Diterpenoids from Tetraclinis a rticulata that Inhibit Various Human Leukocyte Functions

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JULY 2003

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Diterpenoids from *Tetraclinis articulata* that Inhibit Various Human Leukocyte Functions

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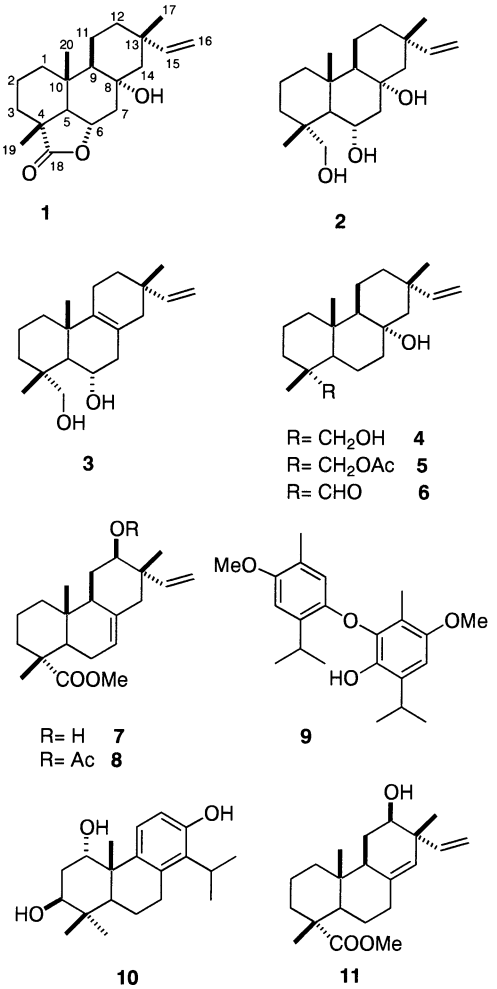
Received October 21, 2002

Ten new compounds, eight of them pimarane derivatives (**1**−**8**), together with a menthane dimer (**9**) and a totarane diterpenoid (**10**), were isolated from the leaves and wood of *Tetraclinis articulata*. The structures of **1**−**10** were established by using spectroscopic techniques, including 2D NMR spectra. Pimaranes **1**−**5** were found to possess an unusual *cis* interannular union of the B and C rings, which, from a biogenetic perspective, could be derived from the hydration of a carbocation at C-8. Compounds **4**−**6** and a mixture of **7** and **11** modulated different human leukocyte functions at a concentration of 10 μM, mainly the degranulation process measured as myeloperoxidase release and, to a lesser extent, the superoxide production measured by chemiluminescence.

The current interest of our group in 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 natural sources of intermediates for the synthesis of high added-value compounds. In this connection we have studied *Tetraclinis articulata* (Vahl) Masters (Cupressaceae), also known as *Thuja articulata* (Vahl) or *Callitris quadrivalvis* Vent., a monospecific species distributed predominantly in North Africa. It has been known since ancient times for its resistance to adverse environmental conditions, including fire and drought, which makes it a useful tree for infertile and nonarable lands. The wood and its veneer are also highly prized in the handicraft industry. In North Africa, different parts of the tree are used in traditional and veterinary medicine, principally targeted at intestinal and respiratory illnesses as well as skin conditions.[1,2] In terms of previous reports on the chemical composition of this plant, apart from two essential oil studies,[2,3] only the isolation of sandarac acids from sandarac gum and totarolone has been described.[4,5] Continuing our research on different coniferous species from Spain and Morocco,[6,7] we report herein the isolation of eight new pimarane diterpenoids (**1**−**8**), a new aromatic menthane dimer (**9**), and a new totaratriol (**10**), together with a number of known compounds, from the leaves and wood of *T. articulata*. We also report here the evaluation of some pimarane derivatives on the inhibition of different human leukocyte functions such as the degranulation process measured as myeloperoxidase and elastase release, and the superoxide production measured by chemiluminescence. These phenomena are involved in a large number of pathophysiological functions,[8,9] and their modulation is considered an interesting strategy in the control of inflammatory disorders.[10]



Results and Discussion

Workup of the hexane extract from the leaves of *T. articulata* led to the isolation of two new pimarane diterpenoids (**1** and **2**), whereas four new natural pimaranes were isolated from the ethyl acetate extract (**4**−**8**). Finally,

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10.1021/np0204949 CCC: $25.00
Published on Web 06/05/2003

Table 1. NMR Data for Compound **1** (δ in ppm, J in Hz)

position	δ ^{1}H	COSY	δ ^{13}C	HMBC
1	α: 0.94 (m) β: 1.81−1.88 (m)	α: H1β, H-2 β: H1β	41.6	β: C-2
2	1.62−1.74 (m)	H1α, H-3α	19.0	
3	α: 1.41 (m) β: 1.81−1.88 (m)	α: H-3β, H-2 β: H-3α	32.8	α: C-4 β: C-2
4			41.3	
5	1.49 (d, 11.4)	H-6	59.4	C-4, C-6, C-7, C-10, C-19
6	4.20 (dt, 4.8, 11.8)	H-5, H-7α, H-7β	73.7	
7	α: 1.61 (t, 11.8) β: 2.37 (dd, 4.8, 11.8)	α: H-7β, H-6 β: H-7α, H-6	47.0	α: C-5, C-8, C-6, C-14 β: C-5, C-8, C-9 C-6, C-14
8			74.0	
9	1.37 (1H, d, 6.6)	H-11α	53.7	C-1, C-8, C-10, C-12, C-14
10			37.0	
11	α: 1.95 (m) β: 1.69 (m)	α: H-9, H-11β, H-12β β: H-11α	17.4	α: C-9, C-10, C-12, β: C-12, C-13
12	α: 1.79 (m) β: 1.50 (dt, 4.9, 14.9)	α: H-12β β: H-11α, H-12α	30.1	α: C-9, C-13, C-14 β: C-13
13			35.9	
14	α: 1.68 (bd, 13.7) β: 1.59 (d, 13.7)	α: H-14β β: H-14α	48.4	C-8, C-13, C-15, C-17
15	5.98 (dd, 10.8, 17.9)	H-16 *cis*, H-16 *trans*	148.0	C-12, C-13
16	*cis*: 5.10 (d, 10.8) *trans*: 5.14 (d, 17.9)	*cis*: H-15 *trans*: H-15	112.4	*cis*: C-13, C-15 *trans*: C-13, C-15
17	0.93 (s)		32.7	C-12, C-13, C-14, C-15,
18			181.3	
19	1.21 (s)		16.5	C-3, C-4, C-5, C-18,
20	1.06 (s)		18.5	C-1, C-5, C-9, C-10

three more new compounds, a pimarane derivative (**3**), a menthane dimer (**9**), and an abietane (**10**), were isolated from the wood chloroform-soluble extract. Together with these new natural products, the following known substances were also isolated from this plant: cedrol,[11] α- and β-acorenol,[12] *p*-methoxythymol,[13] ferruginol,[14] totarol,[15] totarolone,[15] totaradiol,[16] totarolenone,[17] isopimaric acid,[18] sandaracopimaric acid,[19] methyl 12β-hydroxysandaracopimarate (**11**),[4] and the lignans deoxypodophyllotoxin[20] and methyl β-peltatin B.[20]

Compound **1** exhibited the molecular formula $C_{20}H_{30}O_3$, as deduced from its HRFABMS ([M + Na]$^+$, *m*/*z* 341.2095). The most significant bands in the IR spectrum appeared at ν_{max} 1770, 1634, and 3522 cm^{-1}, which can be attributed to a γ-lactone moiety, to a double bond, and to a hydroxyl group. The ^{1}H NMR spectrum showed signals due to three methyl groups, appearing as singlets at δ 0.93, 1.06, and 1.21, a secondary oxygenated group (δ 4.20, dt, J = 4.8, J = 11.8 Hz), and an ABX system corresponding to a vinyl moiety on a quaternary carbon (A: δ 5.10, d, J = 10.8 Hz; B: δ 5.14, d, J = 17.9 Hz; C: δ 5.98, dd, J = 10.8, J = 17.9 Hz). The ^{13}C NMR spectrum confirmed the presence of a lactone (δ 181.3 and 73.7) and indicated the presence of a quaternary oxygenated carbon (δ 74.0). The combined analysis of the HETCOR, COSY, and HMBC spectra (Table 1) permitted the assignment for this compound of a pimarane skeleton,[21,24] possessing a γ-lactone, a tertiary alcohol, and a vinyl group. For the location of the lactone between C-18 and C-6 were the correlations found in the HMBC spectrum between C-18 and Me-19 and between C-6 and H-7 and H-5, with the multiplicities of both H-7 proton signals (H-7α: t, J = 11.8 Hz; H-7β: dd, J = 4.8, J = 11.8 Hz) used to confirm these assignments. Finally, the long-range correlations of C-8 with H-14 and H-9 indicated that the tertiary alcohol was at the C-8 position. To establish the relative configuration, different NOEDIFF experiences were performed (Figure 1). Thus, the NOE correlations of Me-20 with Me-19 and H-6 confirmed the α-disposition of the δ-lactone ring, an orientation that is in agreement with the multiplicity observed for H-6 (two axial and one equatorial coupling). Unexpectedly, a NOE correlation between Me-20 and H-14β was also observed, which suggested a rather unusual *cis* arrangement of the B and C rings. The α-equatorial disposition of the tertiary alcohol was confirmed after observing the small deshielding experienced by Me-20 ($\Delta\delta$: +0.06) when the ^{1}H NMR spectra run in $CDCl_3$ and C_5D_5N were compared. Finally, the NOE correlations between H-15 and H-14α, and between H-16 *trans* and H-11α and H-12α, led to the unambiguous assignment of the relative configuration at C-13.

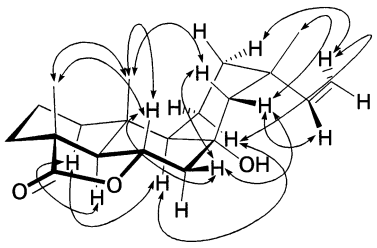

Figure 1. Selected NOEs observed for **1**.

Compound **2** was assigned the molecular formula $C_{20}H_{34}O_3$ through the analysis of its HRFABMS ([M + Na]$^+$, *m*/*z* 345.2404). Its IR spectrum showed a strong absorption due to one or more hydroxyl groups. Its ^{1}H NMR spectrum was very similar to that of **1**, with the major difference being the appearance of an AB system due to a primary alcohol on a quaternary center (A: δ 3.02, dd, J = 7.2 and 11.4 Hz; B: δ 3.47, dd, J = 4.9 and 11.4 Hz),

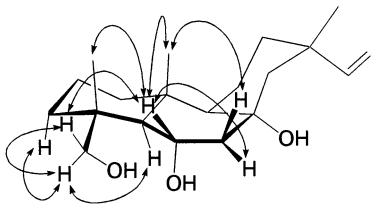

Figure 2. Selected NOEs observed for **2**.

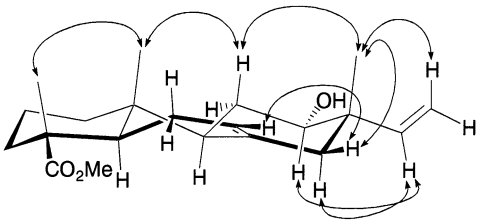

Figure 3. Selected NOEs observed for **7**.

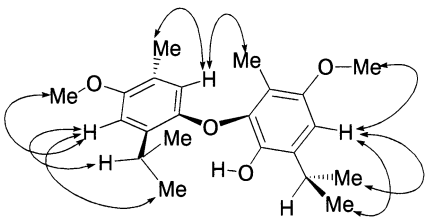

Figure 4. Selected NOEs observed for **9**.

instead of the carbonyl carbon of the γ-lactone as in **1**. The ^{13}C NMR spectrum confirmed the presence of the primary alcohol (δ 67.8), while the carbonyl carbon signal for **1** did not appear. This suggested that compound **2** is the triol resulting from the reduction of **1**, an assignment that was corroborated by 2D NMR studies (COSY-DQF, HETCOR, HMBC) and by chemical correlation. Thus, $LiAlH_4$ reduction of **1** gave **2** as the only reaction product. However, despite the lack of ambiguity of its assignment, the multiplicities observed for H-5 (d, J = 6.5 Hz) and H-7α (dd, J = 2.3 and 15.1 Hz) in compound **2** indicated a deviation from the expected *anti* disposition of H-5, H-6, and H-7α (see Figure 1). The values of the coupling constant measured for these protons could be explained if the B ring adopts a half-chair conformation (Figure 2). To account for this ring conformation change, a relief of steric strain due to the relative *syn* disposition of C-18 and the hydroxyl group at C-6, together with the possible existence of hydrogen bonding in the resulting conformation, was proposed. Finally, the selected NOEs shown in Figure 2 agree with the conformational proposal.

The molecular formula of compound **3** was deduced as $C_{20}H_{32}O_2$ from its HRMS ([M + Na]$^+$, *m/z* 327.2302). Its ^{1}H NMR spectrum was very similar to that of triol **2**, revealing as in **2** the presence of a Δ^{15}-pimarene skeleton, supporting a primary alcohol on a quaternary carbon (AB system, δ_A 3.20, d, J = 11.2 Hz; δ_B 3.54, d, J = 11.2 Hz) and a secondary hydroxyl group (δ 4.14, bdt, J = 7.5 and 10.8 Hz). On comparison of the ^{13}C NMR data of compounds **2** and **3**, it was observed that the latter contained a second double bond (δ 137.1 and 122.5), suggesting that **3** is the result of the dehydration of the hydroxyl group at C-8 on **2**, generating a tetrasubstituted Δ^8-double bond. This assignment was corroborated by 2D NMR techniques.

The spectral data of compound **4** were again consistent with the structure of a Δ^{15}-pimarene-type diterpenoid, but possessing only a primary (^{1}H NMR: AB system, δ_A 3.07, d, J = 10.9 Hz; δ_B 3.37, d, J = 10.9 Hz; ^{13}C NMR: δ 72.2) and a tertiary hydroxyl group (δ 73.2). The location of the oxygenated functions at C-8 and C-18 was corroborated by the analysis of 2D NMR techniques (HMBC and HMQC). Confirmation of these assignments was made by comparing the ^{13}C NMR data of **4** with those of a 8β,19-dihydroxypimarene derivative isolated from the liverwort, *Jungermannia thermarum*.[25] Noting the β-disposition of the tertiary hydroxyl group in this derivative from *J. thermarum*, the interannular junction of the B and C rings in **4** was again carefully studied. As in the case of **1** and **2**, this spatial disposition was determined to be *cis* on the basis of both the correlation observed between Me-20 and H-14β and the deshielding experienced by Me-20 ($\Delta\delta$: −0.04) when comparing the ^{1}H NMR spectra of the natural diol run in both $CDCl_3$ and C_5D_5N.

Compounds **5** and **6** were the corresponding 18-acetate and 18-aldehyde derivatives of compound **4**. The signals due to the acetate moiety in **5** appeared at δ 2.06 (C*H*$_3$CO) in its ^{1}H NMR spectrum and at δ 21.1 (*C*H$_3$CO) and 171.4 (CH$_3$*C*O) in its ^{13}C NMR spectrum. In turn, the NMR data for the aldehyde group in compound **6** appeared at δ 9.18 and 206.3, respectively, in its ^{1}H and ^{13}C NMR spectra. Compounds **5** and **6** were obtained, respectively, by acetylation and PDC/oxidation of diol **4**.

Compound **7** exhibited in its IR spectrum absorptions due to a hydroxyl group (ν_{max} 3480 cm^{-1}), an ester group (ν_{max} 1725 cm^{-1}), and a double bond (ν_{max} 1636 cm^{-1}). Its molecular formula, $C_{21}H_{32}O_3$, was deduced from HRFABMS ([M + Na]$^+$, *m/z* 355.2250). The ^{1}H NMR data were very similar to those of methyl isopimarate, but possessed an additional secondary hydroxyl group (δ 3.52, dd, J = 4.4 and 11.5 Hz). The location of the hydroxyl group at C-12 was confirmed after observing in the HMBC spectrum correlations between C-12 and H-14, H-11, and Me-17. The values of the coupling constants found for H-12 (4.4 and 11.5 Hz) suggested a β disposition for this hydroxyl group. The relative stereochemistry of all the chiral centers present in this compound was confirmed by NOEDIFF experiments (Figure 3).

A comparison of the spectroscopic data of **7** and **8** indicated that **8** is the acetate derivative of alcohol **7**. The generation of **8** by treating **7** with Ac_2O/pyr confirmed this assignment.

Compound **9** was attributed the molecular formula $C_{22}H_{30}O_4$ from its HREIMS ([M]$^+$, *m/z* 358.2134). The analysis of both its ^{1}H and ^{13}C NMR spectra revealed the presence of two aromatic rings. Only three aromatic protons were observed (three singlets at δ 6.24, 6.64, and 6.80), while the substituents observed were the following: two methyl groups, two isopropyl groups, two methoxy groups, and a hydroxyl group (ν_{max} 3559 cm^{-1}). Since two of the aromatic ring substituents remained to be defined, and noticing that only one oxygen atom needed to be located, the presence of an oxygen bridge linking the two aromatic rings was inferred. The final assignment for **9** of a dimeric *p*-menthane structure linked through C-5 and C-2′ was made after the analysis of the correlations observed in both the HMBC and NOEDIFF experiments (Figure 4).

Compound **10** exhibited in its IR spectrum absorptions due to a hydroxyl group (ν_{max} 3413 cm^{-1}) and an aromatic ring (ν_{max} 1585 and 1541 cm^{-1}). Its molecular formula, $C_{20}H_{30}O_3$, was deduced from its HRFABMS ([M + Na]$^+$, *m/z* 341.2097). The ^{1}H NMR spectrum showed signals characteristic of three tertiary methyls [one isopropyl, two oxygenated methines (δ 3.86 and 4.40)] and an aromatic AB system (δ 6.60 and 6.99, J = 8.5 Hz). The multiplicities and chemical shifts in the ^{13}C NMR spectrum indicated

Table 2. ^{1}H NMR Data for Compounds **2**−**6** (δ in ppm, J in Hz)

proton	**2**	**3**	**4**	**5**	**6**
1	α: 0.97 (dt, 3.7, 13.7) *β*: 1.66−1.82 (m)	α: 0.97−1.40 (m) *β*: 1.46−1.92 (m)	α: 0.89 (m) *β*: 1.65−1.89 (m)	α: 0.89 (m) *β*: 1.68−1.92 (m)	α: 0.96 (m) *β*: 1.20−2.01 (m)
2	1.44−1.70 (m)	1.44−1.70 (m)	1.21−1.62 (m)	1.24−1.62 (m)	1.20−2.01 (m)
3	a: 1.21 (m) b: 1.44−1.70 (m)	0.97−1.40 (m)	1.21−1.62 (m)	1.24−1.62 (m)	1.20−2.01 (m)
5	1.63 (d, 6.5)	1.52 (d, 10.8)	1.21−1.62 (m)	1.24−1.62 (m)	1.20−2.01 (m)
6	4.05 (m)	4.14 (dt, 7.5, 10.8)	1.65−1.89 (m)	1.68−1.92 (m)	1.20−2.01 (m)
7	α: 1.74 (dd, 2.3, 15.1) *β*: 1.95 (dd, 5.0, 15.1)	α: 1.97 (dd, 8.5, 16.8) *β*: 2.43 (dd, 8.5, 16.8)	a: 1.21−1.62 (m) b: 1.65−1.89 (m)	a: 1.24−1.62 (m) b: 1.68−1.92 (m)	1.20−2.01 (m)
9	1.44−1.70 (m)		1.21−1.62 (m)	1.24−1.62 (m)	1.20−2.01 (m)
11	1.44−1.70 (m)	1.46−1.92 (m)	1.21−1.62 (m)	1.24−1.62 (m)	1.24−1.62 (m)
12	a: 1.44−1.70 (m) b: 1.66−1.82 (m)	a: 0.97−1.40 (m) b: 1.46−1.92 (m)	1.21−1.62 (m)	1.24−1.62 (m)	1.24−1.62 (m)
14	α: 1.44−1.70 (m) *β*: 1.27 (d, 13.5)	α: 1.73 (d, 16.5) *β*: 1.88 (d, 16.5)	α: 1.21−1.62 (m) *β*: 1.88 (d,14.4)	α: 1.24−1.62 (m) *β*: 1.88 (d,14.3)	α: 1.24−1.62 (m) *β*: 1.88 (d,14.2)
15	6.30 (dd,10.8, 17.7)	5.76 (dd, 10.8, 17.5)	5.76 (dd, 10.9, 17.8)	5.76 (dd, 10.0, 17.8)	5.76 (dd, 10.0, 18.2)
16	*cis*: 4.78 (dd, 1.5, 10.8) *trans*: 4.92 (dd, 1.5, 17.7)	*cis*: 4.93 (dd, 1.4, 10.8) *trans*: 4.89 (dd, 1.4, 17.5)	*cis*: 5.05 (d, 10.9) *trans*: 5.11 (d, 17.8)	*cis*: 5.08 (d, 10.0) *trans*: 5.15 (d, 17.8)	*cis*: 5.12 (dd, 0.9, 10.0) *trans*: 5.17 (d, 0.9, 18.2)
17	1.02 (s)	0.99 (s)	0.93 (s)	0.94 (s)	0.96 (s)
18	a: 3.02 (dd, 7.2, 11.4) b: 3.47 (dd, 4.9, 11.4)	a: 3.20 (d, 11.2) b: 3.54 (d, 11.2)	a: 3.07 (d, 10.9) b: 3.36 (d, 10.9)	a: 3.63 (d, 11.0) b: 3.84 (d, 11.0)	9.18 (s)
19	0.88 (s)	1.00 (s)	0.77 (s)	0.85 (s)	1.09 (s)
20	0.90 (s)	1.02 (s)	1.06 (s)	1.04 (s)	1.09 (s)
*CH$_3$*CO				2.06 (s)	

the presence of three rings in the structure, one of them being aromatic. The combined analysis of the HMQC, COSY-DQF, and HMBC experiments allowed the assignment of a totarane skeleton[26] for this compound, possessing three hydroxyl groups at C-1, C-3, and C-13. The correlations observed in the HMBC experiment between H-1 and C-3 and C-5, between Me-20 and C-1, and between C-3 and Me-18, Me-19, H-1, and H-5 confirmed the location of the oxygenated functions at C-1 and C-3. Finally, the relative configurations at C-1 and C-3 were determined on the basis of the coupling constants observed for the protons at these positions (H-1 bt, J = 2.8 Hz; H-3 dd, J = 4.8 and 12.0 Hz).

Different biological properties have been described for various pimarane derivatives, including antibiotic and spasmolytic activity,[27] antituberculosis activity,[28] inhibitory effects on the mycelial growth of fungi,[29] and the recently reported inhibition of JB6 cell transformation and 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced ornithine decarboxylase activity exhibited by closely related pimaranes from *T. occidentalis*.[24] However, it was the antiinflammatory activity of pimarene derivatives, described in an international patent,[30] that attracted our attention. Besides pimarane diterpenoids, different terpenoids have been reported as potent inhibitors of the inflammatory process.[31,33] In this sense, human neutrophils are mediators of tissue damage in several inflammatory diseases. Among the active substances released by these cells are lysosomal enzymes, such as myeloperoxidase or elastase, which play an important role in tissue destructive events.[34] In this regard, in the present study, we have shown that the tricyclic diterpenoids compounds **4**−**6** and a mixture of **7** and **11** at 10 μM significantly inhibited certain human leukocyte functions such as the degranulation process measured as elastase or myeloperoxidase release and the chemiluminescence response induced by stimulation of neutrophils with TPA (Table 4). Compounds **7** and **11**, sharing a diene group in their structures, were the most interesting and elicited a potent inhibition of cytochalasin B + *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) induced neutrophil degranulation measured as myeloperoxidase release, exerting an inhibitory profile higher than 90% at the concentration of 10 μM. It is interesting to note that compound **6**, which is the only one having an aldehyde group in its tricyclic diterpenoid structure, reduced the chemiluminescence response induced by TPA around 30% at 10 μM. On the other hand, compounds **4**−**6** and the mixture of **7** and **11** were devoid of significant cytotoxic

Table 3. ^{13}C NMR Data for Compounds **2**−**6** (δ in ppm)

carbon	**2**	**3**	**4**	**5**	**6**
1	42.5	36.5	41.0	40.9	40.5
2	19.4	21.4	19.4	19.5	22.0
3	36.9	38.3	35.1	35.6	32.0
4	38.8	38.2	37.5	37.5	37.9
5	55.7	53.0	48.4	49.1	47.6
6	67.8	67.4	17.5	17.4	17.1[a]
7	44.3	43.2	42.5	42.6	42.3
8	74.0	122.5	73.2	72.8	73.0
9	61.5	137.1	54.3	54.3	54.2
10	36.6	40.1	38.9	39.0	39.1
11	18.8	18.4	18.2	18.1	17.3[a]
12	35.0	34.9	30.7	30.6	30.6
13	35.4	35.0	35.5	35.6	35.6
14	49.8	41.6	47.8	47.8	47.7
15	152.6	146.1	148.8	148.8	148.6
16	108.4	111.0	111.7	111.9	112.0
17	28.6	27.6	32.7	32.8	32.8
18	72.6	75.0	72.2	73.0	206.3
19	17.3	18.1	18.0	18.1	14.6
20	18.7	20.5	19.0	19.0	18.9
*C*H$_3$CO				21.1	
CH$_3$*C*O				171.4	

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

Table 4. Effect of Compounds **4**−**6** and a Mixture of **7** and **11** on Human Neutrophil Functions[a]

compound	elastase degranulation release % I (10 μM)	chemiluminescence % I (10 μM)	myeloperoxidase degranulation release % I (10 μM)
4	24.1 ± 5.5	1.8 ± 1.0	46.7 ± 7.3[b]
5	33.5 ± 3.3[b]	6.5 ± 2.2	55.4 ± 5.4[b]
6	17.8 ± 6.4	30.5 ± 2.1[b]	47.8 ± 10.4[b]
7 and **11**	12.5 ± 3.5	11.8 ± 4.2	92.4 ± 7.6[b]

[a] Results show percentages of inhibition at 10 μM. Mean ± SEM (n = 6). [b] $p < 0.01$.

effects on human neutrophils at concentrations up to 10 μM, as assessed by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan (data not shown). These results are very interesting, as neutrophils are recruited into the inflammatory sites in a great variety of chronic diseases. It is known that inhibitors of human neutrophil elastase and myeloperoxidase degranulation may exert potent therapeutic effects on pulmonary emphysema, adult respiratory distress syndrome, and other diseases involving tissue degradation.[35]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer model 141 polarimeter, using $CHCl_3$ as solvent. IR spectra were recorded on a Perkin-Elmer model 983 G spectrometer as NaCl plates (films). NMR studies were performed on a Bruker ARX 400 (^{1}H 400 MHz/^{13}C 100 MHz) spectrometer. Mass spectra were measured on a Hewlett-Packard 5972A mass spectrometer using an ionizing voltage of 70 eV (EIMS) coupled to a Hewlett-Packard 5890A gas chromatograph. HREIMS were obtained on an Autospec-Q VG-Analytical (Fisons) mass spectrometer.

Elastase Release by Human Neutrophils. Leukocytes were obtained and purified as previously described.[36] Neutrophils (2.5 × 10^6/mL) were preincubated with test compounds or vehicle for 5 min and then stimulated with cytochalasin B (10 μM) and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP, 10 nM) for 10 min at 37 °C. After centrifugation at 1200*g* at 4 °C for 10 min, supernatants were incubated with *N*-*tert*-butoxycarbonyl-L-alanine *p*-nitrophenyl ester (200 μM) for 20 min at 37 °C.[37] The extent of *p*-nitrophenol release was measured at 414 nm in a microtiter plate reader. Possible direct inhibitory effects on elastase activity were assessed by preincubating test compounds for 5 min with supernatants of cytochalasin B+fMLP-stimulated human neutrophils, followed by addition of substrate and a 20 min incubation at 37 °C.[38]

Chemiluminescence. Neutrophils (2.5 × 10^6/mL) were mixed with luminol (40 μM) and stimulated with 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 1 μM). The chemiluminescence was recorded in a Microbeta Trilux counter (Wallac, Turku, Finland) after 7 min, previously selected as the time of maximal production.[39]

Myeloperoxidase Release by Human Neutrophils. Aliquots of 1.0 mL of human neutrophils (2.5 × 10^6 cells/mL) were preincubated at 37 °C for 5 min with 10 μL of compounds dissolved in ethanol (or an equivalent volume of ethanol for the controls). After this, the tubes were stimulated for a further 10 min at 37 °C using different stimuli: cytochalasin B (10 μM) and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP, 10 nM). Myeloperoxidase activity was estimated in aliquots of supernatant.[40] The direct effects on myeloperoxidase were also tested using aliquots of supernatants of cytochalasin B+fMLP-stimulated human neutrophils.

Cytotoxicity Assays. The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan[41] was used to assess the possible cytotoxic effects of test compounds on human neutrophils.

Statistical Analysis. The results are presented as means ± SEM. The level of statistical significance was determined by analysis of variance (ANOVA), followed by Dunnett's *t*-test for multiple comparisons.

Plant Material. *Tetraclinis articulata* (Vahl) Masters (Cupressaceae) was collected in April 1998, in the region of Essaouira, Morocco. A voucher specimen is available for inspection at the herbarium of the Scientific Institute of the University of Mohamed V, Rabat.

Extraction and Isolation. The air-dried leaves of *T. articulata* (2.8 kg) were extracted in a Soxhlet apparatus with hexane, resulting in 55 g of crude extract. A 12 g portion was defatted, evaporated under a vacuum, and then dissolved in MeOH and extracted with hexane. The fraction soluble in MeOH (7 g) was subjected to column chromatography over Si gel using mixtures of hexane/*t*-BuOMe of increasing polarity as eluents. Six main fractions were collected. The least polar fraction was constituted by a mixture of esters of fatty acids and sandaracopimaric and isopimaric acids. F_H2 (hexane/*t*-BuOMe, 7:3) consisted of a mixture containing **1**. F_H3 (hexane/*t*-BuOMe, 7:3) was constituted by 58 mg of pure **1**. F_H4 (hexane/*t*-BuOMe, 1:1) was composed of a mixture that repurified by column chromatography over Si gel (hexane/*t*-BuOMe, 2:3) to afford 9 mg of deoxypodophyllotoxin. F_H5 (hexane/*t*-BuOMe, 3:7) was constituted mainly by methyl β-peltatin B. F_H6 (hexane/*t*-BuOMe, 3:7) was flash chromatographed (hexane/*t*-BuOMe, 3:7) to give 24 mg of **2**.

The remaining residue was re-extracted with EtOAc to give 59 g of dried extract. A 14 g portion of this extract was subjected to column chromatography over Si gel eluting with a hexane/*t*-BuOMe/EtOAc gradient system to furnish six main fractions, which were combined after monitoring by TLC. F_{EA}1 (hexane/*t*-BuOMe, 3:1) was mainly constituted by esters of fatty acids. F_{EA}2 (hexane/*t*-BuOMe, 3:2) consisted of a mixture of sandaracopimaric and isopimaric acids. F_{EA}3 (hexane/*t*-BuOMe, 3:2) was rechromatographed to yield 28 mg of sandaracopimaric acid and a mixture that was methylated with diazomethane and then further purified to give 23 mg of **5** and 16 mg of **6**. F_{EA}4 (hexane/*t*-BuOMe, 1:1) was also treated with diazomethane and flash chromatographed (hexane/*t*-BuOMe, 3:2) to yield two fractions. The first was constituted by 62 mg of a mixture of methyl 12β-acetoxysandaracopimarate and **8**. The second fraction was constituted by methyl 12β-hydroxysandaracopimarate (**11**) and **7**, with 6 and 7 mg of these two alcohols isolated after subjecting the mixture to flash chromatography using a 25:1 mixture of toluene/*t*-BuOMe. F_{EA}5 (hexane/*t*-BuOMe, 1:2) was column chromatographed over Si gel to afford 4 mg of 12β-hydroxysandaracopimaric acid and 50 mg of **4**.

The crushed wood (1.4 kg) of *T. articulata* was extracted in a Soxhlet apparatus with chloroform, resulting in 59 g of a dried extract. A 9 g portion was subjected to column chromatography over Si gel using mixtures of hexane/*t*-BuOMe of increasing polarity as eluents. Fractions were combined after monitoring by TLC. F_W1 was subjected to column chromatography over Si gel to give five main fractions, F_W1a−F_W1e. F_W1a (hexane/*t*-BuOMe, 98:2) was constituted by 52 mg of **9**. F_W1b (hexane/*t*-BuOMe, 98:2) was composed by a mixture of 159 mg of totarol and ferruginol. F_W1c (hexane/*t*-BuOMe, 95:5) yielded 47 mg of *p*-methoxythymol. F_W1d (hexane/*t*-BuOMe, 95:5) was flash chromatographed to give 49 mg of α-acorenol and 59 mg of β-acorenol. F_W1e (hexane/*t*-BuOMe, 95:5) was composed mainly by cedrol. F_W2 (hexane/*t*-BuOMe, 3:2) was recrystallized in hexane/dichloromethane to give 130 mg of totarolenone and 24 mg of totarolone. F_W3 (hexane/*t*-BuOMe, 1:1) was also recrystallized in hexane/dichloromethane to give

42 mg of totaradiol. F_W4 (hexane/*t*-BuOMe, 2:3) was composed of 105 mg of **10**.

8α-Hydroxy-13-*epi*-pimar-16-en-6,18-olide (1): white crystals; mp 168−170 °C; $[\alpha]_D$ +22.3° (*c* 0.63, $CHCl_3$); IR (film) ν_{max} 3522, 2940, 2868, 1770, 1634, 1455, 1096, 963, 889 cm^{-1}; EIMS *m/z* 300 $[M - H_2O]^+$ (26), 285 (5), 258 (30), 203 (12), 173 (24), 137 (49), 121 (33), 109 (42), 105 (42), 91 (61), 79 (68), 67 (74), 55 (99), 41 (100); HRFABMS *m/z* 341.2095 (calcd for $C_{20}H_{30}O_3Na$, 341.2093).

13-*epi*-Pimar-16-ene-6α,8α,18-triol (2): white powder; $[\alpha]_D$ +25.2° (*c* 0.56, MeOH); IR (KBr) ν_{max} 3441, 2925, 2868, 1639, 1458, 1382, 1042, 906 cm^{-1}; EIMS *m/z* 304 $[M - H_2O]^+$ (2), 286 (15), 274 (30), 255 (34), 227 (4), 213 (7), 199 (33), 185 (14), 173 (15), 159 (14), 139 (100), 121 (29), 109 (36), 95 (44), 81 (41), 69 (32), 55 (80); HRFABMS *m/z* 345.2404 (calcd for $C_{20}H_{34}O_3Na$, 345.2406).

Reduction of 1 to Obtain 2. To an ice-cooled solution of 15 mg of **1** (0.05 mmol) in 2 mL of THF was added $LiAlH_4$ (8 mg, 0.2 mmol). The mixture was stirred at room temperature for 45 min. After dilution with *t*-BuOMe, a few drops of water were added. The organic layer was then washed with brine, dried with Na_2SO_4, and evaporated under a vacuum. The crude product was column chromatographed to yield 11 mg of **2** after elution with *t*-BuOMe.

13-*epi*-Pimara-8,16-diene-6α,18-diol (3): colorless oil; $[\alpha]_D$ +63.8° (*c* 0.7, $CHCl_3$); IR (film) ν_{max} 3386, 2924, 2871, 1640, 1458, 1374, 1077, 801 cm^{-1}; EIMS *m/z* 304 $[M]^+$ (5), 286 (39), 271 (27), 255 (100), 253 (97), 241 (60), 211 (32), 197 (25), 185 (37), 173 (37), 159 (27), 149 (36), 131 (39), 119 (44), 105 (55), 91 (74), 81 (56), 69 (49), 55 (81); HRFABMS *m/z* 327.2302 (calcd for $C_{20}H_{32}O_2Na$, 327.2300).

13-*epi*-Pimar-16-ene-8α,18-diol (4): colorless oil; $[\alpha]_D$ +58.2° (*c* 0.9, $CHCl_3$); IR (film) ν_{max} 3385, 2921, 2865, 1634, 1450, 1359, 1260, 1101, 1044, 987, 801 cm^{-1}; CIMS *m/z* 305 $[M - 1]^+$ (6), 289 (72), 271 (100), 257 (33), 189 (12), 175 (20), 163 (5), 149 (8), 135 (6), 123 (7), 109 (6), 85 (7); HRFABMS *m/z* 305.2483 (calcd for $C_{20}H_{33}O_2$, 305.2481).

8α-Hydroxy-13-*epi*-pimar-16-en-18-yl acetate (5): colorless syrup; $[\alpha]_D$ +10.0° (*c* 0.6, $CHCl_3$); IR (film) ν_{max} 3560, 3051, 2928, 2858, 1741, 1633, 1451, 1376, 1239, 1037, 801 cm^{-1}; EIMS *m/z* 330 $[M - H_2O]^+$ (8), 315 (6), 288 (3), 270 (7), 257 (19), 255 (39), 241 (6), 227 (5), 213 (7), 199 (9), 187 (20), 173 (11), 161 (15), 145 (17), 135 (30), 119 (25), 105 (43), 91 (56), 81 (70), 67 (62), 55 (100); HRFABMS *m/z* 371.2560 (calcd for $C_{22}H_{36}O_3Na$, 371.2562).

8α-Hydroxy-13-*epi*-pimar-16-en-18-al (6): colorless oil; $[\alpha]_D$ +67.2° (*c* 0.8, $CHCl_3$); IR (film) ν_{max} 3555, 3076, 2924, 2863, 1723, 1633, 1450, 1367, 1260, 1106, 1016, 888 cm^{-1}; CIMS *m/z* 305 $[M + 1]^+$ (9), 287 (92), 273 (57), 257 (100), 243 (26), 231 (11), 215 (6), 189 (9), 175 (8), 163 (7), 149 (9), 135 (7), 123 (8), 109 (8), 85 (10); HREIMS *m/z* 304.2399 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Oxidation of 4 to Obtain 6. To a solution of 23 mg of **4** (0.08 mmol) in 1 mL of DMF was added 77 mg of PDC (0.2 mmol). The mixture was stirred at room temperature for 4 h. Then, a few milliliters of water was added and the mixture extracted with *t*-BuOMe. The organic layer was then dried with Na_2SO_4 and evaporated under a vacuum. The crude product was column chromatographed to yield 13 mg of **6**.

Acetylation of 4 to Obtain 5. To an ice-cooled solution of 20 mg of **4** (0.07 mmol) in 0.2 mL of pyridine were added 0.2 mL of Ac_2O and a catalytic amount of DMAP. The mixture was stirred at 0 °C for 2 h. After the usual workup, the crude product was column chromatographed to give 18 mg of **5** (hexane/*t*-BuOMe, 2:3).

Methyl 12β-hydroxysandaracopimarate (7): colorless oil; $[\alpha]_D$ +8.1° (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 3480, 3079, 2922, 2868, 2849, 1725, 1636, 1435, 1245, 1145, 1065, 913, 808 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, s, H-17), 0.92 (3H, s, H-20), 1.13 (1H, ddd, J = 7.2, 10.1, 13.0 Hz, H-1β), 1.27 (3H, s, H-19), 1.34 (3H, t, J = 12.5 Hz, H-11β), 1.50−1.65 (4H, m, H-2, H-3a, H-6α), 1.72−1.88 (3H, m, H-1α, H-3b, H-11α), 1.95−2.05 (5H, m, H-5, H-6β, H9, H-14), 3.52 (1H, dd, J = 4.4, 11.5 Hz, H-12), 3.65 (3H, s, $COOCH_3$), 5.13 (1H, dd, J = 1.1, 17.5 Hz, H-16a), 5.15 (1H, dd, J = 1.1, 10.8 Hz, H-16b), 5.36 (1H, bd, J = 6.3, H-7), 5.76 (1H, dd, J = 10.8, 17.5 Hz, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.2 (s, $COOCH_3$), 146.6 (d, C-15), 133.7 (s, C-8), 122.1 (d, C-7), 114.4 (t, C-16), 74.2 (d, C-12), 52.0 (q, $COOCH_3$), 50.8 (d, C-9), 46.6 (s, C-4), 45.6 (t, C-14), 45.1 (d, C-5), 42.7 (s, C-13), 38.8 (t, C-1), 37.1 (t, C-3), 35.1 (s, C-10), 27.4 (t, C-11), 25.3 (t, C-6), 18.1 (t, C-2) 17.4 (q, C-19), 15.3 (q, C-20), 13.7 (q, C-17); EIMS *m/z* 332 $[M]^+$ (1), 254 (1), 239 (3), 211 (2), 183 (3), 164 (38), 131 (26), 121 (56), 91 (62), 79 (53), 55 (100); HRFABMS *m/z* 355.2250 (calcd for $C_{21}H_{32}O_3Na$, 355.2249).

Methyl 12β-acetoxysandaracopimarate (8): colorless oil; $[\alpha]_D$ +6.3° (*c* 0.4, $CHCl_3$); IR (film) ν_{max} 3081, 2925, 2852, 1729, 1667, 1640, 1458, 1370, 1240, 1144, 1106, 1031, 970, 918 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (3H, s, H-17), 0.94 (3H, s, H-20), 1.13 (1H, m, H-1β), 1.27 (3H, s, H-19), 1.41 (1H, t, J = 14.2 Hz, H-11β), 1.48−1.63 (4H, m, H-2, H-3a, H-6α), 1.71−1.81 (3H, m, H-1α, H-3b, H-11α), 1.92−2.13 (5H, m, H-5, H-6β, H9, H-14), 2.0 (3H, s, $OCOCH_3$), 3.65 (3H, s, $COOCH_3$), 4.84 (1H, dd, J = 4.6, 11.7 Hz, H-12), 4.98 (1H, dd, J = 1.2, 10.8 Hz, H-16a), 4.99 (1H, dd, J = 1.2, 17.5 Hz, H-16b), 5.37 (1H, bd, J = 6.3, H-7), 5.73 (1H, dd, J = 10.8, 17.5 Hz, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.2 (s, $COOCH_3$), 170.7 (s, $OCOCH_3$), 145.9 (d, C-15), 133.2 (s, C-8), 122.3 (d, C-7), 112.4 (t, C-16), 76.7 (d, C-12), 52.1 (q, $COOCH_3$), 50.4 (d, C-9), 46.6 (s, C-4), 45.5 (t, C-14), 45.0 (d, C-5), 41.0 (s, C-13), 38.8 (t, C-1), 37.0 (t, C-3), 35.1 (s, C-10), 25.6 (t, C-11[a]), 25.3 (t, C-6[a]), 21.3 (s, $OCOCH_3$), 18.0 (t, C-2) 17.4 (q, C-19), 15.5 (q, C-20[b]), 15.2 (q, C-17[b]) ([a,b]assignments with the same superscript letter may be interchanged); HRFABMS *m/z* 397.2352 (calcd for $C_{23}H_{34}O_4Na$, 397.2355).

Acetylation of 7 to Obtain 8. Following the same procedure as described above for the acetylation of **4**, 9 mg of **7** was treated with Ac_2O/DMAP/pyridine to give 8 mg of **8**.

6-Methoxy-2-[(6-methoxymentha-1,3,5-trien-3-yl)oxy]-mentha-1,3,5-trien-3-ol (9): colorless oil; IR (film) ν_{max} 3559, 2959, 2869, 1724, 1656, 1608, 1498, 1460, 1425, 1342, 1260, 1196, 1124, 1087, 1013, 812 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 (6H, d, J = 6.9 Hz, H-8, H-9), 1.36 (6H, d, J = 6.9 Hz, H-8′, H-9′), 1.94 (3H, s, H-10), 2.06 (3H, s, H-10′), 3.35 (1H, sept, J = 6.9 Hz, H-7), 3.56 (1H, sept, J = 6.9 Hz, H-7′), 3.84 (3H, s, OCH_3′), 3.85 (3H, s, OCH_3), 6.24 (1H, s, H-2′), 6.64 (1H, s, H-5), 6.80 (1H, s, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.0 (s, C-5′), 151.5 (s, C-6), 147.7 (s, C-3′), 140.4 (s, C-3), 133.9 (s, C-4′), 132.3 (s, C-4), 124.9 (s, C-1′), 117.5 (C-1), 115.0 (d, C-2′), 109.1 (d, C-3′), 105.3 (d, C-5), 56.2 (q, OCH_3[a]), 56.0 (q, OCH_3[a]), 27.5 (d, C-7), 27.4 (d, C-7′), 23.0 (q, C-8′, C-9′), 22.6 (q, C-8, C-9), 16.0 (q, C-10′), 9.5 (q, C-10) ([a]assignments with the same superscript letter may be interchanged); EIMS *m/z* 358 $[M]^+$ (92), 301 (7), 285 (4), 205 (2), 179 (11), 164 (100), 149 (67), 122 (9), 91 (13), 67 (5); HRFABMS *m/z* 358.2134 (calcd for $C_{22}H_{30}O_4$, 358.2144).

1α,3β-Dihydroxytotarol (10): colorless oil; $[\alpha]_D$ +45.3° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3413, 3050, 2958, 2873, 1702, 1653, 1585, 1541, 1449, 1362, 1281, 1189, 1053, 816 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (3H, s, H-18), 1.13 (3H, s, H-19), 1.25 (3H, s, H-20), 1.35 (3H, d, J = 7.0 Hz, H-16), 1.36 (3H, d, J = 7.0 Hz, H-17), 1.63−1.72 (2H, m, H-5, H-6a), 1.94−2.03 (2H, m, H-2β, H-6b), 2.10 (1H, dt, J = 4.1, 13.8 Hz, H-2α), 2.73 (1H, m, H-7α), 2.97 (1H, dd, J = 4.7, 16.8 Hz, H-7β), 3.28 (1H, sept, J = 6.9 Hz, H-15), 3.86 (1H, dd, J = 4.8, 12.0 Hz, H-3), 4.40 (1H, bt, J = 2.8 Hz, H-1), 6.60 (1H, d, J = 8.5 Hz, H-12), 6.99 (1H, d, J = 8.5, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.8 (s, C-13), 136.7 (s, C-8[a]), 136.6 (s, C-9[a]), 132.5 (s, C-14), 122.4 (d, C-11), 115.3 (d, C-12), 73.7 (d, C-1), 73.2 (d, C-3), 43.7 (s, C-10), 42.7 (d, C-5), 38.9 (s, C-4), 33.0 (t, C-2), 29.6 (t, C-7), 28.2 (q, C-18), 27.4 (d, C-15), 25.5 (q, C-20), 20.3 (q, C-16[b]), 20.2 (q, C-17[b]), 18.7 (t, C-6), 15.4 (q, C-19) ([a,b]assignments with the same superscript letter may be interchanged); HRFABMS *m/z* 341.2097 (calcd for $C_{20}H_{30}O_3Na$, 341.2093).

Acknowledgment. This work was supported by a grant from CICYT (SAF2001-2639), the Spanish Ministerio de Ciencia y Tecnología (Project BQU 2002-03211), from the ESRSFC Ministry of the Moroccan Government (PROTARS

P2T2/07), and from the Junta de Andalucía (Consejería de Presidencia, Programa de Cooperación Andalucía-Marruecos). R.L. was the recipient of a scholarship from the FPI program. The authors also wish to thank Dr. Tony Harris for the translation of the text.

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NP0204949