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Abietane diterpenes from the cones of *Cedrus atlantica*

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Abstract

Five abietanes, three of them isolated as the corresponding acetate derivatives, i.e., 9α,13α-epidioxiabiet-8(14)-en-18-ol, 7α,18-diacetoxy, 9β,13β-epidioxiabiet-8(14)-ene, 7α,18-diacetoxyabiet-8(14)-en-13β-ol, 7α,18-diacetoxy-13β-methoxyabiet-8(14)-ene, and 13β-hydroxyabiet-8(14)-en-7-one, were isolated from the neutral part of the hexane extract of the cones of *Cedrus atlantica* collected in Middle Atlas, Morocco. The structures of these compounds were established by spectroscopic techniques, including 2D NMR spectra, and in the case of **1**, by chemical correlation. The cytotoxicity of these abietane diterpenoids was tested against five cell lines.

Keywords: *Cedrus atlantica*; Pinaceae; Diterpenoids; Abietanes

1. Introduction

Our interest on the phytochemical study of Spanish and Northern Moroccan plants is aimed at finding both new natural compounds with interesting biological activities, and also investigating the occurrence of natural terpenoids which could be used as enantiomerically pure synthons in the synthesis of added-value compounds. In relation with this, we have evaluated the content of the cones of *Cedrus atlantica* Manetti (Pinaceae), as a renewable source of natural products. This species, only distributed in Morocco and Algeria, possesses a high quality wood, used in construction and handicraft industries. The essential oil from this plant has been shown to possess antiinflammatory (Sugita et al., 2004), antifungal (Bouchra et al., 2003), and antimicrobial (Hammer et al., 1999) activities among others. It also proved to be useful in the treatment of hair loss in a combination of aromatherapy oils (Ormerod et al., 2000). In terms of previous reports on the chemical composition of this plant, the oil from the wood of this species has been extensively studied, sesquiterpenoids of the himachalane type being the major components (Plattier and Teisseire, 1974; Teisseire and Plattier, 1974). In addition, Norin and Winell (1971) reported on the composition of the ether extract of the cones of *C. atlantica*. A number of resin acids, namely abietic, neoabietic, dehydroabietic, levopimaric and palustric acid, were present in the acid fraction. However, only 13-*epi*-manool and 7-oxo-dehydroabietinol were found in the neutral part.

In the present paper, we report on the isolation of five new abietane diterpenoids (**1-5**), together with a number of known compounds, from the cones of *C. atlantica*. The structure elucidation of these new abietanes and

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the evaluation of their activity evaluation are the subjects of this article.

2. Results and discussion

Workup of the neutral part of the hexane extract from the cones of *C. atlantica* led to the isolation of five new hydroxyabietanes (**1**–**5**), three of them isolated and characterized as their acetate derivatives (**2**–**4**). Together with these new natural products, the following known substances were also isolated from this plant: abietinal (**6**) (Erdtman and Westfelt, 1963), dehydroabietinal (**7**) (Fetizon et al., 1968), 13-*epi*-manool (**8**) (Barrero et al., 1993), abietinol (**9**) (San Feliciano et al., 1993), dehydroabietinol (**10**) (Miguel Del Corral et al., 1994), palustradien-18-ol (**11**) (Lee et al., 2001), 7-oxo-dehydroabietinol (**12**) (Rowe et al., 1971), 3β-hydroxy-13-*epi*-manool (**13**) (Barrero et al., 1993), 15-hydroxydehydroabietinol (**14**) (Miguel Del Corral et al., 1994), 13β,18-diacetoxyabiet-8(14)-en-7-one (**15**) (Ohtsu et al., 2000), and 7α-hydroxydehydroabietinol (**16**) (Ohmoto et al., 1987).

The molecular formula of compound **1** was determined as $C_{20}H_{32}O_3$, by HRFABMS ($[M + Na]^+$, *m*/*z* 343.2248). The IR spectrum showed a significant absorption band at ν_{max} 3522 cm^{-1} due to a hydroxyl group. This hydroxy group corresponds to a primary alcohol as deduced from the ^1H and ^{13}C NMR signals at δ_H 3.18 (*d*, *J* = 10.9) and 3.32 (*d*, *J* = 10.9), and δ_C 72.5, respectively. In addition, the ^1H NMR spectrum (Table 1) showed signals characteristic of two tertiary methyls (δ 0.94 and 1.09), one isopropyl (δ 0.97, 6H, *d*, *J* = 6.9 Hz) and an olefinic proton signal (δ 6.08, *d*, *J* = 2.1 Hz). The ^{13}C NMR spectrum (Table 2) confirmed the presence of a trisubstituted double bond (δ 126.7 and 144.7), and also showed signals attributed to the presence of two quaternary oxygenated carbons (δ 81.0 and 79.3). Thus, analysis of its ^1H and ^{13}C NMR data suggested that **1** is based on an epidioxyabietene structure. Comparison of the spectroscopical data of **1** with those of endoperoxide derivatives of palustradienes (Barrero et al., 1991; Monaco et al., 1987) led to locate the endoperoxide bridge between the C-9 and C-13 positions. The correlations observed in the HMBC experiment between H-14 (δ_H 6.08) and C-7 (δ_C 24.4), C-9 (δ_C 81.0), C-13 (δ_C 79.3), and C-15 (δ_C 32.2) confirmed this assignment. The position of the primary alcohol at C-4 was determined from correlations between H-18 (δ_H 3.18 and 3.32) and C-4 (δ_C 38.6), C-19 (δ_C 17.9), and C-3 (δ_C 35.7). With respect to the relative stereochemistry, the enhancements experienced both by H-18 (δ_H 3.18) and Me-20 (δ_H 1.09) when Me-19 (δ_H 0.94)

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

Proton	1	2	3	4	5
1	a: 1.38–1.53 (*m*)	a: 1.50–1.74 (*m*)	α: 1.01–1.09 (*m*)	α: 0.97–1.12 (*m*)	α: 1.04–1.13 (*m*)
	b: 1.53–1.86 (*m*)	b: 1.50–1.74 (*m*)	β: 1.40–1.79 (*m*)	β: 1.38–1.82 (*m*)	β: 1.42–1.82 (*m*)
2	a: 1.53–1.86 (*m*)	a: 1.50–1.74 (*m*)	a: 1.40–1.79 (*m*)	a: 1.38–1.82 (*m*)	a: 1.42–1.82 (*m*)
	b: 1.53–1.86 (*m*)	b: 1.50–1.74 (*m*)	b: 1.40–1.79 (*m*)	b: 1.38–1.82 (*m*)	b: 1.42–1.82 (*m*)
3	a: 1.27–1.35 (*m*)	a: 1.37–1.50 (*m*)	a: 1.28–1.40 (*m*)	a: 1.38–1.82 (*m*)	a: 1.14–1.22 (*m*)
	b: 1.38–1.53 (*m*)	b: 1.50–1.74 (*m*)	b: 1.28–1.40 (*m*)	b: 1.38–1.82 (*m*)	b: 1.42–1.82 (*m*)
5	1.87–2.02 (*m*)	1.78 (*dd*, 5.5, 11.6)	1.40–1.79 (*m*)	1.38–1.82 (*m*)	1.42–1.82 (*m*)
6	a: 1.53–1.86 (*m*)	α: 1.55 (*ddd*, 4.4, 5.5, 14.7)	a: 1.40–1.79 (*m*)	a: 1.38–1.82 (*m*)	α: 2.56 (*dd*, 4.9, 18.7)
	b: 1.53–1.86 (*m*)	β: 2.12 (*ddd*, 6.7, 11.6, 14.7)	b: 1.40–1.79 (*m*)	b: 1.38–1.82 (*m*)	β: 2.29 (*dd*, 13.8, 18.7)
7	a: 2.44–2.62 (*m*)	5.90 (*ddd* 1.9, 4.4, 6.7)	5.34 (*bt*, 2.7)	5.42 (*bt*, 3.0)	
	b: 2.44–2.62 (*m*)				
9			1.97–2.06 (*m*)	1.98–2.14 (*m*)	1.91–1.97 (*m*)
11	α: 2.11 (*bd*, 9.8)	α: 1.53 (*dt*, 2.5, 12.6)	a: 1.40–1.79 (*m*)	a: 1.38–1.82 (*m*)	a: 1.42–1.82 (*m*)
	β: 1.38–1.53 (*m*)	β: 2.36 (*ddd*, 3.9, 9.2, 12.6)	b: 1.40–1.79 (*m*)	b: 1.38–1.82 (*m*)	b: 1.42–1.82 (*m*)
12	a: 1.87–2.02 (*m*)	α: 1.50–1.74 (*m*)	a: 1.28–1.40 (*m*)	a: 1.26–1.34 (*m*)	a: 1.42–1.82 (*m*)
	b: 1.38–1.53 (*m*)	β: 2.02–2.19 (*m*)	b: 1.40–1.79 (*m*)	b: 1.38–1.82 (*m*)	b: 1.42–1.82 (*m*)
14	6.08 (*d*, 2.1)	6.50 (*d*, 1.9)	5.78 (*bs*)	5.76 (*bs*)	6.70 (*bs*)
15	1.87–2.02 (*m*)	1.94 (*hp*, 6.9)	1.70 (*hp*, 7.0)	1.38–1.82 (*m*)	1.42–1.82 (*m*)
16	0.97 (*d*, 6.9)	1.01 (*d*, 6.9)	0.81[a] (*d*, 7.0)	0.76[b] (*d*, 6.9)	0.83[c] (*d*, 6.9)
17	0.97 (*d*, 6.9)	1.01 (*d*, 6.9)	0.88[a] (*d*, 7.0)	0.86[b] (*d*, 6.9)	0.94[c] (*d*, 6.9)
18	a: 3.18 (*d*, 10.9)	a: 3.61 (*d*, 11.0)	a: 3.56 (*d*, 11.0)	a: 3.60 (*d*, 11.1)	0.85 (*s*)
	b: 3.32 (*d*, 10.9)	b: 3.89 (*d*, 11.0)	b: 3.87 (*d*, 11.0)	b: 3.91 (*d*, 11.1)	
19	0.94 (*s*)	1.02 (*s*)	0.84 (*s*)	0.87 (*s*)	0.88 (*s*)
20	1.09 (*s*)	1.15 (*s*)	0.79 (*s*)	0.81 (*s*)	0.80 (*s*)
*Me*COOC$_{18}$		2.08 (*s*)	2.04 (*s*)	2.07 (*s*)	
*Me*COOC$_7$		2.08 (*s*)	1.95 (*s*)	1.99 (*s*)	
O*Me*				3.15 (*s*)	

[a–c] 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	31.6	33.9	38.6	38.4	39.0
2	17.6	17.4	18.1	18.1	18.5[e]
3	35.7	36.0	35.9	36.0	41.8
4	38.6	37.2	36.4	36.4	33.2
5	37.3	37.1	41.9	42.1	49.9
6	18.8	27.2	27.0	27.9	37.5
7	24.4	68.0	74.6	74.6	200.8
8	144.7	143.0	139.1	140.9	138.9
9	81.0	81.7	48.0	48.0	51.8
10	39.4	38.9	37.7	38.1	36.1
11	21.7	24.0	17.3	17.1	18.7[e]
12	25.2	27.0	29.3	26.4	29.7
13	79.3	79.8	71.6	75.6	71.9
14	126.7	131.5	134.3	37.9	139.6
15	32.2	32.2	37.9	32.1	37.9
16	17.2[a]	17.2[b]	16.5[c]	17.5[d]	16.3[f]
17	17.5[a]	17.4[b]	17.3[c]	17.7[d]	17.4[f]
18	72.5	72.3	72.4	72.5	32.7
19	17.9	18.4	17.7	16.1	21.3
20	19.5	18.5	14.8	14.4	14.2
CH_3COOC_{18}		21.0	21.0	21.0	
CH_3COOC_7		21.4	21.6	21.6	
CH_3COOC_{18}		170.3	170.9	171.0	
CH_3COOC_7		170.9	169.9	170.0	
OCH_3				49.5	

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

was irradiated, on the one hand; and by H-11β (δ_H 1.38–1.53) after irradiation of Me-20 (δ_H 1.09) on the other, let us assign to **1** the relative configurations shown in Fig. 1. Accordingly, the structure of **1** was determined as 9α,13α-epidioxiabiet-8(14)-en-18-ol. This structural assignment was confirmed by chemical correlation. Thus, oxidation of **1** with PDC/DMF and subsequent treatment with $TMSCHN_2$ led to the corresponding methoxycarbonyl derivative, a compound whose spectroscopical data were coincident with those of methyl 9α,13α-epidioxiabiet-8(14)-en-18-oate, isolated from *Abies marocana* (Barrero et al., 1991).

Compound **2** was isolated after acetylation of the fraction containing the natural alcohol. It was assigned the molecular formula $C_{24}H_{36}O_6$ by HRFABMS ($[M + Na]^+$, *m/z* 443.2410). Its ^{1}H NMR spectrum (Table 1) was similar to that of alcohol **1**, revealing as in **2** the presence of an epidioxyabietene skeleton supporting a primary acetate function on a quaternary carbon. The most significant difference was the presence of an additional secondary acetate group (δ_H 5.90, *ddd*, J = 1.9, 4.4, and 6.7 Hz). The ^{1}H NMR signal at δ_H 2.08 (*s*, 6H), and those appearing in the ^{13}C NMR spectrum (Table 2) at δ_C 21.0, 21.4, 170.3, and 170.9 confirmed the presence of the two acetate functions. The location of the acetoxyl group at C-7 was deduced after observing in the HMBC spectrum correlations between this carbon (δ_C 68.0) and H-14 (δ_H 6.50) and H-6α (δ_H 1.55). In order to establish the relative configuration, different NOEDIFF experiments were performed (Fig. 2). Thus, the configuration at C-7 was assigned to be α on the basis of the enhancements observed at H-14 (δ_H 6.50), H6β (δ_H 2.12) and Me-20 (δ_H 1.15) when H-7 (δ_H 5.90) was irradiated – this α-disposition of the acetate is in agreement with the multiplicity observed for H-7. Also as a result from these experiments, the spatial disposition of the endoperoxide bridge was assigned to be β. In this sense, the enhancement of the signal corresponding to H11α (δ_H 1.53) after irradiating H-5 (δ_H 1.78) was determined (Fig. 2). Then, the structure of **2** proved to be 7α,18-diacetoxy,9β,13β-epidioxiabiet-8(14)-ene.

Compound **3**, as happened with **2**, was isolated as the diacetate derivative of the natural diol. It was attributed the molecular formula $C_{24}H_{38}O_5$ from its HRFABMS ($[M + Na]^+$, *m/z* 429.2631). The most significant bands in the IR spectrum appeared at 3452, 1731 and 1654 cm^{-1}, which can be attributed to a double bond, a carbonyl of ester, and to a hydroxyl group. The NMR data were again consistent with the structure of a $\Delta^{8(14)}$-abietane-type diterpenoid supporting a primary acetate on a quaternary carbon (AB system, δ_A 3.56, *d*, J = 11.0 Hz; δ_B 3.87, *d*, J = 11.0 Hz) and a secondary oxygenated group (δ_H 5.34, *bt*, J = 2.7) (Table 1). Comparison of its ^{13}C NMR with that of **2** revealed the existence of only one tertiary oxygenated carbon (δ_C 71.6), while a new methine group signal can be observed (δ_C 48.0). The multiplicity in ^{1}H NMR of the isopropyl methine signal (*hp*, J = 7.0 Hz), showing coupling only with the

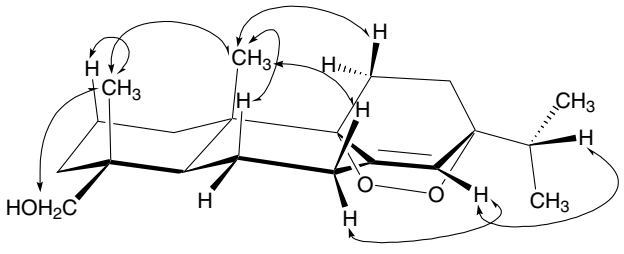


Fig. 1. Selected NOEs observed for **1**.

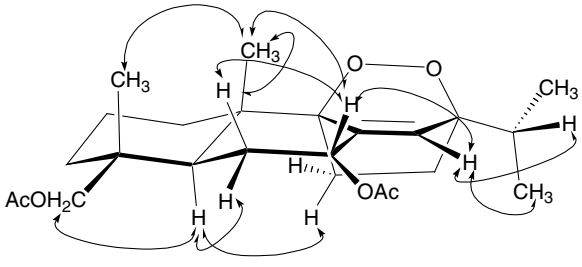


Fig. 2. Selected NOEs observed for **2**.

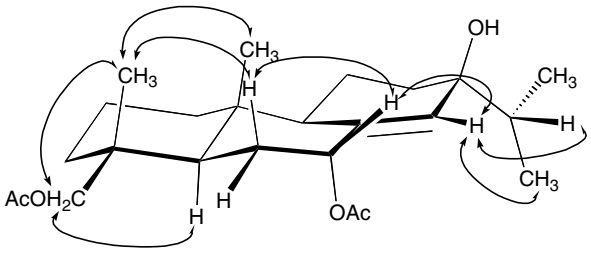


Fig. 3. Selected NOEs observed for **3**.

methyl groups, led to locate the tertiary hydroxyl group at C-13. This location, together with that of the secondary acetate at C-7, was confirmed by HMBC cross-peaks between δ_H 5.78 (H-14) and δ_C 71.6 (C-13), 74.6 (C-7), 48.0 (C-9), and 37.9 (C-15). The multiplicity of the signal corresponding to H-7 (*bt*, $J = 2.7$) suggests an α disposition of the acetate group (Prinz et al., 2002). The relative configuration of this carbon, as well as that of the rest of the chiral centres present in **3**, was confirmed by NOE-DIFF experiences (Fig. 3). Accordingly, the structure of **3** was assigned as 7α,18-diacetoxyabiet-8(14)-en-13β-ol. A natural product derived from **3**, but possessing a forth hydroxyl group at C-15, was isolated from *Larix kaempferi* (Ohtsu et al., 2000).

The molecular formula of compound **4** was deduced as $C_{25}H_{40}O_5$ from its HRFABMS ($[M + Na]^+$, *m/z* 443.2789). A comparison of the spectroscopic data of **4** and **3** suggested that **4** is the methyl ether derivative of alcohol **3**. The ^1H NMR signal at δ_H 3.15 (*s*, 3H), and that appearing in the ^{13}C NMR spectrum at δ_C 49.5, confirmed this assignment.

Compound **5** exhibited the molecular formula $C_{20}H_{32}O_2$, as deduced from its HRFABMS ($[M + Na]^+$, *m/z* 327.2294). The IR spectrum showed absorption bands at ν_{max} 3464 cm^{-1} indicating a hydroxyl group, and at 1682 and 1613 cm^{-1}, which could be assigned to an α,β-unsaturated carbonyl group. This carbonyl assignment was supported by the ^{13}C NMR signal at δ_C 200.8. Compared to those of **3** and **4**, the ^1H NMR spectrum of **5** revealed two major differences: the lack of the signal due to the secondary acetate, and the presence of a new methyl group on a quaternary carbon instead of the primary oxygenated function. Furthermore, the chemical shift observed for H-14 appears in **5** at δ_H 6.70, which together with the HMBC correlation observed between C-7 and H-14 confirmed the presence of an α,β-unsaturated carbonyl group. Thus, the structure of **5** was assigned to be 13β-hydroxyabiet-8(14)-en-7-one.

A number of biological activities have been described for abietane diterpenoids, namely antimicrobial, antiulcer, and cardiovascular activities (Tan et al., 2002), antileishmanial activity (Tan et al., 2002), antioxidant (Wang et al., 2002), antitumor activity (Kotoda et al., 2002), tuberculostatic, antiplatelet agregation activity, antiviral, and several others (Cousins, 1994). However, after noticing the promising results as antitumor promotors obtained by abietanes structurally related to those described herein (Ohtsu et al., 2000, 2001), research on the cytotoxicity of the abietanes **1**–**3** and **5** was carried out. 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. All tested compounds were considered inactive (IC_{50} values higher 5 μg/ml).

3. Conclusion

The neutral fraction of the cones of *C. atlantica* is constituted mainly by diterpenic compounds, most of them possessing an abietane skeleton. Although five new natural compounds have been isolated from this plant, it can be concluded that its content in terpenoid compounds does not differ much from that found in other Pinaceae species.

Since the photo-induced reaction of palustric acid with singlet oxygen has been reported to give the corresponding 9,13-epidioxyabietene (Barrero et al., 1991), it should not be ruled out that compounds **1** and **2** are artefacts of the Soxhlet extraction and/or manipulation of the extract. With respect to compounds **3**–**5**, although the formation of 9,13-dihydroxy-abiet-8(14)-enes from abietic acid under various storage conditions has been reported (Prinz et al., 2002), a mixture of epimers at C-13 was obtained in all cases. The isolation in *C. atlantica* of only the 13β-derivatives seems to indicate that their formation involves an enzymatically-promoted oxidation process.

Finally, these abietane derivatives do not show relevant cytotoxic activity, although closely related derivatives have been shown to possess moderate or potent inhibitory effects on the early antigen activation of Epstein-Barr virus, induced by the tumor promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate.

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 (^1H 400 MHz/^{13}C 100 MHz) spectrometer, and a Bruker AMX

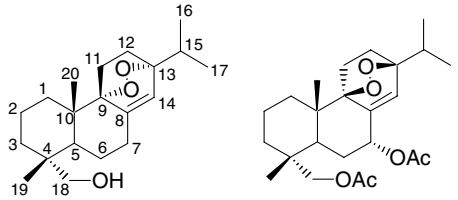
300 (^{1}H 300 MHz/^{13}C 75 MHz) spectrometer. 70 eV Mass spectra (EIMS) were run with a Hewlett–Packard 5972A mass spectrometer coupled to a Hewlett–Packard 5890A gas chromatograph. The accurate mass determination was carried out with an AutoSpec-Q mass spectrometer arranged in an EBE geometry (Micromass Instruments, Manchester, UK) and equipped with a FAB (LSIMS) source. Silica gel SDS 60 (35–70 μm) was used for column chromatography.

4.2. Plant material

The cones of *C. atlantica* Endl (Pinaceae) were collected from the region of Ifrane, Middle Atlas, Morocco, in August 2002. Voucher specimens are available for inspection at the herbarium of the Scientific Institute of the University of Mohamed V, Rabat.

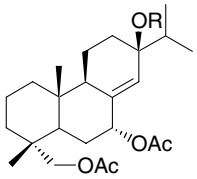
4.3. Extraction and isolation

The plant material (715 g) was powdered and extracted in a Soxhlet apparatus with *n*-hexane, resulting in 50 g of extract. This material was defatted, then dissolved in *t*-BuOMe and fractionated with 1 N NaOH. The organic layer was concentrated under vacuum and 12 g of neutral fraction were obtained. This neutral fraction was subjected to column chromatography over silica gel using mixtures of *n*-hexane/*t*-BuOMe of increasing polarity as eluents to give nine fractions (F-1–F-9). The less polar fraction (350 mg) was eluted with *n*-hexane and was composed by a mixture of hydrocarbons that was not further studied. F-2 (*n*-hexane/*t*-BuOMe, 95:5) was constituted by 1050 mg of a mixture of compounds, abietinal (**6**) and dehydroabietinal (**7**) being the major ones. F-3 (*n*-hexane/*t*-BuOMe, 9:1) contained mainly 13-*epi*-manool (**8**) (160 mg). F-4 (*n*-hexane/*t*-BuOMe, 85:15) was further chromatographed to obtain abietinol (**9**), dehydroabietinol (**10**) and palustradien-18-ol (**11**). F-5 (*n*-hexane/*t*-BuOMe, 7:3) was composed of 495 mg of a mixture that after acetylation and subsequent chromatography on silica gel gave 102 mg of **5**. F-6 (*n*-hexane/*t*-BuOMe, 1:1) was further chromatographed to yield 250 mg of **1** and 191 mg of 7-oxodehydroabietinol (**12**). F-7 (*n*-hexane/*t*-BuOMe, 2:3) consisted of 200 mg containing mainly 3β-hydroxy-13-*epi*-manool (**13**). F-8 (*n*-hexane/*t*-BuOMe, 1:4) (300 mg) was re-subjected to column chromatography to give 95 mg of 15-hydrodehydroabietinol (**14**) and 180 mg of a mixture that after acetylation and flash chromatography yielded 48 mg of 13β,18-diacetoxyabiet-8(14)-en-7-one (**15**). The most polar fraction was eluted with *t*-BuOMe and was constituted by 120 mg of 7α-hydroxydehydroabietinol (**16**), and a mixture that after acetylation and column chromatography gave 24 mg of **4**, 52 mg of **2**, and 47 mg of **3**.

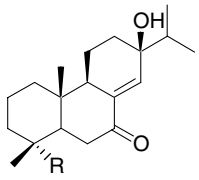


1

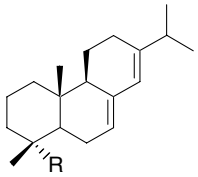
2



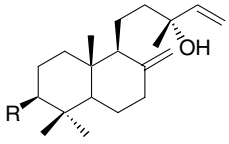
3 R = H
4 R = Me



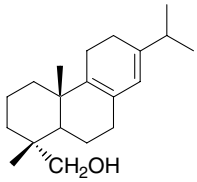
5 R = Me
15 R = CH_2OAc



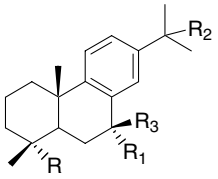
6 R = CHO
9 R = CH_2OH



8 R = H
13 R = OH



11



7 R = CHO, R_1 = H, R_2 = H, R_3 = H
10 R = CH_2OH, R_1 = H, R_2 = H, R_3 = H
12 R = CH_2OH, R_1,R_3 = O, R_2 = H
14 R = CH_2OH, R_1 = H, R_2 = H, R_3 = OH
16 R = CH_2OH, R_1 = OH, R_2 = H, R_3 = H

4.4. 9α,13α-epi-Dioxiabiet-8(14)-en-18-ol (*1*)

Colorless oil, $[\alpha]_D$ −51.8° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3522, 2924, 2855, 1463, 1385, 1261, 1157, 1037, cm^{-1}; HRFABMS *m/z* 343.2248 (calcd for $C_{20}H_{32}O_3Na$, 343.2249).

4.5. 7α,18-Diacetoxy,9β,13β-epi-dioxiabiet-8(14)-ene (2)

Colorless oil, $[\alpha]_D$ +6.2° (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 2932, 2872, 1736, 1465, 1377, 1240, 1037 cm^{-1}; HRFABMS *m/z* 443.2410 (calcd for $C_{24}H_{36}O_6Na$, 443.2409).

4.6. 7α,18-Diacetoxyabiet-8(14)-en-13β-ol (3)

Colorless oil, $[\alpha]_D$ +14.7° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3452, 2929, 2854, 1731, 1654, 1466, 1370, 1242, 1038 cm^{-1}; HRFABMS *m/z* 429.2631 (calcd for $C_{24}H_{38}O_5Na$ 429.2617).

4.7. 7α,18-Diacetoxy-13β-methoxyabiet-8(14)-ene (4)

Colorless oil, $[\alpha]_D$ +43.7° (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 2955, 2927, 2854, 1736, 1456, 1370, 1240, 1038 cm^{-1}; HRFABMS *m/z* 443.2789 (calcd for $C_{25}H_{40}O_5Na$ 443.2773).

4.8. 13β-Hydroxyabiet-8(14)-en-7-one (5)

Colorless oil, $[\alpha]_D$ +45.0° (*c* 0.34, $CHCl_3$); IR (film) ν_{max} 3464, 2952, 2927, 2853, 1682, 1613, 1462, 1386, 1251, 1175 cm^{-1}; HRFABMS *m/z* 327.2294 (calcd for $C_{20}H_{32}O_2Na$ 343.2300).

4.9. Oxidation of 1 to methyl 9α,13α-epidioxiabiet-8(14)-en-18-oate

To a solution of **1** (20 mg, 0.06 mmol) in 1 ml of dry DCM was added 76 mg (0.20 mmol) of PDC were added. The mixture was stirred at room temp. for 5 h. Then, it was filtered trough a pad of 3 cm of SiO_2 using *t*-BuOMe, washed with brine, dried over Na_2SO_4 and concentrated to give 19 mg of aldehyde. A solution of 52 mg of $NaClO_2$ (0.50 mmol), 52 mg of NaH_2PO_4 (0.37 mmol) and 1 ml of H_2O was added over a solution of the aldehyde (19 mg, 0.06 mmol) in 1 ml of *t*-BuOH and 0.5 ml of 2-methyl-2-butene. The mixture was stirred at room temp. for 4 h. Then, after removing *t*-BuOH, 1 ml of H_2O was added, and the resulting mixture extracted with *t*-BuOMe. The extracts were washed with brine, dried over Na_2SO_4 and concentrated to give 15 mg of the corresponding acid. To a solution of 15 mg of this acid (0.05 mmol) in 0.1 ml of dried MeOH and 0.2 ml of dried benzene was added under inert atmosphere a solution of 0.11 ml of $TMSCHN_2$ (0.08 mmol) and 0.2 ml of dried benzene. After stirring for 12 h, the volatile components were evaporated. The crude was cromatographed (*n*-hexane/*t*-BuOMe, 4:1) to give 11 mg (0.03 mmol) of methyl 9α,13α-epidioxiabiet-8(14)-en-18-oate.

4.10. 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 can be measured spectrophotometrically. 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, 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.

Acknowledgements

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