Sesquiterpenes from *Thapsia nitida* var. *meridionalis* and *Thapsia nitida* var. *nitida*

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Nine new eudesmanolides (**1**−**9**), two new guaianolides (**12** and **13**), and a new germacrane (**10**), along with a previously reported guaianolide (**11**), have been isolated from the roots of *Thapsia nitida* var. *meridionalis*. *Thapsia nitida var. nitida* also afforded compound **13** along with a new guaianolide (**14**). The structure of **13** was confirmed by X-ray crystallographic analysis. Compounds **1**, **2**, and **11**−**14** have been tested as potential inhibitors of the sarco- and endoplasmic Ca^{2+}-dependent ATPases (SERCA) pump. None of them showed significant activities.

The medicinal properties of the resins obtained from plants of the genus *Thapsia* (Apiaceae) have been well known for centuries.[1] The last two decades have witnessed the extensive use in cell physiology studies of thapsigargin, a guaianolide isolated from *Thapsia garganica*, due to its capacity to inhibit selectively and irreversibly sarco- and endoplasmatic reticulum Ca^{2+}-dependent ATPases (SERCA).[2] The species *Thapsia nitida* Lacaita is a perennial herb distributed widely in the Iberian peninsula. According to the morphology of the leaf, this species is divided in two varieties frequently found in different zones of Spain and Portugal. While *T. nitida* var. *meridionalis* A. Pujadas grows in uncultured soils of the southwest of the Iberian peninsula, *Thapsia nitida* var. *nitida* Lacaita can be found in the central western area of the same peninsula.[3] The latter species has also been considered as a synonym of *T. maxima* Miller.

As part of our chemotaxonomic studies on the genus *Thapsia*,[4−6] we decided to carry out a comparative investigation of the two above-mentioned varieties of *T. nitida*. In this study, 12 new sesquiterpenes (compounds **1**−**10**, **12**, and **13**) were isolated from *T. nitida* var. *meridionalis* along with a known guaianolide (**11**) previously reported from *Laser trilobum*.[7] In turn, *T. nitida* var. *nitida* also afforded compound **13** along with a new guaianolide (**14**). Herein, we describe the isolation and structural elucidation of these sesquiterpenes. Biological testing as SERCA pump inhibitors of some of these compounds is described.

Results and Discussion

Compound **1** showed in its HREIMS a molecular ion $[M]^+$ at *m*/*z* 344.1617, compatible with the molecular formula $C_{20}H_{24}O_5$, inferring nine degrees of unsaturation. The characteristic features of the ^1H and ^{13}C NMR spectra suggested that **1** is a eudesmane-type sesquiterpene lactone.[8] Thus, the singlet at δ_H 0.75 and the signal at δ_C 14.2 indicated the presence of an angular methyl group. Furthermore, in the ^{13}C NMR spectrum, the carbon signal at δ_C 169.9 was assignable to the carbonyl group of the lactone ring, and the carbon signals at δ_C 136.6 and 120.3 were attributable to a terminal double bond, characteristic of an α-methylene-γ-lactone moiety, which was confirmed by the HMBC correlation between H-13a (δ_H 5.56) and H-13b (δ_H 6.31) and carbons C-11 (δ_C 136.6), C-12 (δ_C 169.9), and C-7 (δ_C 39.0). The proton signal at δ_H 4.83 was assigned to H-6 on the basis of HMBC correlation pairs between H-6/C-12 (δ_C 169.9), H-6/C-11 (δ_C 136.6), H-6/C-5 (δ_C 42.5), and H-6/C-7 (δ_C 39.0). A second terminal double bond was located between C-4 (δ_C 138.5) and C-15 (δ_C 118.2), as shown by the HMBC correlations between H-15b (5.23, d, J = 1.8 Hz) and C-5 (δ_C 42.5) and C-3 (δ_C 69.2). The proton signal at δ_H 5.60 and the carbon signal at δ_C 69.2 were assigned to H-3 and C-3, respectively. The downfield-shifted H-3 proton signal indicated that a senecioyloxy group was attached to C-3, as deduced from the presence of the proton signals at δ_H 5.73, 2.15, and 1.88 and the carbon signals at δ_C 165.9, 157.7, 115.8, 27.4, and 20.3.

The presence of signals at δ_C 61.7 and 52.5 in the ^{13}C NMR spectrum correlated to a ^1H doublet at δ_H 2.88 and to a ^1H triplet at δ_H 3.46, respectively, in the HSQC spectrum and suggested that **1** contains an epoxide ring. This epoxide ring was located at C-1 and C-2 by inspection of $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC correlations.

The relative configuration of **1** was determined via NOESY 1D experiments (Figure 1). Assuming H-7 to be α-oriented, as in all natural eudesmanes isolated from higher plants, the correlation displayed by H-7 with both H-6 (δ_H 4.83, dd, J = 10.4, 7.7 Hz) and CH_3-14 (δ_H 0.87, s, 3H) indicated that H-6 and CH_3-14 also were α-oriented. The correlations between CH_3-14/H-1 and H-1/H-2 suggested that the oxirane ring is β-oriented. Finally, the ester group attached to C-3 was also β-oriented, as was inferred from the correlation between H-2 and H-3. Thus, the structure of **1** was elucidated unambiguously as 1β,2β-epoxy-3β-senecioyloxy-5βH,6αH,7αH,10αMe-eudesma-4(15),11(13)-dien-6,12-olide.

Compound **2** differs from **1** only in the nature of an ester residue, and its ^1H NMR data showed typical signals of a 3-methylbutanoate side chain attached at C-3 instead of those from a senecioate ester unit. The HREIMS data of compound **2** showed a molecular ion $[M]^+$ at *m*/*z* 346.1778, confirming **2** as the 3-methylbutanoate analogue of **1**.

Compound **3** gave the molecular formula $C_{20}H_{24}O_6$ as determined by HREIMS at *m*/*z* 360.1572, indicating nine degrees of unsaturation. The signals of the ^1H and ^{13}C NMR spectra (Table 1) suggested that the structures of compounds **1** and **3** are closely related. They differed mainly in the presence of proton signals at δ_H 3.26 and 3.10 and the presence of carbon signals at δ_C 56.5 and 53.0, attributable to an epoxide ring, instead of the signals attributable to the α-methylene-γ-lactone moiety present in **1**. HMBC correlations confirmed that this oxirane ring was attached to carbons C-11 and C-13. The NOESY 1D experiments conducted were almost identical to those of **1**. In addition, a correlation between H-9β (δ_H 1.72) and H-13a indicated that the epoxide ring was α-oriented (Figure 1). The structure of **3** was thus elucidated as 1β,2β;11α,-13-diepoxy-3β-senecioyloxy-5βH,6αH,7αH,10αMe-eudesm-4(15)-en-6,12-olide.

Compound **4** gave a molecular formula of $C_{20}H_{26}O_5$, as determined by HREIMS at *m*/*z* 346.1768 $[M]^+$ (calcd 346.1780),

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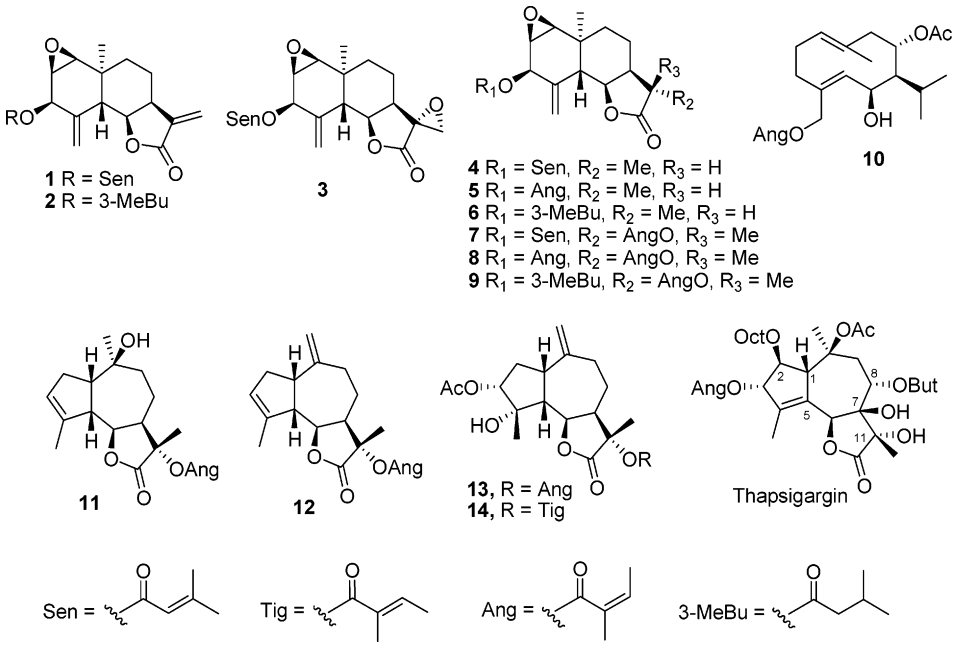
Chart 1



indicating eight degrees of unsaturation. The NMR spectra of **4** suggested that its structure is also closely related to **1**. The ^{1}H and ^{13}C NMR data were recorded in d_6-benzene since less overlap was observed with this solvent. The presence of a 3H doublet at δ_H 0.80 correlated to C-7 (δ_C 41.7) and C-12 (δ_C 177.4) indicated that **4** differs from **1** only in the presence of an α-methyl-γ-lactone moiety instead of the α-methylene-γ-lactone moiety present in **1**. The methyl group attached to C-11 was found to be α-oriented, as determined by the NOESY 1D correlations between H-11 and H-5 and between CH_3-13 and H-7. Thus, compound **4** was elucidated as 1β,2β-epoxy-3β-senecioyloxy-5βH,6αH,7αH,10αMe,11αMe-eudesm-4(15)-en-6,12-olide.

Comparison of the ^{1}H and ^{13}C NMR data of **4** with those of compounds **5** and **6** indicated that both compounds have the same sesquiterpenoid backbone, the only difference occurring at the ester group attached to C-3. The structure of **5** was therefore determined as 3β-angeloyloxy-1β,2β-epoxy-5βH,6αH,7αH,10αMe,11αMe-eudesm-4(15)-en-6,12-olide, while the structure 1β,2β-epoxy-3β-(3-methylbutanoyloxy)-5βH,6αH,7αH,10αMe,11αMe-eudesm-4(15)-en-6,12-olide was proposed for compound **6**.

Compound **7** showed in its HREIMS a molecular ion $[M]^+$ at *m*/*z* 444.2126, compatible with the molecular formula $C_{25}H_{32}O_7$, representing 10 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra showed that the structure of **7** was related to those of **1**−**6**. An analysis of its NMR data (Table 1) permitted the establishment of the backbone of this sesquiterpenoid, which is practically identical to that of **1** and **4**. An ester group attached to C-11, instead of the exomethylene or methyl group found in **1** and **4**, respectively, was the main difference found from the ^{1}H NMR spectrum of **7**. This ester group was identified as an angeloyl moiety from the presence of the proton signals at δ_H 6.15, 1.98, and 1.86 and the carbon signals at δ_C 166.9, 140.7, 127.0, 20.3, and 16.1. HMBC correlations confirmed that this ester group is attached to C-11, and NOESY 1D correlations determined that it is α-oriented. Thus,

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

position	**1**	**2**	**3**[a]	**4**[b]	**5**[b]	**6**[b]
1	2.88 d (3.9)	2.88 d (3.8)	2.92 d (3.9)	2.33 d (3.8)	2.26 d (3.9)	2.33 d (3.7)
2	3.46 dd (3.9, 4.2)	3.42 dd (4.0, 3.8)	3.51 dd (4.2, 3.9)	3.34 dd (4.2, 3.8)	3.23 dd (4.2, 3.9)	3.21 dd (4.4, 3.7)
3	5.69 d (4.2)	5.60 d (4.0)	5.66 d (4.2)	5.63 d (3.8)	5.53 d (4.2)	5.58 d (4.4)
5	2.23 d (10.2)	2.23 m	2.37 d (11.2)	2.50 d (11.0)	2.38 d (11.0)	2.44 d (11.0)
6	4.83 dd (10.4, 7.7)	4.81 dd (10.6, 7.7)	4.96 dd (11.2, 7.8)	4.05 dd (11.0, 6.5)	3.98 dd (11.0, 7.0)	4.02 dd (11.0, 7.3)
7	3.30 m	3.30 m	3.13 m	1.57 m	1.53 m	1.57 m
8	2.10 m 2H	2.10 m 2H	1.87 m 2H	α 1.20 m	α 1.13 m	α 1.17 dddd (14.7, 6.1, 4.9, 2.0)
				β 1.06 m	β 1.05 m	β 1.06 ddt (14.7, 4.9, 2.0)
9	α 1.49 ddd (14.3, 4.6, 3.1)	α 1.48 ddd (13.1, 4.4, 2.7)	α 1.60 m	α 0.88 ddd (13.4, 4.7, 2.0)	α 0.86 m	α 0.88 ddd (13.4, 4.0, 2.0)
	β 1.78 dt (13.2, 5.5)	β 1.78 dt (13.2, 5.5)	β 1.72 dt (14.9, 5.6)	β 1.44 dt (13.4, 4.7)	β 1.40 m	β 1.44 dt (13.4, 4.9)
11				1.80 m	1.83 m	1.80 m
13	a 6.31 dd (3.6, 0.6)	a 6.31 d (3.6)	a 3.26 dd (6.5, 0.7)	0.82 d (7.0)	0.80 d (6.8)	0.82 d (6.8)
	b 5.56 d (3.6)	b 5.56 d (3.6)	b 3.10 dd (6.5, 0.7)			
14	0.87 s	0.87 s	0.91 s	0.29 s	0.23 s	0.28 s
15	a 5.40 dd (1.8, 0.7)	a 5.38 d (1.8)	a 5.47 dd (2.0, 0.7)	a 5.29 d (1.8)	a 5.19 d (1.6)	a 5.31 d (1.6)
	b 5.23 d (1.8)	b 5.25 d (1.8)	b 5.26 d (2.0)	b 5.08 d (1.8)	b 4.97 d (1.6)	b 5.08 d (1.6)
2′	5.73 m	2.23 d (7.0) 2H	5.75 m	5.70 m		2.04 d (7.3) 2H
3′		2.07 m			5.52 qq (7.4, 1.7)	2.10 m
4′	2.15 d (1.5)	0.95 d (6.6)	2.17 d (1.5)	2.10 s	1.96 dq (7.4, 1.7)	0.82 d (7.3)
5′	1.88 d (1.5)	0.95 d (6.6)	1.90 d (1.5)	1.30 s	1.82 m	0.83 d (7.3)

[a] NMR recorded at 600 MHz. [b]C_6 D_6.

compound **7** was elucidated as 11α-angeloyloxy-1β,2β-epoxy-3β-senecioyloxy-5βH,6αH,7αH,10αMe-eudesm-4(15)-en-6,12-olide.

A detailed study of the NMR data of compounds **8** and **9** determined that their structures were identical to those of **7**, with the only differences occurring at the C-3 ester group position. The HREIMS data, chemical shifts, coupling constants, ^{1}H−^{1}H COSY, HSQC, and HMBC data, and NOESY 1D correlations confirmed **8** as the angelate analogue at C-3 of **7**, while compound **9** turned out to be the 3-methylbutanoate analogue.

The DEPT spectra and HSQC correlations of compound **10** showed the presence of six methyl groups, four secondary carbons, seven tertiary carbons, and five quaternary carbons (Table 1). The ^{13}C NMR spectrum displayed seven signals that could be readily assigned to an acetate unit (δ_C 172.8 and 21.3) and an angelate moiety (δ_C 170.0, 138.5, 127.5, 21.2, and 15.8). The remaining 15 carbons suggested the sesquiterpenoid nature of the molecule. Analysis of the ^{1}H and ^{13}C NMR data established the presence of an isopropyl group attached to carbon C-7, as was confirmed by HMBC correlations. The ^{1}H−^{1}H COSY spectrum of compound **10** was very useful since only two spin systems were detected, with the first fragment running from H-1 to H_2-3 and the second connecting H-5 to H_2-9 and encompassing two oxymethines (C-6 and C-8) as well as an isopropyl branched at C-7. Analysis of the HMBC spectrum revealed that these two fragments are mutually joined, with eventual merging into a 10-membered carbocycle typical of a germacrane skeleton. The carbon signals at δ_C 138.7, 131.7, 131.3, and 130.1 were assignable to the carbons C-5, C-1, C-4, and C-10, respectively, as a result of the HSQC and HMBC correlations, showing that carbons C-1/C-10 and C-4/C-5 were connected by double bonds. Finally, three oxygenated carbon signals at δ_C 74.5, 66.7, and 59.7 were assigned to C-8, C-6, and C-15, respectively, by HMBC correlations with the nearby proton signals, thereby establishing that the acetate group and the angelate unit were attached to C-8 and C-15, respectively, and a hydroxyl group was present at C-6. The relative stereochemistry of compound **10** was established using *J* values and NOE data derived from its ^{1}H NMR and NOESY 1D spectra. NOE interactions (H-15a/H-7; H_2-15/CH_3-14; H-1/H-5) were consistent with the conformation of [^{1}D$_{14}$, ^{5}D$_{15}$]-type[9] usually preferred by germacranes isolated from species in the Umbelliferae (Figure 2). The occurrence of a NOE correlation between H-6 and H-7 indicated that the hydroxyl group attached to C-6 was in the β-orientation. Comparison of the values of $J_{6,7}$ and $J_{7,8}$ with those reported in the literature for related compounds supported this assignment and indicated a β-orientation for H-8.[10,11] Although the molecular ion was not observed, the HREIMS gave a fragment ion peak at *m*/*z* 335.1860, compatible with the molecular formula $C_{19}H_{27}O_5$, corresponding to the loss of an isopropyl group. Compound **10** was therefore elucidated as 1βH,5βH,7αH,8α-acetyl-15-angeloyloxy-6β-hydroxygermacrane.

All spectroscopic data of compound **11** were fully coincident with those reported in the literature for 10β-hydroxy-11α-angeloyloxyslov-3-enolide, a guaianolide previously isolated from *Laser trilobum*.[7] Comparison of the ^{1}H and ^{13}C NMR spectra of compounds **12**−**14** with those of **11** established that their structures were closely related. Thus, the main difference between compounds **12** and **11** was the presence of an exomethylene group instead of a hydroxyl group attached to carbon C-10, as was confirmed by the presence of two doublets at δ_H 4.87 and 4.97 in the ^{1}H NMR spectrum, assignable to H-14a and H-14b, respectively, and the presence of the signals at δ_C 147.0 (C-10) and 112.0 (C-14) in the ^{13}C NMR spectrum. HSQC, HMBC, and NOE correlations confirmed the structure of compound **12** as 1βH,5βH,6αH,7αH-11α-angeloyloxyguaia-3(4),10(14)-dien-6,7-olide.

Compound **13** showed in its ^{1}H and ^{13}C NMR spectra signals similar to those of **12**. Instead of the double bond between carbons C-3/C-4 evident in **12**, compound **13** showed in its ^{1}H NMR spectrum a singlet at δ_H 1.47 attributable to CH_3-15, attached to the oxygenated carbon C-4 (δ_C 78.2), and a multiplet at δ_H 4.60 assignable to an oxygenated methine (C-3, δ_C 77.8), as confirmed by HSQC correlations. HMBC correlations confirmed the presence of an acetate moiety attached to carbon C-3. NOE effects were similar to those observed in compound **12**, inferring the same relative configurations at carbons C-1, C-5, C-6, C-7, and C-11. Finally, NOE correlations between H-3, H-1, and CH_3-15 confirmed a α-orientation for the acetate and the hydroxyl group attached at C-3 and C-4, respectively (Figure 1). The molecular ion peak $[M]^+$ at *m*/*z* 406.1972 in the HREIMS confirmed the structure of compound **13** as 1βH,5βH,6αH,7αH-3α-acetyloxy-11α-angeloyloxy-4α-hydroxyguaia-3(4),10(14-dien-6,7-olide. The structure of **13** was finally confirmed by X-ray crystallography, verifying the suggested structure (Figure 2).[12]

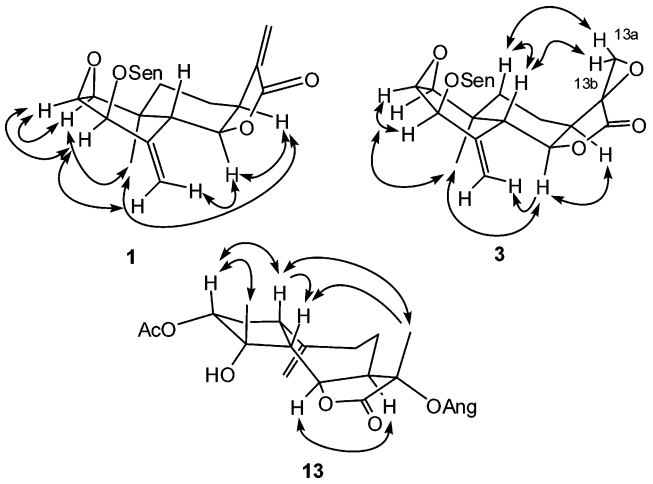


Figure 1. Selected NOE correlations of compounds **1**, **3**, and **13**.

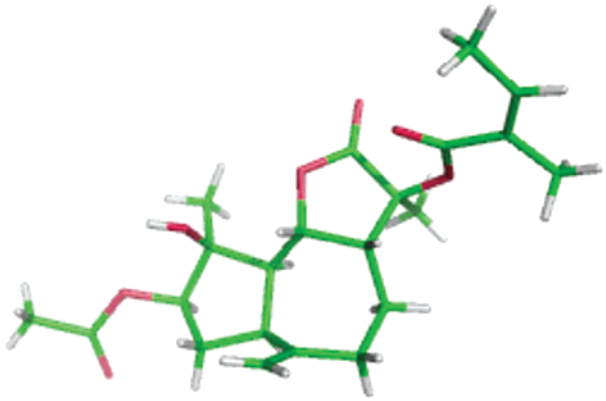


Figure 2. Structure of guaianolide **13**.

Compound **13** was also isolated from *Thapsia nitida* var. *nitida*. In addition to **13**, a compound with almost identical ^{13}C NMR and ^{1}H NMR data was isolated from the same plant. The major difference between the two products was the absence of a signal near δ_H 6.2 characteristic of the vinylic proton in angelic acid. Instead, a very similar signal was found at δ_H 6.87. Accordingly, the two signals of the allylic methyl groups were found at δ_H 1.78 and 1.76. These findings suggest that the angelic acid has been replaced with a tiglic acid in compound **14**.

The structural relationships between compounds **1** and **2** and **11**−**14** encouraged an evaluation of their activity as inhibitors of the SERCA pump. However, none of the compounds showed activity up to a concentration of 100 μM. The solubility in the assay medium allowed testing of compounds **1**, **2**, **11**, and **12**, in concentrations up to 10 μM. No activity was observed even at these high concentrations.

In summary, nine new eudesmanolides (**1**−**9**), two new guaianolides (**12** and **13**), and a new germacrane (**10**), along with a

Table 2. ^{1}H NMR Data for Compounds **7**−**10** and **12**−**14** (δ_H, *J* in Hz)

position	7	8[a]	9	10	12	13	14
1	2.90 d (3.9)	2.91 d (3.9)	2.90 d (3.9)	5.07 d (8.3)	3.16 ddd (11.5, 9.0, 2.4)	2.68 m	2.64 m
2	3.48 dd (4.0, 3.9)	3.54 dd (4.0, 3.9)	3.48 dd (4.0, 4.0)	a 2.36 m	α 2.63 m	α 2.26 m	α 2.18 m
				b 2.14 m	*β* 2.40 ddt (16.5, 9.0, 2.0)	*β* 1.94 ddd (11.9, 11.2, 6.4)	*β* 1.95 ddd (11.1, 11.2, 6.3)
3	5.60 d (4.0)	5.60 d (4.0)	5.89 d (4.0)	a 2.49 m	5.30 br s	4.71 dd (11.9, 6.2)	4.68 dd (12.0, 6.3)
				b 1.92 m			
5	2.44 d (12.2)	2.46 d (12.2)	2.41 d (11.4)	5.44 br d (12.5)	2.85 dd (11.7, ss11.5)	2.11 m	2.10 m
6	4.97 dd (12.2, 9.2)	4.97 dd (12.2, 9.2)	4.96 dd (11.4, 9.3)	4.58 dd (8.0, 8.0)[b]	4.71 dd (11.7, 9.5)	5.10 dd (11.9, 9.7)	5.06 dd (12.0, 9.9)
7	3.36 m	3.38 m	3.37 m	1.15 d (10.5)	2.94 ddd (13.2, 9.5, 3.5)	3.00 ddd (11.9, 9.7, 4.6)	2.95 ddd (12.0, 9.6, 4.2)
8	1.95 m 2H	1.80 m 2H	1.80 m 2H	5.11 dd (12.5, 5.3)	α 1.88 m	1.80 m 2H	1.80 m, 2H
					β 1.98 m		
9	α 1.92 dt (14.7, 6.5)	α 1.90 dt (14.7, 6.5)	α 1.78 m	α 1.92 m	α 2.31 ddd (14.5, 11.9, 6.8)	α 2.24 m	α 2.24 m
	β 1.55 m	*β* 1.57 m	*β* 1.51 m	*β* 2.60 dd (12.5, 5.3)	*β* 2.51 ddd (14.5, 6.8, 1.3)	*β* 2.41 dd (14.4, 4.8)	*β* 2.35 m
11				1.60 m			
12				1.05 d (6.5)			
13	1.61 s	1.61 s	1.61 s	1.05 d (6.5)	1.52 s	1.48 s	1.44 s
14	0.85 s	0.85 s	0.85 s	1.64 br s	a 4.87 d (1.3)	a 4.95 br s	a 4.93 br s
					b 4.97 d (1.3)	b 4.90 br s	b 4.89 br s
15	a 5.41 d (1.8)	a 5.41 d (1.8)	a 5.41 d (1.3)	a 4.50 d (12.7)	1.84 br s	1.32 s	1.30 s
	b 5.28 d (1.8)	b 5.28 d (1.8)	b 5.29 d (1.3)	b 4.38 d (12.7)			
2′	5.74 m		2.24 d (7.0) 2H				
3′		6.18 qq (7.4, 1.5)	2.10 m	6.06 qq (7.2, 1.5)	6.17 qq (7.2, 1.5)	6.14 qq (7.4, 1,5)	6.86 m
4′	2.15 d (1.7)	1.95 dq (7.4, 1.5)	0.95 d (6.6)	1.97 dq (7.2, 1.5)	2.00 dq (7.2, 1.5)	1.95 dq (7.4, 1.5)	1.72 m
5′	1.88 d (1.7)	1.87 m	0.95 d (6.6)	1.88 m	1.90 m	1.85 m	1.75 s
2″							
3″	6.15 qq (7.3, 1.3)	6.10 qq (7.2, 1.5)	6.15 qq (7.4, 1.5)				
4″	1.98 dq (7.3, 1.3)	1.98 dq (7.2, 1.5)	1.98 dq (7.4, 1.5)				
5″	1.86 m	1.89 m	1.87 m				
AcO				2.10 s		2.11 s	2.09

[a] NMR recorded at 600 MHz. [b] ^{1}H NMR shows 1H signal O*H* at δ 3.40 d (10.4) that disappeared when D_2O was added.

known guaianolide (**11**), have been isolated from the roots of *Thapsia nitida* var. *meridionalis*. Lactones **13** and **14** were encountered in *Thapsia nitida* var *nitida*. To the best of our knowledge, this is the first time that eudesmane-type sesquiterpenolides have been described from the genus *Thapsia*.

Experimental Section

General Experimental Procedures. Melting points are uncorrected and were measured using a Reichert-Jung apparatus and a Büchi SMP-20 apparatus. Optical rotations were measured with a Perkin-Elmer model 341 digital polarimeter. IR spectra were measured in a Perkin-Elmer Spectrum BX spectrophotometer and a Perkin-Elmer 1600 spectrophotometer. ^{1}H (1D, DQF-COSY, NOESY 1D) and ^{13}C (1D, gHSQC, HMBC) NMR spectra were recorded using a Varian Inova 400 spectrometer, a Varian Inova 600 spectrometer, and a Varian Gemini 2000 spectrometer. Mass spectra were recorded at the Mass Spectral Facilities of the Universidad de Alicante (Spain) with a JEOL AX505 W instrument. Solvents were distilled prior to use, and spectroscopic grade solvents were used. TLC was performed on plates precoated with silica gel F_{254} (Merck, Germany).

Plant Material. The roots of *T. nitida* var. *meridionalis* were collected in La Algaida (Puerto Real, Cádiz, Spain) in May 2004. A voucher specimen has been deposited in the Departamento de Ciencias y Recursos Agrícolas y Forestales, University of Cordoba collection (COA 33946).

The roots of *T. nitida* var. *nitida* were collected 8 km from El Santuario de la Virgen de la Cabeza (Andújar, Jaén, Spain) in June 1987. A voucher specimen has been deposited at the Herbarium at the University of Copenhagen (UWS 87-33).

Extraction and Isolation (*Thapsia nitida* var. *meridionalis*). The dried roots (1500 g) were ground and extracted with CH_2Cl_2 (3 L) in a Soxhlet apparatus, yielding 20 g of an oily residue, which was purified by column chromatography with EtOAc/hexanes mixtures of increasing polarity. The 3:7 EtOAc/hexanes-eluted fraction was further purified by column chromatography with EtOAc/toluene mixtures. The 1:9 EtOAc/toluene-eluted fraction was subjected to subsequent column purification, yielding compounds **1** (40 mg), **2** (40 mg), **4** (2 mg), **5** (2 mg), **6** (8 mg), **7** (4 mg), **8** (1 mg), and **9** (2 mg). The 1:19 EtOAc/toluene-eluted fraction yielded after further purification by column chromatography compounds **10** (10 mg), **11** (20 mg), and **12** (40 mg). Finally, the 3:17 EtOAc/toluene-eluted fraction yielded after additional column purification compounds **3** (2 mg) and **13** (200 mg).

Extraction and Isolation (*Thapsia nitida* var. *nitida*). The dried roots (600 g) were ground and extracted with ethyl acetate (600 mL) to give 19 g of an oily residue, half of which was purified by column chromatography over silica gel (150 g) using EtOAc/MeOH/toluene (4:1:15) as an eluent. The fractions eluted with 62 to 100 mL of the eluent contained **13** and **14**. This fraction was subjected to further column chromatography over silica gel (30 g) using EtOAc/hexane (7:2, 50 mL) and EtOAc/hexane (7:3, 50 mL) as eluents to give 111 mg of pure **14** (eluted with 38 to 48 mL) and 58 mg of pure **13** (eluted with 62 to 72 mL).

Compound 1: amorphous solid; $[\alpha]^{20}_D$ −8.8 (*c* 0.24, $CHCl_3$); IR (NaCl) ν_{max} 2970, 1765, 1712, 1647, 1445, 1222, 1143, 990 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100

Table 3. ^{13}C NMR Data for Compounds **1**−**10** and **12**−**14**

position	1	2	3	4	5	6	7	8	9	10	12	13	14
1	61.7	61.5	61.9	61.9	61.3	61.7	61.8	61.8	62.2	131.7	44.2	37.2	37.4
2	52.5	52.3	53.0	52.2	52.5	52.5	52.8	53.2	52.7	24.7	35.0	30.3	30.4
3	69.2	69.8	69.4	70.1	70.1	70.6	69.3	70.1	69.3	34.0	126.6	78.2	77.7
4	138.5	138.4	138.4	139.2	139.2	139.2	138.7	138.5	138.8	131.3	140.3	77.8	78.5
5	42.5	42.6	43.7	41.2	40.4	41.2	41.3	41.2	41.6	138.7	52.0	48.2	48.4
6	75.5	75.5	75.0	74.2	73.8	75.5	74.5	74.1	74.5	66.7	78.6	78.6	78.2
7	39.0	38.9	35.0	41.5	41.7	41.9	37.7	37.4	38.1	56.3	42.8	42.5	42.2
8	19.3	19.3	20.6	19.2	18.8	19.4	18.0	18.5	18.3	74.5	22.6	23.6	22.6
9	29.8	29.9	31.3	29.6	29.9	29.9	29.8	32.8	32.1	42.1	35.7	35.7	35.8
10	35.7	35.6	36.0	36.0	35.3	36.1	35.4	35.6	35.6	130.1	147.0	144.5	144.3
11	136.6	136.5	56.5	35.8	35.3	35.3	79.5	79.2	79.5	26.8	78.9	78.5	78.6
12	169.9	169.9	173.0	177.4	177.4	177.4	174.0	174.0	175.1	21.4	175.0	174.6	174.7
13	120.3	120.2	53.0	13.5	13.5	13.7	20.3	20.6	21.0	23.4	20.6	20.7	20.8
14	14.2	14.2	15.1	14.5	14.2	14.8	15.9	15.4	16.2	20.6	112.0	111.8	111.9
15	118.2	118.5	119.0	117.2	116.9	117.9	118.5	118.8	118.9	59.7	17.2	26.0	26.4
1′	165.9	172.7	166.1	166.0	167.7	172.5	160.0	167.8	172.8	170.0	166.7	166.2	166.2
2′	115.8	43.3	116.0	117.5	127.8	43.5	115.0	127.0	43.7	127.5	126.8	126.6	127.5
3′	157.7	25.8	158.3	156.2	140.0	26.2	159.0	138.2	26.0	138.5	140.5	140.6	139.3
4′	20.3	27.4	20.6	20.2	20.5	22.4	20.6	20.6	21.8	21.2	20.4	20.1	14.5
5′	27.4	27.4	27.7	26.8	15.7	22.4	27.4	15.9	21.8	15.8	15.9	15.8	11.8
1″							166.9	166.8	166.9				
2″							127.0	126.7	127.0				
3″							140.7	140.8	140.9				
4″							20.3	21.1	20.7				
5″							16.1	15.9	15.8				
CH_3CO										172.8		170.8	170.6
CH_3CO										21.3		20.9	21.1

MHz) data, see Table 3; HREIMS *m/z* 344.1617 (calcd for $C_{20}H_{24}O_5$, 344.1624); EIMS 70 eV, *m/z* 344 (1), 262 (2), 244 (5), 215 (4), 173 (4), 83 (100), 55 (15).

Compound 2: amorphous solid; $[\alpha]^{20}_D$ −66.0 (*c* 0.24, $CHCl_3$); IR (NaCl) ν_{max} 2961, 2874, 1766, 1731, 1103, 991 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 346.1778 (calcd for $C_{20}H_{26}O_5$, 346.1780); EIMS 70 eV, *m/z* 346 (2), 262 (35), 244 (40), 215 (35), 173 (32) 85 (100), 57 (93).

Compound 3: yellow oil; $[\alpha]^{20}_D$ −41.6 (*c* 0.06, $CHCl_3$); IR (NaCl) ν_{max} 2924, 1784, 1712, 1644, 1225, 1143, 991 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Table 3; HREIMS *m/z* 360.1572 (calcd for $C_{20}H_{24}O_6$, 360.1573); EIMS 70 eV, *m/z* 320 (2), 242 (10), 85 (11), 83 (100), 57 (10), 55 (16), 43 (11).

Compound 4: yellow oil; $[\alpha]^{20}_D$ −7.0 (*c* 0.09, $CHCl_3$); IR (NaCl) ν_{max} 2931, 1773, 1710, 1641, 1224, 1141, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 346.1768 (calcd for $C_{20}H_{28}O_5$, 346.1780); EIMS 70 eV, *m/z* 346 (1), 247 (30), 173 (59), 145 (22) 83 (100), 55 (34).

Compound 5: yellow oil; $[\alpha]^{20}_D$ −7.2 (*c* 0.09, $CHCl_3$); IR (NaCl) ν_{max} 2928, 1773, 1230, 1164 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 346.1722 (calcd for $C_{20}H_{26}O_5$, 346.1780); EIMS 70 eV, *m/z*: 346 (1), 247 (33), 173 (40), 83 (100), 55 (40).

Compound 6: yellow oil; $[\alpha]^{20}_D$ −15.0 (*c* 0.12, $CHCl_3$); IR (NaCl) ν_{max} 2962, 1773, 1730, 1167, 1011, 989 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 348.1938 (calcd for $C_{20}H_{28}O_5$, 348.1937); EIMS 70 eV, *m/z* 348 (2), 264 (18), 248 (27), 247 (24), 195 (18), 191 (20), 173 (100), 145 (28).

Compound 7: yellow oil; $[\alpha]^{20}_D$ −6.1 (*c* 0.18, $CHCl_3$); IR (NaCl) ν_{max} 2924, 1784, 1712, 1644, 1225, 1143, 991 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 444.2126 (calcd for $C_{25}H_{32}O_7$, 444.2148); EIMS 70 eV, *m/z* 444 (1), 244 (26), 83 (100), 55 (26).

Compound 8: yellow oil; $[\alpha]^{20}_D$ −3.0 (*c* 0.06, $CHCl_3$); IR (NaCl) ν_{max} 2920, 1785, 1710, 1228, 1144 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Table 3; HREIMS *m/z* 444.2148 (calcd for $C_{25}H_{32}O_7$, 444.2148); EIMS 70 eV, *m/z* 444 (1), 244 (35), 83 (100), 55 (37).

Compound 9: yellow oil; $[\alpha]^{20}_D$ −2.9 (*c* 0.20, $CHCl_3$); IR (NaCl) ν_{max} 2959, 1786, 1728, 1162, 991 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 446.2302 (calcd for $C_{25}H_{34}O_7$, 446.2304); EIMS 70 eV, *m/z* 446 (1), 244 (78), 83 (100), 55 (39).

Compound 10: yellow oil; $[\alpha]^{20}_D$ −6.2 (*c* 0.16, $CHCl_3$); IR (NaCl) ν_{max} 3445, 2958, 1731, 1243, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 335.1860 (calcd for $C_{19}H_{27}O_5$ $[M - C_3H_7]^+$, 335.1858); EIMS 70 eV, *m/z* 299 (5), 239 (22), 221 (17), 83 (100), 55 (39), 43 (58).

Compound 12: yellow oil; $[\alpha]^{20}_D$ −83.3 (*c* 0.15, $CHCl_3$); IR (NaCl) ν_{max} 2918, 1781, 1715, 1234, 1092, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 330.1854 (calcd for $C_{20}H_{26}O_4$, 330.1831); EIMS 70 eV, *m/z* 331 (2), 230 (40), 202 (17), 105 (16), 83 (100), 55 (47).

Compound 13: colorless crystals, mp 156−158 °C ($MeOH/H_2O$); $[\alpha]^{20}_D$ −34.0 (*c* 0.25, $CHCl_3$); IR (NaCl) ν_{max} 3093, 2970, 2936, 2879, 1765, 1712, 1647, 1445, 1226, 1143, 990 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 3; HREIMS *m/z* 406.1972 (calcd for $C_{22}H_{30}O_7$, 406.1992); EIMS 70 eV, *m/z* 406 (1), 331 (2), 230 (40), 202 (17), 105 (16), 83 (100), 55 (47).

Compound 14: colorless crystals, mp 159−160 C; $[\alpha]^{20}_D$ −20.0 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3470, 3070, 2970, 2936, 2879, 1765, 1709, 1640, 1445, 1370, 1350, 1330, 1250, 1230, 1190, 1160, 970 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 3; HRMS (electrospray) *m/z* 407.2092 (calcd for $C_{22}H_{31}O_7$, 407.2070).

X-ray Crystallographic Analysis of Compound 13. Crystal Data. Single crystals suitable for X-ray diffraction studies were grown from a solution in $MeOH/H_2O$, $C_{22}H_{30}O_7$, M_r 406.46, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 10.1786(7) Å, b = 14.1332(11) Å, c = 14.6231(19) Å, V = 2103.6(3) $Å^3$, Z = 4, D_c = 1.283 Mg/m^3, $F(000)$ = 872, μ(Mo Kα) = 0.095 mm^{-1}, crystal size 0.4 × 0.23 × 0.22 mm.

Data Collection and Reduction. A single crystal was mounted and immersed in a stream of nitrogen gas [T = 122.0(5) K]. Data were collected, using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a KappaCCD diffractometer. Data collection and cell refinement were performed using COLLECT[13] and DIRAX.[14] Data reduction was performed using EvalCCD.[15] Correction for absorption was performed using Gaussian integration[16] as included in maXus.[17]

Structure Solution and Refinement. Positions of all non-hydrogen atoms were found by direct methods.[18,19] Full-matrix least-squares refinements[18,20] were performed on F^2, minimizing $\sum w(F_o^2 - kF_c^2)^2$, with anisotropic displacement parameters of the non-hydrogen atoms. The position of the hydrogen atoms were located in subsequent

difference electron density maps and refined with fixed isotropic displacement parameters ($U_{iso} = 1.2U_{eq}$ for CH and CH_2, $U_{iso} = 1.5U_{eq}$ for OH and CH_3). Refinement (352 parameters, 4834 unique reflections) converged at $R_F = 0.033$, $wR_F^2 = 0.082$ [4638 reflections with $F_o > 4\sigma(F_o)$; $w^{-1} = (\sigma^2(F_o^2) + (0.0435P)^2 + 0.6636P)$, where $P = (F_o^2 + 2F_c^2)/3$; $S = 1.063$]. The residual electron density varied between −0.17 and 0.45 e Å^{-3} (non-centrosymmetric space group but the absolute configuration cannot be determined (Flack = 0.0(6)). Complex scattering factors for neutral atoms were taken from International Tables for Crystallography as incorporated in SHELXL97.[20,21] Fractional atomic coordinates, a list of anisotropic displacement parameters, and a complete list of geometrical data have been deposited in the Cambridge Crystallographic Data Centre (No. CCDC 611640).

Measurement of ATPase Activity. The rate of ATP hydrolysis was determined essentially as previously described.[22,23] Briefly after 5 min of incubation the OD_{340} was measured kinetically at room temperature ($n = 3$) for at least 10 min using the standard buffers. Typically a 1 mM DMSO solution of inhibitor was diluted 1:100 in buffer A before making serial dilutions in buffer A. The amount of DMSO present did not influence the measured ATPase activity. The total ATPase activity was 7.0 μmol ATP/mg SR protein/min.

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Supporting Information Available: X-ray data tables for **13** are available free of charge via the Internet at http://pubs.acs.org.

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(12) Crystallographic data for compound **13** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 611640). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
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