoglycemic, whereas the leaves are used against bronchopulmonary diseases and as a diuretic (Bellakhder, 1997). The essential oils of the leaves and cones have been proven to possess antimicrobial activity (Stassi et al., 1996). The essential oil of the wood *J. thurifera* var. *africana* has been used as an abortive and menstruation regulator (Bellakhder, 1997), whereas the wood tar is used in veterinary science. The hexane and chloroform extracts of the leaves of European *J. thurifera* have been tested on neoplastic KB cells, and show considerable cytostatic activity (San Feliciano et al., 1989). Both plants have been shown to accumulate terpenoids,

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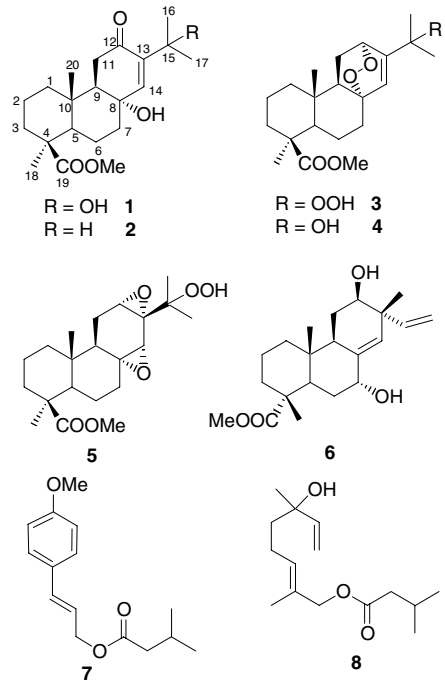
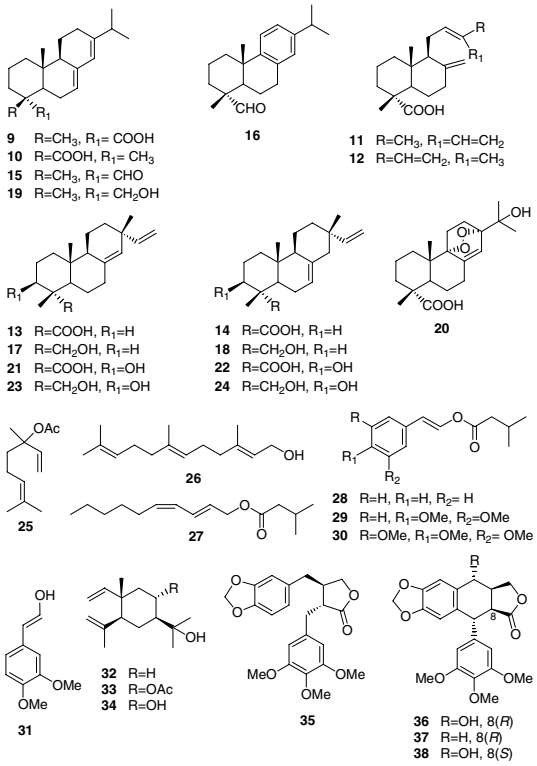
phenylpropanoids and lignans (Hussein et al., 2003; Comte et al., 1997; San Feliciano et al., 1991, 1993a–c 1986, 1988; Cairnes et al., 1980; Barrero et al., 2000) we have not found any article describing the composition of the African trees. We report herein the isolation of five new abietane diterpenoids (**1**–**5**), a new pimarane derivative (**6**), and two new isovalerate derivatives (**7**–**8**) from the hexane extracts of the leaves of *J. phoenicea* and *J. thurifera* var. *africana*. We also report the in vitro cytotoxicity of the new abietane derivatives.

2. Results and discussion

Workup of the acidic part of the hexane extract from the leaves of *J. phoenicea* led, after esterification with diazomethane, to the isolation of three new abietanes and one new pimarane diterpenoids as their methyl esters (**1**–**2**, **4** and **6**, respectively). Two new abietane acid derivatives, also isolated after esterification with diazomethane (**3** and **5**), together with the two new lynaloyl and cinnamyl isovalerates (**7**–**8**) were isolated from the hexane extract of the leaves of *J. thurifera* var. *africana*. In addition to the new compounds, a number a known compounds were also identified from these species; abietic (**9**) (Smith, 1978), 4-*epi*-abietic acid (**10**) (Tabacik and Laporthe, 1971), abietinal (**15**) (Erdtman and Westfelt, 1963), dehydroabietinal (**16**) (Fetizon et al., 1968), abietinol (**19**) (San Feliciano et al., 1993a), 15-hydroxy-9α,13α-epidioxyabiet-8(14)-en-18-oic acid (**20**) (Sy and Brown, 1988), *cis*-(**11**) (Barrero et al., 1987) and *trans*-communic (**12**) acids (Lee et al., 1987), sandaraco (**13**) (Comte et al., 1995), and isopimaric (**14**) acids (Antkowiak et al., 1962), pimara-8(14),15-dien-18-ol (**17**) (Wenkert and Buckwalter, 1972), pimara-7,15-dien-18-ol (**18**) (Wenkert and Buckwalter, 1972), 3β-hydroxysandaracopimaric acid (**21**) (Doi and Kawamura, 1972), 3β-hydroxyisopimaric acid (**22**) (Bruno et al., 1986), pimara-8(14),15-dien-3β,18-diol (**23**) (San Feliciano et al., 1988), pimara-7,15-dien-3β,18-diol (**24**) (San Feliciano et al., 1988), linaloyl acetate (**25**) (Kaiser and Lamparsky, 1977), phytol (**26**) (Sims and Pettus, 1976), (*E*,*Z*)-2,4-decadienyl isovalerate (**27**) (Rickards and Weiler, 1978), cinnamyl isovalerate (**28**), 3′,4′-dimethoxycinnamyl isovalerate (**29**) (San Feliciano et al., 1986), 3′,4′,5′-trimethoxycinnamyl isovalerate (**30**) (San Feliciano et al., 1986), 3′,4′-dimethoxycinnamol (**31**) (San Feliciano et al., 1986), elemol (**32**), 8α-acetoxyelemol (**33**) (De Pascual-Teresa et al., 1977), 8α,11-elemdiol (**34**) (De Pascual-Teresa et al., 1977), yatein (**35**) (Harmatha et al., 1982), podophyllotoxin (**36**) (Andrews et al., 1988), deoxypodophyllotoxin (**37**) (San Feliciano et al., 1990), and picropodophyllotoxin (**38**) (Fonseca et al., 1980).





Compound **1** exhibited the molecular formula $C_{21}H_{32}O_5$, as deduced from its HRFABMS ($[M + Na]^+$, *m/z* 387.2148). The IR spectrum showed

absorption bands at ν_{max} 3399 cm^{-1} indicating a hydroxyl group and at 1724 and 1659 cm^{-1}, attributable to an ester group and to an α,β-unsaturated carbonyl group, respectively. These carbonyl assignments were supported by ^{13}C NMR signals at δ 201.7 and 177.4. In the ^{1}H NMR spectrum (Table 1), four tertiary methyl signals at δ 0.47 (3H), 1.21 (3H), and 1.40 (6H), the latter ones attached to an oxygenated carbon, an oxygenated methyl signal (δ 3.60), and an olefinic proton signal (δ 6.59) were observed. The ^{13}C NMR spectrum (Table 2) showed 21 resonances and confirmed the presence of a trisubstituted double bond (δ 145.8 and 146.3). The presence of two quaternary oxygenated carbons (δ 68.6 and 71.9) was also indicated. The combined analysis of the HETCOR, COSY, HMBC spectra, and NOEDIFF experiments, permitted the assignment of an abietane skeleton for this compound (Mei et al., 2002; Lee and Cheng, 2001; Ohtsu et al., 2001): methyl 12-oxo-8α,15-dihydroxyabiet-13-en-19-oate. The location of the α,β-unsaturated ketone in **1** was established according to its HMBC spectrum, in which cross-peaks were observed between δ_H 2.17 (H-7) and δ_C 146.3 (C-14), and between δ_H 6.59 (H-14) and δ_C 71.9 (C-15). The long-range correlations of δ_C 68.6 (C-8) with δ_H 6.59 (H-14), δ_H 2.50 (H-11), and δ_H 2.00 (H-6) indicated that the tertiary alcohol was at the C-8 position. Finally, the location of the methoxycarbonyl group at the C-4 position was confirmed by the cross-peaks observed between δ_C 177.4 (C-19) and δ_H 1.21 (Me-18), and δ_H 1.01 (H-3). In order to establish the relative configuration of the stereogenic centers, different NOEDIFF experiments were performed (Fig. 1). The configuration of C-4 and C-8 were assigned based upon the enhancements observed at δ_H 6.59 (H-14), and at δ_H 3.60 (CH_3OCO) when Me-20 (δ_H 0.47) was irradiated. Furthermore, the very high field chemical shift observed for Me-20 in the ^{1}H NMR spectrum (δ 0.47) was explained on the basis of the shielding effect exerted towards this methyl by both, the double bond at C-13 and the methoxycarbonyl group at C-19.

The HRFABMS of compound **2** showed a signal at *m/z* 371.2195, indicating a molecular formula of $C_{21}H_{32}O_4$. The ^{1}H and ^{13}C NMR spectra of this product were very similar to those of **1** (Tables 1 and 2), the only difference being the lack of the oxygenated function at C-15 in **2**. The ^{1}H NMR signals corresponding to the isopropyl group appeared at δ_H 1.01 (3H, *d*, *J* = 6.9 Hz), 1.05 (3H, *d*, *J* = 6.9 Hz) and 2.87 (1H, *hp*, *J* = 6.9 Hz). A new methine was observed at δ_C 26.3 in the ^{13}C NMR spectrum. Accordingly, the structure of **2** was determined to be methyl 12-oxo-8α-hydroxyabiet-13-en-19-oate. An epimer of **2** at C-4 was isolated form *Pinus sylvestris* needles (Buratti et al., 1990).

The molecular formula of compound **3** was deduced as $C_{21}H_{32}O_6$ from its HRMS ($[M + Na]^+$, *m/z* 403.2093). Its ^{1}H NMR spectrum was again similar to that of ester **1**, revealing – as in **1** – the presence of a Δ^{13}-abietane skeleton supporting a methyl ester on a quaternary carbon (δ_H 3.63, *s*, 3H) and an oxygenated function at C-15 (δ_H 1.34, *s*, 3H; δ_H 1.45, *s*, 3H). Besides, a new secondary oxygenated carbon was observed (δ_H 4.92, *m*). After comparing the ^{13}C NMR data of

Table 1
^{1}H NMR data for compounds **1**–**5** (δ in ppm, *J* in Hz)

Proton	1	2	3	4	5
1	α: 0.96–1.08 (*m*)	α: 0.85–1.10 (*m*)	α: 0.93–1.08 (*m*)	α: 0.95–1.11 (*m*)	α: 1.68–1.97 (*m*)
	β: 1.81–1.88 (*m*)	β: 1.80–1.87 (*m*)	β: 0.93–1.08 (*m*)	β: 0.95–1.11 (*m*)	β: 0.97–1.13 (*m*)
2	α: 1.43–1.52 (*m*)	α: 1.42–1.52 (*m*)	α: 1.34–1.47 (*m*)	α: 1.20–1.46 (*m*)	a: 1.42–1.60 (*m*)
	β: 1.76 (*btt*, 3.4, 13.9)	β: 1.75 (*btt*, 3.6, 14.0)	β: 1.72 (*tq*, 3.5, 13.9)	β: 1.75 (*tq*, 3.5, 13.9)	b: 1.68–1.97 (*m*)
3	a: 0.96–1.08 (*m*)	a: 0.85–1.10 (*m*)	a: 1.34–1.47 (*m*)	a: 1.20–1.46 (*m*)	a: 0.97–1.13 (*m*)
	b: 2.16–2.24 (*m*)	b: 2.16–2.24 (*m*)	b: 2.18 (*bd*, 3.2)	b: 2.22 (*bd*, 13.1)	b: 2.25 (*bd*, 13.2)
5	1.81–1.88 (*m*)	1.80–1.87 (*m*)	1.18–1.27 (*m*)	1.18–1.27 (*m*)	1.22–1.33 (*m*)
6	a: 1.95–2.03 (*m*)	α: 1.95–2.02 (*m*)	a: 1.82–1.97 (*m*)	a: 1.20–1.46 (*m*)	a: 1.68–1.97 (*m*)
	β: 1.51–1.66 (*m*)	β: 1.53–1.66 (*m*)	b: 2.08 (*m*)	b: 2.09 (*m*)	b: 2.00–2.12 (*m*)
7	α: 2.17 (*b dd*, 3.5, 13.2)	α: 2.10–2.18 (*m*)	α: 1.34–1.47 (*m*)	α: 1.20–1.46 (*m*)	α: 1.42–1.60 (*m*)
	β: 1.51–1.66 (*m*)	β: 1.53–1.66 (*m*)	β: 1.82–1.97 (*m*)	β: 1.85–1.99 (*m*)	β: 1.68–1.97 (*m*)
9	1.81–1.88 (*m*)	1.80–1.88 (*m*)	1.82–1.97 (*m*)	1.85–1.99 (*m*)	1.68–1.97 (*m*)
11	α: 2.98 (*dd*, 6.4, 18.4)	α: 2.92 (*dd*, 6.4, 18.3)	α: 2.25 (*ddd*, 4.3, 9.4, 13.5)	α: 2.30 (*ddd*, 4.3, 9.3, 13.6)	a: 1.68–1.97 (*m*)
	β: 2.50 (*d*, 18.4)	β: 2.52 (*d*, 18.3)	β: 1.25 (*dd*, 5.1, 13.5)	β: 1.17 (*ddd*, 1.5, 5.5, 13.6)	b: 2.00–2.12 (*m*)
12			4.92 (*m*)	4.92 (*m*)	3.22 (*bd*, 7.2)
14	6.59 (*bs*)	6.38 (*bs*)	6.18 (*d*, 1.8)	6.14 (*d*, 1.8)	3.35 (*s*)
15		2.87 (*hp*, 6.9)			
16	1.40 (*s*)	1.01[a] (*d*, 6.9)	1.34[b] (*s*)	1.42[c] (*s*)	1.34[d] (*s*)
17	1.40 (*s*)	1.05[a] (*d*, 6.9)	1.45[b] (*s*)	1.44[c] (*s*)	1.49[d] (*s*)
18	1.21 (*s*)	1.21 (*s*)	1.21 (*s*)	1.23 (*s*)	1.26 (*s*)
20	0.47 (*s*)	0.44 (*s*)	0.35 (*s*)	0.39 (*s*)	0.69 (*s*)
*Me*OCO	3.60 (*s*)	3.60 (*s*)	3.63 (*s*)	3.66 (*s*)	3.67 (*s*)

[a–d] Assignments with the same superscript letter may be interchanged.

Table 2
^{13}C NMR data for compounds **1**–**5** (δ in ppm)

Carbon	1	2	3	4	5
1	39.6	39.7	37.9	38.0	40.0
2	18.9	19.0	18.7	18.7	19.3
3	37.6	37.7	37.9	38.0	38.1
4	43.7	43.7	43.9	43.9	44.1
5	55.1	55.3	55.5	55.6	55.0
6	21.1	21.3	20.6	20.6	22.6
7	42.7	43.1	32.6	32.7	35.5
8	68.6	68.9	76.9	76.8	59.2
9	55.4	55.6	49.6	49.7	47.1
10	38.9	39.0	37.4	37.3	39.4
11	35.7	34.9	25.0	25.6	20.5
12	201.7	198.6	72.2	72.6	49.8
13	145.8	148.2	146.3	149.6	62.1
14	146.3	144.5	129.0	124.6	56.7
15	71.9	26.3	81.9	70.8	81.7
16	28.9[a]	21.2[b]	22.7[c]	28.2[d]	21.2[e]
17	29.0[a]	21.9[b]	23.0[c]	28.3[d]	21.6[e]
18	28.2[a]	28.9	28.8	28.8	29.0
19	177.4	177.5	177.5	177.5	177.5
20	15.5	15.3	13.0	13.1	14.8
CH_3OCO	51.4	51.3	51.4	51.5	51.4

[a–e] Assignments with the same superscript letter may be interchanged.

compounds **1** and **3** (Table 2), the most significant data were the confirmation of the presence of a tertiary oxygenated carbon (δ 72.2) in the latter; the two quaternary oxygenated carbon corresponding to C-8 and C-15 were again observed (δ 76.9 and 81.9, respectively), while no ketone carbonyl group signal was detected in **3**. According to the molecular formula one unit of unsaturation remained to be defined. As endoperoxide derivatives of abietane have been reported as natural products, the presence of a peroxide function between C-8 and C-12 was taken into account. The correlations observed in the HMBC experiment between H-14 (δ_H 6.18) and C-7 (δ_C 32.6), C-9 (δ_C 49.6), C-12 (δ_C 72.2), C-8 (δ_C 76.9), and C-15 (δ_C 81.9) confirmed this assignment. Finally, the anomalous deshielding experienced by C-15 compared with that of **1** and other 15-hydroxyabietane derivatives could be explained if a hydroperoxide function is located at this position, which, in addition, would also lead to assign the sixth oxygen atom present in this molecule. Treatment of **3** with LAH gave compound, **4**, with ^1H and ^{13}C NMR spectra practically identical to those of **3**. The only remarkable differences being the shielding experienced by C-15, now appearing at δ 70.8, and the deshielding experienced by C-16 and C-17 (signals at δ 28.2 and 28.3). Compound **4** was confirmed to be the result of the chemoselective reduction of the hydroperoxide function of **3** after observing a peak at *m/z* 387.2145 ($[M + Na]^+$) in its HRFABMS, which indicated a molecular formula of $C_{21}H_{32}O_5$. With respect to the relative stereochemistry, the spatial disposition of the endoperoxide moiety was determined to be α, while the methoxycarbonyl group was determined to be β. These assignments were reached after noticing the enhancement experienced by H-14 (δ 6.18) when Me-20 was irradiated and the anomalously high field chemical shift observed for Me-20 (δ 0.35). The rest of selected NOEs shown in Fig. 2 confirmed these assignments. Compound **4** was also isolated in the present study on *J. phoenicea*, and hence must be considered as a new natural product. The C-4 epimer of **4** was isolated by us from the wood of *Abies marocana* (Barrero et al., 1994).

The spectral data for compound **5** (Tables 1 and 2) suggest that, as **3**, this compound is an abietane methyl

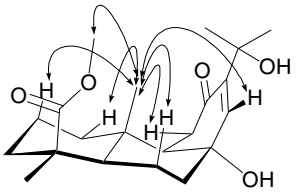


Fig. 1. Selected NOEs observed for **1**.

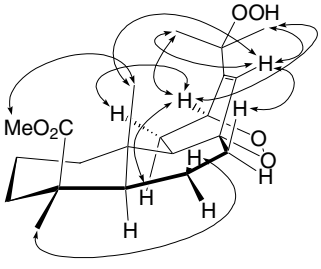


Fig. 2. Selected NOEs observed for **3**.

ester derivative (ν_{max} 1722 cm^{-1}; δ_C 177.5 and 51.4; δ_H 3.67, 3H, *s*) possessing a hydroperoxide function at C-15 (δ_C 81.7; δ_H 1.34 and 1.49, both 3H, *s*). The rest of the functionalities present in this molecule could be determined to be two trisubstitued oxirane rings, as inferred from the signals in their ^{1}H [1H, δ 3.22, *bd* (J = 7.2); 1H, δ 3.35, *s*], and ^{13}C (two tertiary carbons at δ 49.8 and 56.7, and two quaternary carbons at 59.2 and 62.1) NMR spectra. The location of the epoxide rings was established upon HMBC by observing correlations between C-13 (δ_C 62.1) and Me-16 and Me-17 (δ_H 1.34 and 1.49); and between C-8 (δ_C 59.2) and H-6 and H-11. Accordingly, the structure of **5** was determined to be methyl 15-hydroperoxy-8α,14α,12α,13α-diepoxiabietan-13-en-19-oate. However, the molecular ion was not observed in the HR mass spectrum, the fragment presenting the highest *m/z* appeared at 387. This fragment corresponds to a loss of an oxygen atom from the molecular ion, which indicated a molecular formula of $C_{21}H_{32}O_5$. Finally, the relative stereochemistry of the chiral centers was confirmed by NOEDIFF experiments (Fig. 3).

Compound **6** exhibited the molecular formula $C_{20}H_{30}O_3$, as deduced from its HRFABMS ([M + Na]$^+$, *m/z* 371.2199). The most significant absorptions in the IR spectrum appeared at ν_{max} 3416, 1723, and 1635 cm^{-1}, which can be attributed to a hydroxyl group, an ester carbonyl and a double bond. The ^{1}H NMR spectrum shows signals due to three methyl groups, which appear as singlets at δ 0.87, 1.07, and 1.25, two secondary oxygenated carbons (δ 3.61, *dd*, J = 4.0, J = 13.2 Hz, and δ 4.42, *t*, J = 2.5 Hz), an ABX system corresponding to a vinyl moiety attached to a quaternary carbon (A: δ 5.13, *dd*, J = 1.0, J = 17.4 Hz; B: δ 5.17, *dd*, J = 1.0, J = 10.8 Hz; X: δ 5.81, *dd*, J = 10.8, J = 17.4 Hz), and an olefinic proton (δ 5.57, *d*, J = 2.2 Hz). Complete analysis of its ^{1}H and ^{13}C NMR spectra suggested a pimarane skeleton for **6** (Barrero et al., 2003; Chang et al., 2000). The location of the ester group was assigned according to the correlations found in the HMBC spectrum between C-18 (δ_C 180.3) and Me-19 (δ_H 1.25). The position of the olefinic proton at C-14 was determined from correlations between δ_H 1.07 (Me-17) and δ_C 137.7 (C-14), and between δ_H 5.57 (H-14) and δ_C 48.6 (C-9), 73.0 (C-12), and 84.9 (C-7). The latter two cross-peaks suggested the presence of two hydroxyl groups at C-7 and C-12. The following correlations confirmed this assignment: cross-peaks were observed between δ_C 73.0 (C-12) and δ_H 1.49 (H-11) and 1.07 (Me-17); while the signal corresponding to C-7 (δ_C 84.9) showed correlations with the signals of H-6 (δ_H 1.89), and H-14 (δ_H 5.57). The relative configuration was assigned on the basis of the enhancements observed in NOEDIFF experiments (Fig. 4). Enhancement of the signals corresponding to Me-19 and Me-17, observed after irradiating Me-20, were fundamental for the assignment of the stereochemistry at C-4, C-10, and C-13. The ^{1}H NMR coupling constant values measured for H-7 (*t*, J = 2.5 Hz), and H-12 (*dd*, J = 4.0, J = 13.2 Hz) confirmed the spatial disposition attributed to these protons.

Compound **7** showed a signal of the molecular ion at *m/z* 248.1413 in the HRMS, indicating a molecular formula of $C_{15}H_{20}O_3$. The ^{1}H NMR spectrum showed signals due to the presence of a *para*-substituted aromatic ring at δ 7.36 (2H, *d*, J = 8.7 Hz) and 6.88 (2H, *d*, J = 8.7 Hz), two olefinic proton at δ 6.63 (1H, *bd*, J = 15.8 Hz) and 6.18 (1H, *dt*, J = 15.8, 6.6 Hz), an oxygenated allylic methylene at 4.73 (2H, *bd*, J = 6.6 Hz), and a methoxy group at 3.83 (3H, *s*), all of them attributable to a 4′-methoxylcinnamyl moiety. The ^{13}C NMR signals are in agreement with this assignment. On the other hand, the signals appearing at δ_H 1.00 (6H, *d*, J = 6.5 Hz), 2.17 (1H, *m*), and 2.26 (2H, *d*, J = 7.5 Hz), together with the signal at δ_C 173.0 confirmed the presence of an isovalerate residue. Thus, the structure of **7** was assigned to be of 4′-methoxycinnamyl isovalerate.

As in **7**, the NMR spectral data for compound **8** confirmed, the presence of an isovalerate moiety. The rest of the signals could be attributed to a linaloyl residue which has been oxygenated on one of the geminal methyl groups (Tschesche et al., 1977). The assignment of the double bond geometry was achieved on the basis of the shielded ^{13}C chemical shift observed for the vinyl methyl (δ 13.9), which suggested a *syn* disposition of this methyl and the hydrocarbon chain. Accordingly, the structure of **8** was assigned as 8-isovaleroyloxylinalool.

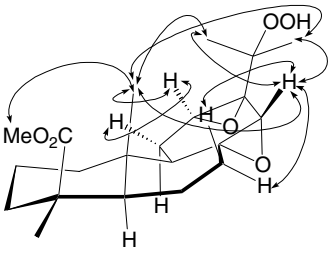


Fig. 3. Selected NOEs observed for **5**.

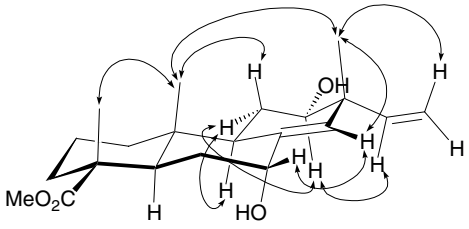


Fig. 4. Selected NOEs observed for **6**.

Table 3
Cytotoxic activity of compounds **1–5**, IC_{50} (μg/ml)

	A-549	H-116	PSN1	T98G	SKBR3
1	>5	2.5	5	5	>5
2	>5	2.5	>5	>5	>5
3	5	2.5	5	>5	>5
4	>5	>5	>5	>5	>5
5	>5	>5	>5	>5	>5
Cycloheximide	0.1	0.1	0.01	2.5	0.05

Abietane diterpenoids have been reported to possess several biological properties such as antimicrobial, antiulcer, and cardiovascular activities (Tan et al., 2002), activity as antitumor promoters (Ohtsu et al., 2001), antileishmanial activity (Tan et al., 2002), antioxidant (Wang et al., 2002), tuberculostatic, antiplatelet agregation activity, antiviral, and several others (Cousins, 1994). However, it has been the remarkable antitumor activity of abietane quinone derivatives recently described in a patent (Kotoda et al., 2002), which has encouraged us to test the cytotoxicity of our abietanes **1–5**. Five cell lines, A-549 (human lung carcinoma), H-116 (human colon carcinoma), PSN1 (human pancreatic adenocarcinoma), T98G (human caucasian gioblastoma), and SKBR3 (human breast carcinoma) were tested following established methods. The results are summarized in Table 3. Compounds **1–3** showed an inhibitory activity against H-116 at 2.5 μg/ml.

3. Conclusion

Although the study of *J. thurifera* var. *africana* and *J. phoenicea* led to the isolation of eight new natural products as minor components, it can be concluded from a comparative analysis with previously reported studies that the content of terpenoid compounds in the European and African species does not generally present significant quantitative variations (San Feliciano et al., 1993a; Tabacik and Laporthe, 1971). Sandaracopimaric acid and various 4-*epi*-abietic acid derivatives are the main components of both, European and North Africa grown, *J. phoenicea*. On the other hand, both, the components of *J. thurifera* var. *africana* have been shown to be diterpenic acids, phenylpropanoids and lignans, a composition very similar to that found in the European variety.

Since the photoinduced reaction of abietadienes with singlet oxygen is a known process (Barrero et al., 1991), it should not be ruled out that compounds **3** and **5** are artefacts of the Soxhlet extraction and/or manipulation of the extract.

Finally, three of the abietane derivatives show cytotoxic activity at 2.5 μg/ml.

4. Experimental

4.1. General

Optical rotations were determined with a polarimeter Perkin–Elmer model 141, using $CHCl_3$ as the solvent. IR spectra were recorded with a Perkin–Elmer model 983 G instrument as NaCl plates (films). NMR studies were performed with a Bruker ARX 400 (^{1}H 400 MHz/^{13}C 100 MHz) spectrometer, and a Bruker AMX 300 (^{1}H 300 MHz/^{13}C 75 MHz) spectrometer. Mass spectra (EIMS, 70 eV) were run with a Hewlett–Packard 5972A mass spectrometer coupled to a Hewlett–Packard 5890A gas chromatograph. The accurate mass determination was carried out with an AutoSpec-Q mass spectrometer arranged in an EBE geometry (Micromass Instruments, Mancester, UK) and equipped with a FAB (LSIMS) source. Silica gel SDS 60 (35–70 μm) was used for column chromatography.

4.2. Plant material

Juniperus thurifera L. var. *africana* Maire (Cupressaceae) was collected in August 2002, in the region of Tamahdit, Middle Atlas, Morocco. *J. phoenicea* L. (Cupressaceae) was collected in May 1998, in the region of Ouarzzazzat, High Atlas, Morocco. Voucher specimens are available for inspection at the herbarium of the Scientific Institute of the University of Mohamed V, Rabat.

4.3. Extraction and isolation

The air-dried leaves of *J. phoenicea* (0.5 kg) were extracted in Soxhlet apparatus with hexane resulting in 23 g of crude extract. This crude material was extracted with 1 N NaOH, the aqueous layer acidified with HCl and then extracted with *t*-BuOMe to give 2.9 g of an acid fraction after removing the solvent. This acid fraction was esterified with diazomethane and subsequently subjected to column chromatography over silica gel using mixtures of hexane/*t*-BuOMe of increasing polarity as eluents. The less polar fraction (P1, hexane/*t*-BuOMe, 1:2) was composed of 1250 mg of a mixture of methyl ester derivatives of diterpenic acids, methyl sandaracopimarate being the major one. The presence

of the methyl esters of 4-*epi*-abietic acid (**10**) and *cis*- and *trans*-communic (**11**)–(**12**) acids as minor compounds in this mixture was determined by comparison with standards. The six following fractions were eluted with hexane/*t*-BuOMe, 1:2. P2 (83 mg) was rechromatographed to isolate 7 mg of **6**. P3 was containing 44 mg of **2**. P4 (65 mg) was a mixture with **2** as the main component. Repeated column chromatography of P5 (57 mg) over silica gel led to the isolation of 10 mg of **4**. P6 (95 mg) was a mixture containing **1**. P7 consisted of 99 mg of **1**.

The air-dried leaves of *J. thurifera* var. *africana* (1 kg) were extracted in a Soxhlet apparatus with hexane resulting in 81 g of crude extract. A 20 g portion was subjected to column chromatography over silica gel using mixtures of hexane/EtOAc of increasing polarity as eluents. Eight main fractions were collected, T1–T8. A mixture of 5.2 g of abietic acid (**9**), *trans*-communic acid (**12**), and isopimaric acid (**14**) in a 7:2:1 ratio was obtained by crystallization from T1 using a mixture of hexane–CH_2Cl_2 (20:1). The mother liquors were concentrated and chromatographed repeatedly to afford 43 mg of (*E*,*Z*)-2,4-decadienyl isovalerate (**27**), 173 mg of abietinal (**15**), 90 mg of linaloyl acetate (**25**), 10 mg of dehydroabietinal (**16**), 10 mg of cinnamyl isovalerate (**28**), 30 mg of elemol (**32**), and 190 mg of **7**. T2 was re-subjected to silica gel column chromatography to yield a mixture (41 mg) of pimara-8(14),15-dien-18-ol (**17**) and pimara-7,15-dien-18-ol (**18**), and 32 mg of abietinol (**19**). T3 was again subjected to column chromatography to give 110 mg of 3′,4′-dimethoxycinnamyl isovalerate (**29**), 36 mg of **8**, 15 mg of 15-hydroxy-9α,13α-epidioxyabiet-8(14)-en-18-oic acid (**20**), and 92 mg of an equimolecular mixture of 3β-hydroxysandaracopimaric acid (**21**) and 3β-hydroxyisopimaric acid (**22**). T4 was rechromatographed over silica gel to yield 14 mg of 4-*epi*abietic acid (**10**), 90 mg of phytol (**26**), 130 mg of 3′,4′,5′-trimethoxycinnamyl isovalerate (**30**), and 250 mg of 8α-acetoxyelemol (**33**). T5 was constituted of 476 mg of 8α,11-elemdiol (**34**). T6 was composed mainly of yatein (**35**) (225 mg). T7 was subjected to repeated column chromatography and various chemical derivatizations including esterification with $TMSCHN_2$, acetylation and saponification to yield 95 mg of **3**, 4.5 mg of **4**. In addition, 75 mg of yateine (**35**), 312 mg of deoxypodophyllotoxin (**37**), 75 mg of a mixture of pimara-8(14),15-dien-3β,18-diol (**23**) and pimara-7,15-dien-3β,18-diol (**24**), as well as 10 mg of 3′,4′-dimethoxycinnamol (**31**) could be obtained. Rechromatography of T8 led to the isolation of podophyllotoxin (**36**) (248 mg) and picropodophyllotoxin (224 mg) (**38**).

4.4. Methyl 12-oxo-8α,15-dihydroxyabiet-13-en-19-oate (*1*)

Colorless oil. $[\alpha]_D$ +20.1° (ca. 0.66, CH_2Cl_2); IR (film) ν_{max} 3399, 2953, 2875, 1724, 1659, 1462, 1237, 1159, 1096, 997 cm^{-1}; EIMS *m*/*z* 349 $[M - CH_3]^+$ (52), 328 (77), 268 (42), 253 (100), 197 (44), 149 (82), 121 (97), 107 (59), 91 (75), 79 (77), 67 (60), 59 (80), 55 (87); HRFABMS *m*/*z* 387.2148 (calc. for $C_{21}H_{32}O_5Na$, 387.2147).

4.5. Methyl 12-oxo-8α-hydroxyabiet-13-en-19-oate (*2*)

Colorless oil. $[\alpha]_D$ +18.3° (ca. 0.91, CH_2Cl_2); IR (film) ν_{max} 3430, 2957, 2931, 1724, 1667, 1464, 1236, 1156, 1015, 977 cm^{-1}; HRFABMS *m*/*z* 371.2195 (calcd for $C_{21}H_{32}O_4Na$, 371.2198).

4.6. Methyl 15-hydroperoxy-8α,12α-epidioxyabiet-13-en-19-oate (*3*)

White powder. $[\alpha]_D$ +9.7° (ca. 1.03, $CHCl_3$); IR (film) ν_{max} 3401, 2948, 1724, 1465, 1237, 1151, 1035, 971, cm^{-1}; EIMS *m*/*z* 348 (13), 332 (6), 254 (28), 181 (9), 133 (21), 121 (58), 109 (29), 91 (45), 81 (40), 67 (35), 59 (39), 55 (44), 43 (100); HRFABMS *m*/*z* 403.2093 (calcd for $C_{21}H_{32}O_6Na$, 403.2097).

4.7. Methyl 15-hydroxy-8α,12α-epidioxyabiet-13-en-19-oate (*4*)

Colorless oil. $[\alpha]_D$ +72.4° (ca. 0.46, $CHCl_3$); IR (film) ν_{max} 3444, 2932, 1723, 1649, 1463, 1235, 1170, 971 cm^{-1}; EIMS *m*/*z* 349 $[M - CH_3]^+$ (10), 332 (13), 271 (8), 253 (17), 195 (14), 149 (83), 121 (100), 107 (52), 91 (84), 79 (76), 67 (77); HRFABMS *m*/*z* 387.2145 (calc. for $C_{21}H_{32}O_5Na$, 387.2147).

4.8. Reduction of *3* with LAH

To a solution of **3** (28 mg, 0.08 mmol) in 6 ml of dry THF was added 8 mg (0.20 mmol) of LAH. The mixture was stirred at room temperature overnight. Std. NH_4Cl soln was added and the mixture extracted with *t*-BuOMe. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated to give 24 mg of **4**.

4.9. Methyl 15-hydroperoxy-8α,14α,12α,13α-diepoxyabietan-13-en-19-oate (*5*)

White powder. $[\alpha]_D$ +0.25° (ca. 0.40, $CHCl_3$); IR (film) ν_{max} 3387, 2934, 2854, 1722, 1452, 1241, 1163 cm^{-1}; EIMS *m*/*z* 348 (6), 332 (5), 269 (9), 254 (27), 223 (16), 163 (25), 133 (28), 121 (99), 109 (50), 91 (53), 79 (65), 67 (78), 59 (100), 55 (88); HRFABMS *m*/*z* 387.2143 (calcd for $C_{21}H_{32}O_5Na$, 387.2147).

4.10. Methyl 7α,12β-dihydroxysandaracopimarate (*6*)

Colorless oil. $[\alpha]_D$ +14.3° (ca. 1.2, $CHCl_3$); IR (film) ν_{max} 3416, 2927, 2853, 1723, 1635, 1440, 1253, 1160, 1068, 994, 843 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ

0.87 (3H, *s*, H-20), 1.07 (3H, *s*, H-17), 1.17–1.25 (1H, *m*, H-1β), 1.25 (3H, *s*, H-19), 1.42–1.52 (1H, *m*, H-6β), 1.49 (1H, *bq*, $J = 13.7$ Hz, H-11β), 1.52–1.81 (6H, *m*, H-1α, H-2, H-3, H-11α), 1.89 (1H, *bdt*, $J = 2.3$, 14.7 Hz, H-6 α); 2.28–2.34 (1H, *m*, H-9), 2.38 (1H, *dd*, $J = 2.2$, 13.0 Hz, H-5), 3.61 (1H, *dd*, $J = 4.0$, 13.2 Hz, H-12), 3.68 (3H, *s*, $COOCH_3$), 4.42 (1H, *t*, $J = 2.5$ Hz, H-7), 5.13 (1H, *dd*, $J = 1.0$, 17.4 Hz, H-16 *trans*), 5.17 (1H, *dd*, $J = 1.0$, 10.8 Hz, H-16 *cis*), 5.57 (1H, *d*, $J = 2.2$, H-14), 5.81 (1H, *dd*, $J = 10.8$, 17.4 Hz, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3 (*s*, $COOCH_3$), 145.0 (*d*, C-15), 137.7 (*d*, C-14), 133.1 (*s*, C-8), 114.6 (*t*, C-16), 84.9 (*d*, C-7), 73.0 (*d*, C-12), 52.5 (*q*, $COOCH_3$), 48.6 (*d*, C-9), 46.1 (*s*, C-4), 43.4 (*s*, C-13), 40.4 (*d*, C-5), 38.2 (*t*, C-1), 37.5 (*s*, C-10), 37.2 (*t*, C-3), 27.4 (*t*, C-6), 25.8 (*t*, C-11), 18.0 (*t*, C-2), 17.3 (*q*, C-17[a]), 17.5 (*q*, C-19[a]), 15.0 (*q*, C-20) ([a]assignments with the same superscript letter may be interchanged); HRFABMS *m/z* 371.2199 (calcd for $C_{21}H_{32}O_4Na$, 371.2198).

4.11. 4′-Methoxycinnamyl isovalerate (7)

Obtained as colorless oil; IR (film) ν_{max} 2960, 2872, 1735, 1608, 1513, 1464, 1379, 1293, 1251, 1174, 1118, 904 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.99 (6H, *d*, $J = 6.5$, H-4′ and H-5″), 2.18 (1H, *m*, H-3″), 2.26 (2H, *d*, $J = 7.0$, H-2″), 3.84 (3H, *s*, O*Me*), 4.73 (2H, *dd*, $J = 1.2$, 6.6, H-1), 6.18 (1H, *dt*, $J = 6.6$, 15.8, H-2); 6.63 (1H, *bd*, $J = 15.8$, H-3), 6.88 (2H, *d*, $J = 8.7$, H-3′ and H-5′), 7.36 (2H, *d*, $J = 8.7$, H-2′ and H-6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.0 (*s*, C-1″), 159.7 (*s*, C-4′), 134.0 (*d*, C-3), 128.9 (*s*, C-1′), 127.9 (*d*, C-2 ′ and C-6′), 121.2 (*d*, C-2), 114.1 (*d*, C-3′ and C-5′), 65.1 (*t*, C-1), 55.4 (*q*, OCH_3), 43.6 (*t*, C-2″), 25.8 (*d*, C-3″), 22.5 (*q*, C-4″ and C-5″); EIMS *m/z* 248 (40), 164 (38), 147 (87), 131 (24), 103 (32), 91 (37), 85 (100), 77 (18), 57 (87), 49 (97); HRFABMS *m/z* 248.1413 (calcd for $C_{15}H_{20}O_3$, 248.1412).

4.12. Isovaleroyloxylinalool (**8**)

Colorless oil. $[\alpha]_D$ −1.4° (ca. 0.3, CH_2Cl_2); IR (film) ν_{max} 3429, 2958, 2920, 2851, 1733, 1644, 1609, 1463, 1372, 1294, 1251, 1184, 1119 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.93 (6H, *d*, $J = 6.5$, H-4′ and H-5′), 1.27 (3H, *s*, H-10), 1.50–1.65 (2H, *m*, H-4), 1.62 (3H, *bs*, H-9), 1.98–2.15 (3H, *m*, H-5, H-3′), 2.18 (2H, *d*, $J = 7.0$, H-2′), 4.42 (2H, *s*, H-8), 5.05 (1H, *dd*, $J = 1.1$, 10.7, H-1 *cis*), 5.20 (1H, *dd*, $J = 1.1$, 17.3, H-1 *trans*); 5.44 (1H, *bt*, $J = 7.2$, H-6), 5.89 (1H, *dd*, $J = 10.7$, 17.3, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1 (*s*, C-1′), 144.9 (*d*, C-2), 130.4 (*d*, C-6), 111.9 (*t*, C-1), 73.2 (*d*, C-3), 69.9 (*t*, C-8), 43.5 (*t*, C-2′), 41.6 (*t*, C-5), 27.9 (*q*, C-10), 25.8 (*d*, C-3′), 22.5 (*t*, C-4), 22.4 (*q*, C-4′ and C-5′), 13.9 (*q*, C-9); EIMS *m/z* 254 (2), 187 (11), 159 (29), 134 (87), 119 (76), 105 (39), 91 (72), 79 (50), 67 (48), 57 (92), 43 (100); HRFABMS *m/z* 277.1784 (calc. for $C_{15}H_{26}O_3Na$, 277.1779).

4.13. Cell proliferation assay

The 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was used, essentially as described (Mosmann, 1983). The assay is dependent on the reduction of MTT by mitochondrial dehydrogenases of viable cell to a blue formazan product which come be measured spectofhotometrically. BAE cells (8×10^3 cells in a total volume of 200 μl of DMEM/20% FBS) and tumor cells (4×10^3 A-549 cells or 6×10^3 H-116, 6×10^3 PSN1, 6×10^3 SKBR3 and 6×10^3 T98G cells in a total volume of 200 μl of complete medium) were incubated in each well with serial dilutions (5, 2.5, 1, 0.5, 0.1, 0.05 and 0.01 μg/ml) of the tested compounds. After 2 days of incubation (37 °C, 5% CO_2 in a humid atmosphere) 50 μl of MTT (5 mg/ml in PBS) were added to each well and the plate was incubated for a further 2 h (37 °C). The resulting formazan was dissolved in 100 μl DMSO and read at 490 nm. All determinations were carried out in triplicate. IC_{50} value was calculated as the concentration of drug yielding a 50% of cell survival.

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References

Andrews, R.C., Teague, S.J., Meyers, A.I., 1988. Asymmetric total synthesis of (−)-podophyllotoxin. J. Am. Chem. Soc. 110, 7854–7858.

Antkowiak, W., Apsimon, J.W., Edwards, O.E., 1962. Structure of isopimaric acid. J. Org. Chem. 27, 1930–1931.

Barrero, A.F., Quílez del Moral, J.F., Lucas, R., Payá, M., Akssira, M., Akaad, S., Mellouki, F., 2003. Diterpenoids from *Tetraclinis articulata* that inhibit various human leukocyte functions. J. Nat. Prod. 66, 844–850.

Barrero, A.F., Quilez del Moral, J., Lara, A., 2000. Sesquiterpenes from *Juniperus thurifera* L. Stereochemistry in unusual cedrane and duprezianane series. Tetrahedron 56, 3717–3723.

Barrero, A.F., Sanchez, J.F., Alvarez-Manzaneda, E.J., Muñoz Dorado, M., Haidour, A., 1994. Terpenoids of the wood of *Abies marocana*. Phytochemistry 35, 1271–1274.

Barrero, A.F., Sanchez, J.F., Alvarez-Manzaneda, E.J., Muñoz Dorado, M., Haidour, A., 1991. Endoperoxide diterpenoids and other terpenoids of the wood of *Abies marocana*. Phytochemistry 35, 1271–1274.

Barrero, A.F., Sanchez, J.F., Altarejos, J., 1987. Resin acids in the woods of *Juniperus sabina* L. and *Juniperus oxycedrus* L. Ars Pharm. 28, 449–457.

Bellakhder, J., 1997. La Pharmacopée Marocaine Traditionnelle. Ibis Press, Paris, pp. 271–272.

Bruno, M., Savona, G., Fernandez-Gadea, F., Rodriguez, B., 1986. Diterpenoids from *Salvia greggii*. Phytochemistry 25, 475–477.

Buratti, L., Allais, J.P., Barbier, M., 1990. A resin acid from *Pinus sylvestris* needles. Phytochemistry 29, 2708–2709.

Cairnes, D.A., Ekundayo, O., Kingston, D.G.I., 1980. Plant anticancer agents. X. Lignans from *Juniperus phoenicea*. J. Nat. Prod. 43, 495–497.

Chang, L.C., Song, L.L., Park, E.J., Luyengi, L., Lee, K.J., Farnsworth, N.R., Pezzuto, J.M., Kinghorn, A.D., 2000. Bioactive constituents of *Thuja occidentalis*. J. Nat. Prod. 63, 1235–1238.

Comte, G., Allais, D., Chulia, A.J., Vercauteren, J., Pinaud, N., 1997. Three phenylpropanoids from *Juniperus phoenicea*. Phytochemistry 44, 1169–1173.

Comte, G., Allais, D.P., Simon, A., Es-Saady, D., Chulia, A.J., Delage, C., Saux, M., 1995. Crystal structure of sandaracopimaric acid, a lipoxygenase inhibitor from *Juniperus phoenicea*. J. Nat. Prod. 58, 239–423.

Cousins, D.J., 1994. Medicinal, Essential Oil, Culinary Herb and Pesticidal Plant of the Labietae, Parts I and II. CAB International, Wallingford.

De Pascual Teresa, J., San Feliciano, A., Egido, T., Barrero, A., 1977. Components of *Juniperus thurifera* L. berries. II. 8α-Acetoxyelemol. An. Qúm. 73, 151–152.

Doi, K., Kawamura, T., 1972. New diterpene from *Juniperus rigida*. Phytochemistry 11, 841–842.

Erdtman, H., Westfelt, L., 1963. Neutral diterpenes from pinewood resins. Acta Chem. Scand. 17, 1826–1827.

Fetizon, M., Moreau, G., Moreau, N., 1968. Determination of the configuration of aldehydes in the 4β-position of the di- or triterpenic series. Bull. Soc. Chim. France 8, 3295–3301.

Fonseca, S.F., Ruveda, E.A., McChesney, J.D., 1980. Carbon-13 NMR analysis of podophyllotoxin and some of its derivatives. Phytochemistry 19, 1527–1530.

Harmatha, J., Budesinsky, M., Trka, A., 1982. Plant substances. XLV. The structure of yatein. Determination of the positions and configurations of benzyl groups in lignans of the 2,3-dibenzylbutyrolactone type. Collect. Czech. Chem. Commun. 47, 644–663.

Hussein, S.A.M., Merfort, I., Nawwar, M.A.M., 2003. Lignans and other phenylpropanoids from leaves of *Juniperus phoenicea*. J. Saudi Chem. Soc. 7, 105–110.

Kaiser, R., Lamparsky, D., 1977. New mono- and sesquiterpenoid constituents from lavender and lavandine oil. Tetrahedron Lett. 7, 665–668.

Kotoda, H., Suzuki, K., Uchida, S., 2002. Antitumor abietane diterpene quinone and its production from conifer bark. JP 2002193869 A2 20020710.

Lee, C., Cheng, Y., 2001. Diterpenoids from the leaves of *Juniperus chinensis* var. *kaizuka*. J. Nat. Prod. 64, 511–514.

Lee, G.H., Lin, C.C., Cheng, Y.S., Peng, S.M., 1987. Structure of methyl *trans*-communate. Acta Crystallogr., Sect. C 43, 1382–1384.

Mei, S., Jiang, B., Niu, X., Li, M., Yang, H., Na, Z., Lin, Z., Li, C., Sun, H., 2002. Abietane diterpenoids from *Coleus xanthanthus*. J. Nat. Prod. 65, 633–637.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Ohtsu, H., Tanaka, R., In, Y., Matsunaga, S., Tokuda, H., Nishino, H., 2001. Abietane diterpenoids from the cones of *Larix kaempferi* and their inhibitory effects on Epstein–Barr virus activation. Planta Med. 67, 55–60.

Rickards, G., Weiler, L., 1978. Stereoselective synthesis of 1-substituted (*E*,*E*)- and (*E*,*Z*)-2,4-decadienyl derivatives. J. Org. Chem. 43, 3607–3609.

San Feliciano, A., Miguel del Corral, J.M., Gordaliza, M., Salinero, M.A., 1993a. Carbon-13 NMR data for abieta-7,13-diene diterpenoids. Magn. Reson. Chem. 31, 841–844.

San Feliciano, A., Miguel del Corral, J.M., Lopez, J.L., de Pascual-Teresa, B., 1993b. Further diterpene acids from *Juniperus thurifera*. Phytochemistry 33, 1165–1167.

San Feliciano, A., Miguel del Corral, J.M., Gordaliza, M., Salinero, M.A., Del Rey, B., 1993c. Acidic constituents of *Juniperus phoenicea* subsp. *turbinata* leaves. Fitoterapia 64, 185–186.

San Feliciano, A., Miguel del Corral, J.M., Lopez, J.L., de Pascual-Teresa, B., 1991. Lignans from polar extracts of *Juniperus thurifera*. Phytochemistry 31, 267–270.

San Feliciano, A., Miguel del Corral, J.M., Gordaliza, M., Castro, A., 1990. Lignans from *Juniperus sabina*. Phytochemistry 29, 1335–1338.

San Feliciano, A., Medarde, M., López, J.L., Miguel del Corral, J.M., Puebla, P., Barrero, A.F., 1989. Lignans from *Juniperus thurifera*. Phytochemistry 28, 2863–2866.

San Feliciano, A., Medarde, M., Lopez, J.L., Miguel del Corral, J.M., Puebla, P., Barrero, A.F., 1988. Terpenoids from leaves of *Juniperus thurifera*. Phytochemistry 27, 2241–2248.

San Feliciano, A., Medarde, M., Lopez, J.L., Miguel del Corral, J.M., Barrero, A.F., 1986. Two new cinnamyl isovalerate derivatives from *Juniperus thurifera* leaves. J. Nat. Prod. 49, 677–679.

Sims, J.J., Pettus, J.A., 1976. Isolation of free *cis* and *trans*-phytol from the red alga *Gracilaria andersoniana*. Phytochemistry 15, 1076–1077.

Smith, W.B., 1978. The carbon-13 NMR spectrum of abietic acid and its methyl ester. Org. Magn. Reson. 11, 427–428.

Stassi, V., Verykokidou, A., Loukis, A., 1996. The antimicrobial activity of the essential oils of four *Juniperus* species growing wild in Greece. Flav. Fragr. J. 11, 71–74.

Sy, L.K., Brown, G.D., 1988. Abietane diterpenes from *Illicium angustisepalum*. J. Nat. Prod. 61, 907–912.

Tabacik, C., Laporthe, Y., 1971. Diterpenes of *Juniperus phoenicea*. Major constituents. Phytochemistry 10, 2147–2153.

Tan, N., Kaloga, M., Radtke, O.A., Kiderlen, A.F., Oksuz, S., Ulubelen, A., Kolodziej, H., 2002. Abietane diterpenoids and triterpenoic acids from *Salvia cilicica* and their antileishmanial activities. Phytochemistry 61, 881–884.

Tschesche, R., Ciper, F., Breitmaier, E., 1977. Monoterpenoid glucosides from the leaves of *Betula alba* and the fruits of *Chaenomeles japonica*. Chem. Ber. 110, 3111–3117.

Wang, S., Wu, J., Shyur, L., Kuo, Y., Chang, S., 2002. Antioxidant activity of abietane-type diterpenes from heartwood of *Taiwania cryptomerioides* Hayata. Holzforschung 56, 487–492.

Wenkert, E., Buckwalter, B.L., 1972. Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances. X. Pimaradienes. J. Am. Chem. Soc. 94, 4367–4369.