Neoclerodane Diterpenoids from *Teucrium massiliense*

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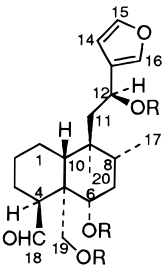
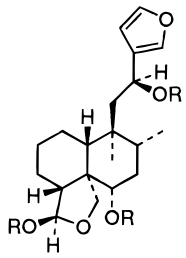
A reinvestigation of the diterpene metabolites of *Teucrium massiliense* L. allowed the isolation of four new neoclerodane derivatives, teumassilenins A−D, together with all the diterpenoids previously reported as constituents of this plant. The structures of the new compounds (**1**−**4**) were established by chemical and spectroscopic means. A plausible biogenetic relationship between several of these substances is briefly discussed, and some unpublished physical and spectroscopic data of the previously known diterpenoid teumassin (**5**) are now reported.

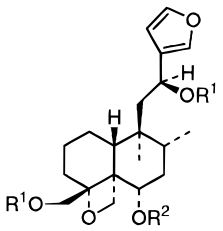
A large number of neoclerodane diterpenoids[1] have been isolated from natural sources in the past few years.[2,3] These compounds have attracted interest owing to their biological activities, especially as insect antifeedants[4−9] and as antifungal, antimicrobial, and moluscicidal agents.[2,3] More recently, interest in neoclerodanes has increased due to their hepatotoxic[10−12] and tumoricidal[13,14] actions. The species belonging to the genus *Teucrium* (family Labiatae) are the most abundant natural source of neoclerodane diterpenoids.[2−8,15] In a continuation of our studies on *Teucrium* plants,[16,17] we have reinvestigated *T. massiliense* L., a species already studied by us some years ago.[18,19] We report here the structural elucidation of four new neoclerodanes found in this plant, as well as some unpublished physical and spectroscopic data of an already known diterpenoid.



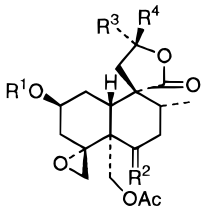
1 R=H
9 R=Ac



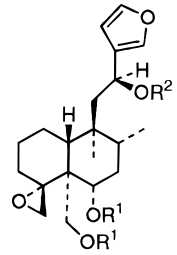
2 R=H
11 R=Ac



	R^1	R^2
3	H	H
12	Ac	Ac
13	Ac	H



	R^1	R^2	R^3	R^4
4	H	αOAc,βH	β-Furyl	H
5	H	O	H	β-Furyl
8	H	αH,βOH	H	β-Furyl
14	Ac	αOAc,βH	β-Furyl	H
15	Ac	αH,βOAc	H	β-Furyl



	R^1	R^2
6	H	H
7	Ac	H
10	Ac	Ac

Results and Discussion

Repeated chromatography of an Me_2CO extract of the aerial parts of *T. massiliense* (see Experimental Section) led to the isolation of the new neoclerodanes teumassilenins A−D (**1**−**4**) along with teumassin (**5**), teumassilin (**6**), 6,-19-diacetylteumassilin (**7**), teumarin (**8**), deacetylajugarin II, montanin C, and teucjaponin A, which were previously found in this plant.[18,19] Teumassin (**5**), which has been described[19] as an amorphous solid, was isolated as a crystalline substance, and its melting point and ^{13}C NMR data (not previously reported[19]) are included in the Experimental Section and Table 2, respectively. The structures of the new diterpenoids were established as follows.

Teumassilenin A (**1**) had the molecular formula $C_{20}H_{30}O_5$, and its IR spectrum showed hydroxyl (3370 cm^{-1}, br), furan (3160, 3130, 1600, 1510, 875 cm^{-1}), and aldehyde (2720, 1720 cm^{-1}) absorptions. Treatment of **1** with Ac_2O−pyridine yielded a triacetyl derivative (**9**, $C_{26}H_{36}O_8$) for which the IR spectrum was devoid of any hydroxyl absorption, thus establishing that teumassilenin A possessed three alcohol functions. The ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 2, respectively) were very similar to those of teumassilin[18] (**6**), showing almost identical signals for a β-substituted furan, a hydroxymethylene group at the C-19 position, an equatorial hydroxyl group attached to the C-6α position, and another secondary alcohol at C-12. The observed differences between the ^1H and ^{13}C NMR spectra of these diterpenoids were consistent with the presence in **1** of a C-18β aldehyde [δ_{H-18} 9.98, $\delta_{H-4\alpha}$ 3.35; δ_C 46.8 d (C-4) and 208.2 d (C-18)] instead of the 4α,18-oxirane of **6** [C-18 protons at δ 2.40 d, J_{gem} = 4 Hz, and 3.15 dd, $J_{18B,3\alpha}$ = 2.4 Hz; δ_C 67.7 s (C-4) and 48.1 t (C-18)].[18] The configuration of the C-18 aldehyde of **1** must be β, as was revealed by the coupling values of the H-4α proton with both of the C-3 methylene protons ($J_{3\alpha,4\alpha}$ = 2.6 Hz, $J_{3\beta,4\alpha}$ = 5.1 Hz), which precluded an axial−axial relationship between these protons. This conclusion was strongly supported by NOE experiments because irradiation at δ 9.98 (aldehyde proton) caused NOE enhancement, among others, in the signal of the H-10β (δ 2.03) axial proton.

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**3** and **14**

proton(s)	**1**	**2**	**3**	**14**	$J_{H,H}$ (Hz)	**1**	**2**	**3**	**14**
H-1α	1.28 (dddd)	*b*	∼2.15[a]	∼1.90[a]	1α,1*β*	12.7	*b*	*b*	*b*
H-1*β*	∼1.75[a]	*b*	∼1.80[a]	∼1.90[a]	1α,2α	3.9	*b*	*b*	3.4
H-2α	∼1.70[a]	*b*	∼1.65[a]	5.17 (quint)	1α,2*β*	12.5	*b*	*b*	
H-2*β*	1.48 (ddddd)	*b*	∼2.10[a]		1α,10*β*	12.5	*b*	*b*	*b*
H-3α	∼1.75[a]	*b*	∼1.45[a]	2.50 (ddd)	1*β*,2α	*b*	*b*	*b*	2.5
H-3*β*	1.88 (dddd)	*b*	∼1.65[a]	1.30 (ddd)[c]	1*β*,2*β*	4.5	*b*	*b*	
H-4α	3.35 (ddd)				1*β*,10*β*	2.7	*b*	*b*	*b*
H-4*β*		*b*			2α,2*β*	13.6	*b*	*b*	
H-6*β*	3.98 (br dd)	3.53 (dd)	3.76 (dd)	4.83 (ddd)	2α,3α	*b*	*b*	*b*	3.5
H-7α	∼1.50[a]	*b*	1.29 (ddd)	∼2.10[a]	2α,3*β*	3.2	*b*	*b*	2.4
H-7*β*	∼1.50[a]	*b*	∼1.65[a]	1.50 (*b*r dt)	2*β*,3α	13.3	*b*	*b*	
H-8*β*	∼1.58[a]	*b*	∼1.70[a]	1.84 (ddq)	2*β*,3*β*	4.5	*b*	*b*	
H-10*β*	2.03 (dd)	*b*	∼1.80[a]	∼1.95[a]	3α,3*β*	12.8	*b*	*b*	14.8
H_A−11	1.54 (dd)	*b*	1.47 (dd)	2.19 (dd)	3α,18B			0	2.2
H_B−11	1.79 (dd)	*b*	1.94 (dd)	2.44 (dd)	3α,4α	2.6			
H-12	4.62 (dd)	4.77 (dd)	4.78 (dd)	5.35 (*b*r t)	3α,4*β*		*b*		
H-14	6.31 (dd)	6.39 (dd)	6.38 (dd)	6.35 (dd)	3*β*,4α	5.1			
H-15	7.30 (t)	7.37[a]	7.35 (t)	7.45 (t)	3*β*,4*β*		*b*		
H-16	7.31 (m)	7.37[a]	7.34 (m)	7.41 (m)	4α,18	2.4			
Me-17	0.72 (3H, d)	0.74 (3H, d)	0.72 (3H, d)	1.11 (3H, d)	4*β*,18α		0		
H_A−18	9.98 (d)	4.89 (s)	3.42 (d)	2.25 (d)[d]	6*β*,7α	11.0	11.4	11.4	11.2
H_B−18			3.81 (d)	3.02 (dd)[e]	6*β*,7*β*	4.0	4.0	4.8	4.2
H_A−19	3.75 (br d)	3.84 (d)	4.58 (d)	4.47 (dd)	7α,7*β*	*b*	*b*	13.0	13.7
H_B−19	4.17 (d)	4.05 (d)	4.66 (d)	5.27 (d)	7α,8*β*	*b*	*b*	11.9	11.1
Me-20	0.57 (3H, s)	0.51 (3H, s)	0.49 (3H, s)		7*β*,8*β*	*b*	*b*	*b*	3.8
OAc				2.06 (3H, s)	8*β*,17	6.1	6.6	6.6	6.9
				2.01 (3H, s)	11A,11B	15.6	*b*	15.5	14.1
				1.96 (3H, s)	11A,12	3.9	3.4	2.6	8.2
					11B,12	7.8	8.0	9.0	8.9
					14,15	1.8	1.8	1.6	1.7
					14,16	0.8	0.9	0.6	0.9
					15,16	1.7	*b*	1.6	1.7
					18A,18B			13.0	4.1
					19A,19B	11.7	9.1	6.0	13.3
					19A,6*β*	<0.5	0	0	1.0

[a] This is an overlapped signal. For compounds **1**, **3**, and **14**, the *δ* values of the overlapped signals were determined from the HMQC spectra. [b] Value not measured due to strong overlapping of the signal. [c] This proton shows a W-type coupling $^4J_{3\beta,1\beta} = 1.7$ Hz. [d] This is the exo hydrogen with respect to ring B. [e] This is the endo hydrogen with respect to ring B.

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**3**, **5**, and **14**

carbon	**1**	**2**	**3**	**5**	**14**
C-1	21.28 (t)[a]	21.4 (t)	20.1 (t)	30.8 (t)	27.1 (t)
C-2	22.5 (t)	23.3 (t)	17.3 (t)	65.3 (d)	69.5 (d)
C-3	21.33 (t)[a]	28.5 (t)	31.4 (t)	40.5 (t)	36.6 (t)
C-4	46.8 (d)	53.9 (d)	91.3 (s)	58.0 (s)	61.3 (s)
C-5	45.9 (s)	50.6 (s)	50.9 (s)	53.9 (s)	45.1 (s)
C-6	74.6 (d)	77.9 (d)	70.1 (d)	206.5 (s)	71.8 (d)
C-7	35.8 (t)	37.0 (t)	36.2 (t)	43.6 (t)	32.7 (t)
C-8	34.8 (d)	35.1 (d)	34.4 (d)	41.3 (d)	40.7 (d)
C-9	38.8 (s)	39.4 (s)	39.7 (s)	51.2 (s)	50.6 (s)
C-10	42.9 (d)	44.3 (d)	40.1 (d)	47.9 (d)	44.7 (d)
C-11	44.5 (t)	43.9 (t)	43.2 (t)	43.0 (t)	43.0 (t)
C-12	62.6 (d)	63.0 (d)	63.0 (d)	72.1 (d)	71.5 (d)
C-13	130.8 (s)	130.8 (s)	131.0 (s)	124.8 (s)	124.7 (s)
C-14	108.3 (d)	108.3 (d)	108.3 (d)	107.9 (d)	107.9 (d)
C-15	143.3 (d)	143.6 (d)	143.5 (d)	144.4 (d)	144.5 (d)
C-16	138.4 (d)	138.4 (d)	138.3 (d)	139.5 (d)	139.6 (d)
C-17	15.6 (q)	15.6 (q)	15.6 (q)	17.1 (q)	16.6 (q)
C-18	208.2 (d)	104.4 (d)	65.2 (t)	49.3 (t)	48.5 (t)
C-19	60.6 (t)	68.3 (t)	70.2 (t)	61.3 (t)	61.5 (t)
C-20	18.5 (q)	16.6 (q)	15.7 (q)	177.0 (s)	175.7 (s)
OAc				170.9 (s)	170.4 (s)
				20.9 (q)	170.1 (s)
					169.9 (s)
					21.2 (q)
					21.2 (q)
					21.1 (q)

[a] These assignments may be interchanged.

From all the above data, it was evident that teumassilenin A possessed the structure depicted in **1**, except for its absolute configuration and the stereochemistry of the C-12 stereogenic center.

Thermal treatment of 6,12,19-tri-*O*-acetylteumassilin[18] (**10**) at 210 °C for 15 min in the absence of solvent[20] yielded a compound identical in all respects (mp, mixed mp, $[\alpha]_D$, ^{1}H NMR, TLC) with the tri-*O*-acetyl derivative **9** of teumassilenin A (see Experimental Section[21]). This correlation established a neoclerodane absolute configuration[1,18] and a 12*S* stereochemistry[18] for teumassilenin A (**1**).

Teumassilenin B (**2**, $C_{20}H_{30}O_5$) possessed a C-18, C-19-hemiacetal grouping instead of the C-18 aldehyde and the C-19 hydroxyl functions of **1**. That structural moiety of **2** was supported by its ^{1}H- and ^{13}C NMR spectral data [$\delta_{H-18\alpha}$ 4.89 s, $J_{18\alpha,4\beta} = 0$ Hz, C-19 protons at *δ* 3.84 and 4.05, both d, $J_{gem} = 9.1$ Hz; δ_C 53.9 d (C-4), 104.4 d (C-18), and 68.3 t (C-19), see Tables 1 and 2] and by those of its tri-*O*-acetyl derivative **11** ($\delta_{H-18\alpha}$ 5.89 d, $J_{18\alpha,4\beta} = 1.7$ Hz, $\delta_{H-4\beta}$ 2.24 m, $W_{1/2} = 9$ Hz), which were almost identical with those reported[22−25] for other (4*R*,18*R*)-neoclerodane-18,19-hemiacetal derivatives previously isolated from *Teucrium* species. The 12*S* configuration of **2** was in agreement with the variation of the molecular rotation values of **2** and **11** ($[M]_D$ −19° and −169°, respectively) because it is known[26] that acetylation of a 12*S* hydroxyl group in this kind of compounds produces a negative increment in the $[M]_D$ value, whereas in the case of the 12*R* epimer this transformation causes a positive increment in the molecular rotation of the 12-*O*-acetyl derivative. Although this comparison should be taken cautiously due to the additional esterification of the C-6α and C-18*β* hydroxyl groups in **11**, the acetylation at C-12 seems to be the greatest contributor to the change of the molecular rotation,

taking into account that the furan is the most polarizable group.[26] This is also supported by the behavior of several (12*S*)-12-hydroxyfuroneoclerodane diterpenoids and their peracetyl derivatives,[26] including **1** and **9** ($\Delta[M]_D$ −264°).

The ^{1}H and ^{13}C NMR spectra of teumassilenin C (**3**, $C_{20}H_{30}O_5$) established for this diterpenoid a neoclerodane framework possessing two secondary hydroxyl groups at the C-6α and C-12 positions and a β-substituted furan in which the C-13−C-16 carbon atoms are involved (see Tables 1 and 2). In addition, compound **3** had two methylene carbons and a quaternary carbon, all of them bearing oxygen atoms (δ_H 3.42 and 3.81 d, J_{gem} = 13.0 Hz, and 4.58 and 4.66 d, J_{gem} = 6.0 Hz; δ_C 65.2 t, 70.2 t, and 91.3 s). Treatment of **3** with Ac_2O−pyridine gave a tri-*O*-acetyl derivative (**12**, $C_{26}H_{36}O_8$), in addition to minor quantities of a diacetate (**13**, $C_{24}H_{34}O_7$) in which the C-6α hydroxyl group was not esterified because its geminal proton resonated at an almost identical field in **3** and **13** ($\delta_{H-6\beta}$ 3.76 dd and 3.74 dd, respectively). Since the IR spectrum of **12** was devoid of hydroxyl absorptions and its ^{1}H NMR spectrum showed paramagnetically shifted H-6β ($\Delta\delta$ +1.04 ppm), H-12 ($\Delta\delta$ +1.14 ppm), and two methylene protons ($\Delta\delta$ +0.52 and +0.57 ppm) with respect to those of **3** ($\delta_{H-6\beta}$ 3.76, δ_{H-12} 4.78, and the doublets at δ 3.42 and 3.81, J_{gem} = 13.0 Hz), it was evident that **3** possessed an ether bridge in which a quaternary carbon and a methylene grouping are involved (see above). This ether must be an oxetane between the C-4α and C-19 positions because the resonances of the C-4 and C-19 carbons of **3** (δ 91.3 s and 70.2 t, respectively) as well as the chemical shift of the C-19 methylene protons (at δ 4.58 and 4.66 d in **3** and at δ 4.59 and 4.74 d in **12**), and especially their geminal coupling value (J_{gem} = 6.0, 6.3 Hz for **3** and **12**, respectively), are almost identical to those reported[27−29] for several neoclerodane derivatives having a 4α,19-oxetane grouping. Moreover, the HMBC spectrum of **3** showed connectivities between the C-4 carbon (δ 91.3 s) and the H_A-18 (δ 3.42), H_B-18 (δ 3.81), and H_A-19 (δ 4.58) protons, whereas the ROESY spectrum evidenced NOE interactions between both C-19 protons (δ 4.58 and 4.66) and those of the Me-20 group (δ 0.49). These results further supported structure **3** for teumassilenin C. The stereochemistry at the C-12 asymmetric center of this diterpenoid must be *S** because the chemical shifts of the H-8β, H-10β, H_A-11, H_B-11, H-12, and Me-17 protons (**3**, Table 1) were almost identical to those reported[30] for 6,19-diacetylteumassilin (**7**), and **3** and **7** displayed the same behavior under NOE experiments (e.g., strong NOE's between H-12 and H_A-11, H-8β, and Me-17 protons and weak NOE's between H-12 and H_B-11 and Me-17 protons), thus establishing an identical C-12 configuration and the same preferred rotamer of the C-9 side chain in both compounds.[30]

Teumassilenins A−C (**1**−**3**) could be biogenetically derived from teumassilin (**6**) as shown in Figure 1. A heterolytic cleavage of the C-4α−oxygen bond of the 4α,18-oxirane of **6** (intermediate **a**) followed by a deprotonation at C-18 could produce the enolic form (**b**) of both C-18 aldehydes teumassilenin A (**1**) and its 4α-epimer (intermediate **c**). The 4α-aldehyde (**c**) may generate teumassilenin B (**2**) by formation of the more stable 18*R*-exo hemiacetal with the C-19 hydroxyl group. Finally, if there is an attack of the C-19 hydroxyl group on the C-4 carbocation of the intermediate **a**, teumassilenin C (**3**) could also be originated.

Although compounds **1**−**3** should be considered as artifacts of **6** formed in the course of the extraction and/or isolation (e.g., as a consequence of an acid catalysis of the silica gel used in the chromatographic process), we suppose that they are natural products in their own right because the opening of a 4α,18-oxirane in neoclerodanes to the intermediate **a** (Figure 1) requires drastic reaction conditions.[20,31] In agreement with this assumption, it is of interest to indicate that although a large number of 4α,-18-epoxyneoclerodane derivatives have been isolated from many *Teucrium* plants[2−4,15] only in a few species have 4α,-19-oxetane- (such as **3**) and/or (18*R*)-neoclerodane-18,19-hemiacetal derivatives (like **2**) been found. Moreover, teumassilenin A (**1**) is the first example of a 18β-aldehyde from *Teucrium* species, while a neoclerod-3-en-18-al derivative (teuscorodal[32]) and a (18*S*)-neoclerodane-18β,6β-hemiacetal (teuchamaedryn C[33]) have already been isolated from *T. scorodonia* and *T. chamaedrys*, respectively. In any case, it is noteworthy that in the present study on *T. massiliense* we have isolated low quantities of teumassilin (**6**, 0.012% on dry plant material) with respect to our previous investigations on the same species (0.32%[18] and 0.44%[19]), but this could be due to natural variations in the diterpene contents, since the three studies on this plant have been made with different material collections.

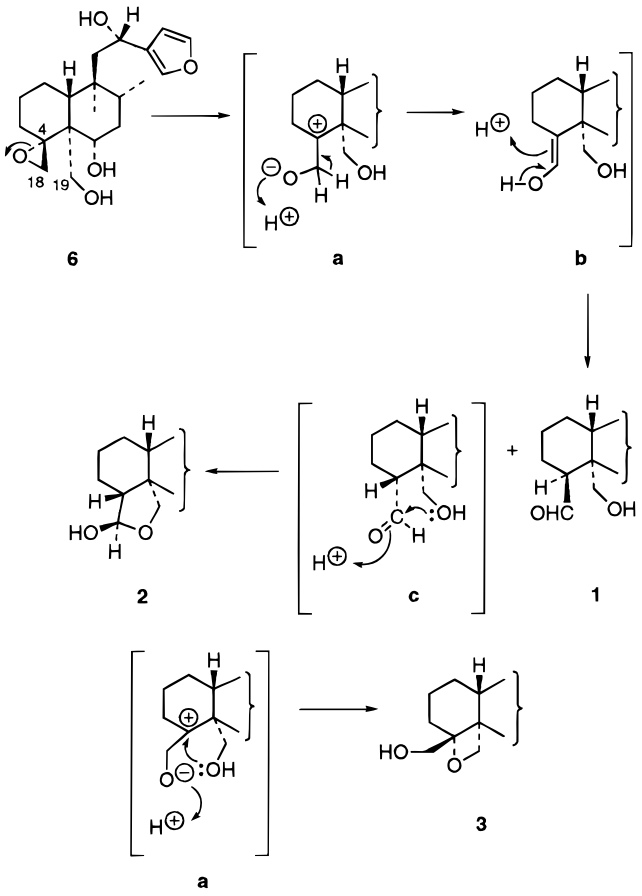

Figure 1. Proposed biogenetic pathway for the formation of teumassilenins A−C (**1**−**3**) from teumassilin (**6**)

The last of the new diterpenoids found in this study was teumassilenin D (**4**), isolated as an impure substance that was purified as its acetyl derivative **14** ($C_{26}H_{32}O_{10}$, see the Experimental Section). The ^{1}H and ^{13}C NMR spectra of **14** (Tables 1 and 2) were very similar to those of the peracetyl derivative (**15**) of teumarin[34] (**8**), except for the C-6 acetoxyl group, which is β-oriented in **15** ($\delta_{H-6\alpha}$ 5.05 t, $J_{6\alpha,7\alpha} = J_{6\alpha,7\beta}$ = 3.5 Hz)[34] and α-oriented in **14** ($\delta_{H-6\beta}$ 4.85 dd, $J_{6\beta,7\alpha}$ = 11.2 Hz, $J_{6\beta,7\beta}$ = 4.2 Hz). Moreover, the ^{13}C NMR chemical shift of the C-1−C-3, C-5, C-7, C-9, C-11−C-17, C-19, and C-20 carbons of **14** and **15** were almost

identical, and the observed differences in the remaining carbons [$\Delta\delta = \delta$(**14**) − δ(**15**): +3.0 (C-4), +2.8 (C-6), +7.4 (C-8), +2.8 (C-10), and −3.4 (C-18) ppm] were easily explained on account of the change in the configuration of the C-6 acetoxyl group in both compounds. The ROESY spectrum of **14**, as well as monodimensional NOE experiments, clearly revealed that this substance possessed a 12*R** configuration because irradiation at the Me-17 protons (δ 1.11) caused, among others, a positive NOE enhancement (4%) in the signal of the H-12 proton (δ 5.35) and not in those of the H-14 and H-16 furan protons.[35] From all the above data, it was evident that **14** was the diastereomer of **15**[34,36] at the C-6 and C-12 positions. As impure teumassilenin D showed signals for two acetoxyl groups (δ 1.98 and 2.07, both 3H, s) and at δ 4.29 (1H, br, $W_{1/2}$ = 6 Hz), 4.85 (1H, dd, J = 11.0, 4.3 Hz), and 5.27 and 4.46 (1H each, both d, J_{gem} = 13.4 Hz) for the H-2α, H-6β, and C-19 methyl-ene protons, respectively, and they resonated at δ 5.17, 4.83, and 5.27 and 4.47 in the acetyl derivative **14** (see Table 1), it was clear that natural teumassilenin D possessed the structure depicted in **4** with a free hydroxyl group at the C-2β position. The absolute stereochemistry of **14**, and therefore of **4**, was not ascertained. However, on biogenetic grounds, we suppose that these compounds belong to the neoclerodane series,[1] like the other diterpenoids isolated from *Teucrium* plants.[2,3,5,6,15−20,22−30,32−36]

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. IR spectra (KBr or NaCl) were obtained on a Perkin-Elmer 681 spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ solution using a Varian Unity-500 or Varian INOVA-300 apparatus at 500 (compounds **1**−**3** and **14**) or 300 MHz (compounds **4**, **9**, and **11**−**13**), respectively, and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25). ^{13}C NMR spectra were recorded in $CDCl_3$ at 125.7 MHz, and chemical shifts are reported with respect to solvent signals (δ_{CDCl3} 77.00). ^{13}C NMR assignments were determined by HMQC and HMBC spectra. MS were recorded in the positive EI mode on a Hewlett-Packard HP 5989A instrument, and no fragments below *m*/*z* 50 were registered. Elemental analyses were made with a Carlo Erba EA 1108 apparatus. Merck Si gel no. 7734 (70−230 mesh) deactivated with 15% H_2O, w/v, was used for column chromatography.

Plant Material. *T. massiliense* L. was collected in August 1993 in the Gennargentu Mountains, Sardinia, Italy, and voucher specimens are deposited in the Herbarium of the Dipartimento di Biologia of the University of Milan, Italy.

Extraction and Isolation. Dried and finely powdered aerial parts of *T. massiliense* (850 g) were extracted with Me_2CO (5 L × 3) at room temperature for 5 days. The extract (125 g) was chromatographed on a Si gel column (1.5 kg) eluted with petroleum ether (bp 50−70 °C), a petroleum ether−EtOAc gradient from 10% to 100%, and finally EtOAc−MeOH (9:1), yielding the following compounds in order of increasing chromatographic polarity: 6,19-diacetylteumassilin[18] (**7**, 700 mg), montanin C[18] (3.3 g), teucjaponin A[18] (2.1 g), a mixture of compounds (2 g), deacetylajugarin II[18] (4 g), and crude teumarin[18,34,36] (**8**, 5 g). Repeated column chromatography [Si gel, petroleum ether−EtOAc (7:3−2:3) as eluent] of the mixture of compounds allowed the isolation of teumassilenin A (**1**, 200 mg, less polar constituent), teumassilenin B (**2**, 250 mg), teumassilenin C (**3**, 125 mg), and teumassilin[18] (**6**, 100 mg). Rechromatography of crude teumarin [4.8 g, Si gel column, $CHCl_3$−MeOH (99:1) as eluent] gave teumassin[19] (**5**, 300 mg) and pure teumarin[18,34,36] (**8**, 3.7 g, most polar constituent), together with crude teumassilenin D (**4**, 200 mg, less polar constituent). Attempts at isolating pure **4** were unsuccessful, and this substance was characterized as its 2-*O*-acetyl derivative **14** (42 mg), which was obtained after treating crude **4** (100 mg) with Ac_2O−pyridine in the usual manner, followed by chromatographic purification [column chromatography, Si gel, petroleum ether−EtOAc (1:1) as eluent].

All the previously known diterpenoids were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (^{1}H NMR, IR, MS) data and by comparison (mixed mp, TLC) with authentic samples.

Teumassin (**5**), which was previously described as an amorphous solid,[19] was crystallized (mp 188−189 °C, from EtOAc − *n*-hexane), and its unreported ^{13}C NMR spectral data[19] are included in Table 2.

Teumassilenin A [(12*S*)-15,16-Epoxy-6α,12,19-trihydroxyneocleroda-13(16),14-dien-18β-al] (1): mp 187−190 °C (EtOAc−*n*-hexane); $[\alpha]^{20}_D$ +2.0° [*c* 0.855, $CHCl_3$−MeOH (9:1)]; IR (KBr) ν_{max} 3370 br (OH), 3160, 3130, 1600, 1510, 875 (furan), 2720, 1720 (aldehyde), 2940, 2860, 1450, 1390, 1290, 1160, 1080, 1030, 1020, 1000, 800, 735 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; EIMS *m*/*z* (rel int) 350 $[M]^+$ (1), 335 (4), 332 (16), 320 (4), 314 (21), 302 (15), 247 (10), 236 (18), 220 (83), 208 (89), 190 (100), 175 (82), 173 (55), 161 (60), 149 (40), 148 (42), 135 (30), 123 (32), 121 (35), 119 (38), 107 (34), 105 (38), 97 (84), 95 (69), 91 (45), 81 (48), 79 (45), 69 (68), 55 (48); *anal.* C 68.43%, H 8.66%, calcd for $C_{20}H_{30}O_5$, C 68.54%, H 8.63%.

Teumassilenin B [(12*S*,18*R*)-15,16-Epoxy-6α,12-dihydroxyneocleroda-13(16),14-diene-18,19-hemiacetal] (2): mp 65−70 °C, amorphous solid; $[\alpha]^{21}_D$ −5.4° (*c* 0.718, $CHCl_3$); IR (KBr) ν_{max} 3350 br (OH), 1500, 870 (furan), 2930, 2860, 1460, 1380, 1160, 1100, 1080, 1010, 750 cm^{-1}; ^{1}H NMR seeTable 1; ^{13}C NMR see Table 2; EIMS *m*/*z* (rel int) 350 $[M]^+$ (1), 332 (8), 314 (7), 221 (10), 219 (100), 173 (94), 159 (37), 145 (26), 119 (25), 97 (73), 95 (49), 91 (31), 81 (36), 79 (29), 69 (50), 55 (42); *anal.* C 68.73%, H 8.51%, calcd for $C_{20}H_{30}O_5$, C 68.54%, H 8.63%.

Teumassilenin C [(12*S*)-4α,19;15,16-Diepoxyneocleroda-13(16),14-diene-6α,12,18-triol] (3): mp 125−127 °C (EtOAc−*n*-hexane); $[\alpha]^{20}_D$ −33.4° (*c* 0.605, $CHCl_3$); IR (KBr) ν_{max} 3400, 3300, 3200 (OH), 1510, 875 (furan), 2960, 2880, 1470, 1385, 1310, 1155, 1140, 1060, 1035, 1020, 1000, 970, 840 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR seeTable 2; EIMS *m*/*z* (rel int) 350 $[M]^+$ (1), 335 (7), 332 (8), 319 (61), 301 (12), 283 (21), 238 (67), 220 (75), 202 (34), 189 (82), 174 (41), 173 (57), 161 (63), 147 (58), 137 (89), 121 (54), 119 (49), 107 (47), 105 (42), 97 (100), 95 (72), 91 (50), 81 (57), 79 (48), 69 (86), 55 (63); *anal.* C 68.38%, H 8.59%, calcd for $C_{20}H_{30}O_5$, C 68.54%, H 8.63%.

2-*O*-Acetylteumassilenin D [(12*R*)-2β,6α,19-Triacetoxy-4α,18;15,16-diepoxyneocleroda-13(16),14-dien-20,12-olide] (14): mp 88−96 °C; amorphous solid; $[\alpha]^{20}_D$ +1.3° (*c* 0.226, $CHCl_3$); IR (KBr) ν_{max} 3140, 3130, 1505, 875 (furan), 1760 (γ-lactone), 1740, 1730 br, 1240 (OAc), 2960, 2940, 2880, 1450, 1370, 1190, 1145, 1085, 1050, 1025, 980, 925 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; EIMS *m*/*z* (rel int) 505 $[MH]^+$ (1), 445 (3), 444 $[M - AcOH]^+$ (3), 413 (7), 401 (9), 372 (30), 371 (37), 342 (53), 329 (90), 324 (42), 312 (100), 294 (30), 267 (38), 251 (46), 218 (39), 202 (42), 185 (36), 157 (42), 133 (28), 105 (34), 95 (66), 81 (50), 55 (26); *anal.* C 61.73%, H 6.51%, calcd for $C_{26}H_{32}O_{10}$, C 61.89%, H 6.39%.

Teumassilenin A Triacetate (9). Treatment of **1** (30 mg) with Ac_2O−pyridine (1:1, 6 mL) at room temperature for 20 h yielded **9** (20 mg after crystallization from EtOAc−*n*-hexane): mp 163−165 °C; $[\alpha]^{20}_D$ −56.9° (*c* 0.195, $CHCl_3$); IR (KBr) ν_{max} 3140, 1500, 870 (furan), 2740, 1710 (aldehyde), 1740, 1730, 1250, 1230 (OAc), 2940, 2880, 1470, 1370, 1160, 1020, 810, 770 cm^{-1}; ^{1}H NMR δ 9.83 (1H, d, $J_{18,4\alpha}$ = 1.0 Hz, H-18), 7.47 (1H, m, H-16), 7.36 (1H, t, $J_{15,16} = J_{15,14}$ = 1.8 Hz, H-15), 6.38 (1H, dd, $J_{14,16}$ = 0.8 Hz, H-14), 5.89 (1H, dd, $J_{12,11A}$ = 3.6 Hz, $J_{12,11B}$ = 8.5 Hz, H-12), 5.29 (1H, br dd, $J_{6\beta,7\alpha}$ = 8.3 Hz, $J_{6\beta,7\beta}$ = 7.6 Hz, H-6β), 4.74 (1H, d, $J_{19B,19A}$ = 12.2 Hz, H_B-19), 4.17 (1H, br d, $J_{19A,6\beta}$ < 0.6 Hz, H_A-19), 3.01 (1H, m, $W_{1/2}$ = 7 Hz, H-4α), 2.07 (3H, s, OAc), 2.01 (3H, s, OAc), 1.99 (3H, s, OAc), 0.78 (3H, d, $J_{17,8\beta}$ = 6.5 Hz, Me-17), 0.69 (3H, s, Me-20); EIMS *m*/*z* (rel int) $[M]^+$ absent, 416 $[M - AcOH]^+$ (3), 374 (3), 356

(3), 322 (20), 263 (31), 262 (33), 219 (25), 203 (100), 202 (61), 174 (39), 173 (48), 159 (27), 119 (21), 95 (20), 91 (15), 81 (17), 79 (11), 69 (11), 55 (13); *anal.* C 65.69%, H 7.43%, calcd for $C_{26}H_{36}O_8$, C 65.53%, H 7.61%.

Teumassilenin B Triacetate (11). Treatment of **2** (50 mg) with Ac_2O−pyridine (1:1, 8 mL) at room temperature for 24 h gave **11** [41 mg, after chromatographic purification, Si gel column, petroleum ether−EtOAc (2:1) as eluent]: thick oil; $[\alpha]^{21}_D$ −35.5° (*c* 0.287, $CHCl_3$); IR (NaCl) ν_{max} 3140, 1500, 875 (furan), 1735 br, 1250 br (OAc), 2950, 2880, 1460, 1370, 1160, 1135, 1050, 1020, 950, 920 cm^{-1}; ^1H NMR δ 7.40 (1H, m, H-16), 7.34 (1H, t, $J_{15,16} = J_{15,14} = 1.6$ Hz, H-15), 6.36 (1H, dd, $J_{14,16} = 0.8$ Hz, H-14), 5.92 (1H, dd, $J_{12,11A} = 3.2$ Hz, $J_{12,11B} = 8.4$ Hz, H-12), 5.89 (1H, d, $J_{18\alpha,4\beta} = 1.7$ Hz, H-18α), 4.74 (1H, dd, $J_{6\beta,7\alpha} = 11.8$ Hz, $J_{6\beta,7\beta} = 4.3$ Hz, H-6β), 4.18 (1H, d, $J_{19B,19A} = 9.1$ Hz, H_B-19), 4.05 (1H, d, H_A-19), 2.24 (1H, m, $W_{1/2} = 9$ Hz, H-4β), 2.10 (3H, s, OAc), 2.02 (3H, s, OAc), 2.01 (3H, s, OAc), 0.73 (3H, d, $J_{17,8\beta} = 6.7$ Hz, Me-17), 0.62 (3H, s, Me-20); EIMS *m*/*z* (rel int) 476 $[M]^+$ (1), 416 $[M - AcOH]^+$ (100), 373 (11), 314 (14), 297 (15), 262 (38), 241 (78), 223 (13), 221 (20), 219 (30), 203 (75), 202 (58), 175 (33), 173 (40), 159 (21), 147 (20), 145 (20), 133 (20), 121 (28), 119 (25), 105 (22), 97 (24), 95 (33), 94 (36), 91 (28), 81 (41), 79 (21), 55 (20); *anal.* C 65.43%, H 7.49%, calcd for $C_{26}H_{36}O_8$, C 65.53%, H 7.61%.

Teumassilenin C Triacetate (12) and 12,18-Di-*O*-acetylteumassilenin C (13) from Teumassilenin C (3). Treatment of **3** (60 mg) with Ac_2O−pyridine (1:1, 10 mL) at room temperature for 20 h yielded a mixture of **12** and **13** (61 mg), which was easily separated by column chromatography [Si gel, petroleum ether−EtOAc (1:1) as eluent], yielding pure **12** (33 mg, less polar constituent) and **13** (22 mg).

Compound 12: mp 100−102 °C (EtOAc−*n*-hexane); $[\alpha]^{21}_D$ −14.0° (*c* 0.428, $CHCl_3$); IR (KBr) ν_{max} 3140, 3120, 1500, 875 (furan), 1740, 1730, 1250, 1230 (OAc), 2970, 2890, 1380, 1160, 1020, 985, 790, 730 cm^{-1}; ^1H NMR δ 7.40 (1H, m, H-16), 7.36 (1H, t, $J_{16,15} = J_{16,14} = 1.7$ Hz, H-15), 6.37 (1H, dd, $J_{14,16} = 0.8$ Hz, H-14), 5.92 (1H, dd, $J_{12,11A} = 2.9$ Hz, $J_{12,11B} = 8.9$ Hz, H-12), 4.80 (1H, dd, $J_{6\beta,7\alpha} = 11.6$ Hz, $J_{6\beta,7\beta} = 4.5$ Hz, H-6β), 4.74 (1H, d, $J_{19B,19A} = 6.3$ Hz, H_B-19), 4.59 (1H, d, H_A-19), 4.38 (1H, d, $J_{18B,18A} = 11.1$ Hz, H_B-18), 3.94 (1H, d, H_A-18), 2.11 (3H, s, OAc), 2.02 (3H, s, OAc), 2.01 (3H, s, OAc), 0.70 (3H, d, $J_{17,8\beta} = 6.5$ Hz, Me-17), 0.57 (3H, s, Me-20); EIMS *m*/*z* (rel int) $[M]^+$ absent, 417 $[M - AcO]^+$ (100), 357 (76), 280 (22), 279 (10), 263 (10), 213 (11), 203 (12), 185 (27), 146 (28), 119 (23), 97 (16), 95 (14), 91 (14), 81 (14), 79 (10), 55 (12); *anal.* C 65.43%, H 7.48%, calcd for $C_{26}H_{36}O_8$, C 65.53%, H 7.61%.

Compound 13: mp 124−126 °C (EtOAc−*n*-hexane); $[\alpha]^{21}_D$ −23.6° (*c* 0.195, $CHCl_3$); IR (KBr) ν_{max} 3440 (OH), 3150, 3120, 1600, 1505, 875 (furan), 1750, 1730, 1250 (OAc), 2970, 1435, 1375, 1160, 1060, 1020, 960, 950, 855, 810, 740 cm^{-1}; ^1H NMR δ 7.40 (1H, m, H-16), 7.36 (1H, t, $J_{15,16} = J_{15,14} = 1.8$ Hz, H-15), 6.38 (1H, dd, $J_{14,16} = 0.9$ Hz, H-14), 5.92 (1H, dd, $J_{12,11A} = 2.3$ Hz, $J_{12,11B} = 9.2$ Hz, H-12), 4.64 (1H, d, $J_{19B,19A} = 6.6$ Hz, H_B-19), 4.61 (1H, d, $J_{18B,18A} = 13.1$ Hz, H_B-18), 4.48 (1H, d, H_A-18), 4.43 (1H, d, H_A-19), 3.74 (1H, dd, $J_{6\beta,7\alpha} = 11.1$ Hz, $J_{6\beta,7\beta} = 4.3$ Hz, H-6β), 2.77 (1H, br, disappeared after addition of D_2O, 6α-OH), 2.08 (3H, s, OAc), 2.01 (3H, s, OAc), 0.75 (3H, d, $J_{17,8\beta} = 6.4$ Hz, Me-17), 0.50 (3H, s, Me-20); EIMS *m*/*z* (rel int) $[M]^+$ absent, 416 $[M - H_2O]^+$ (1), 403 (18), 375 (52), 374 (25), 357 (44), 356 (30), 315 (83), 301 (30), 296 (29), 280 (47), 239 (30), 202 (37), 189 (68), 173 (50), 161 (80), 147 (85), 133 (67), 119 (85), 105 (68), 97 (78), 95 (100), 94 (75), 91 (78), 81 (88), 69 (70), 55 (81); *anal.* C 66.20%, H 7.82%, calcd for $C_{24}H_{34}O_7$, C 66.34%, H 7.89%.

Thermal Rearrangement of Teumassilin Triacetate (10) into Teumassilenin A Triacetate (9). Compound **10**[18] (20 mg) in a round-bottom flask (2 mL) was heated for 15 min under Ar in a silicone oil bath preheated at 210 °C. The reaction mixture was allowed to cool to room temperature and then subjected to column chromatography [Si gel, EtOAc−petroleum ether (1:1) as eluent], giving **9** (4 mg, less polar constituent) and starting material (**10**, 11 mg). Attempts at improving the yield of **9** were unsuccessful.[21] Compound **9** was identical in all respects (mp, mixed mp, $[\alpha]_D$, ^1H NMR, TLC) to 6,12,19-tri-*O*-acetylteumassilenin A described above.

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References and Notes

(1) Although the hydrocarbon skeleton of these diterpenoids is biogenetically derived from an *ent*-labdane, and they should be named *ent*-clerodanes, we prefer to use the term neoclerodane proposed by Rogers et al. (Rogers, D.; Unal, G. G.; Williams, D. J.; Ley, S. V.; Sim, G. A.; Joshi, B. S.; Ravindranath, K. R. *J. Chem. Soc., Chem. Commun.* **1979**, 97−99) because it is the nomenclature used in the majority of the articles published on this subject since 1979.
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(21) Attempts at obtaining **1** from **6** by thermal treatment[20] were unsuccessful and always caused total decomposition of **1**. The best conditions for the preparation of **9** from **10** are those reported in the Experimental Section; at temperatures over 220 °C, **10** partially decomposed without increasing the yield of **9**.
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