Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 67 (2006) 2651–2658
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cytotoxic phenylpropanoids and an additional thapsigargin analogue isolated from *Thapsia garganica*

Huizhen Liu [a], Kent Gunnertoft Jensen [a], Linh My Tran [a], Ming Chen [b], Lin Zhai [b], Carl Erik Olsen [c], Helmer Søhoel [a], Samuel R. Denmeade [d], John T. Isaacs [d], S. Brøgger Christensen [a,*]

[a] *Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark*
[b] *Department of Clinical Microbiology 7602, Tagensvej 20, DK-2100 Copenhagen Ø, Denmark*
[c] *Department of Natural Sciences, The Royal Veterinary and Agricultural University, Bülowsvej 17, DK-1870 Frederiksberg C, Denmark*
[d] *The Sidney Kimmel Comprehensive Cancer Center at Johns, Hopkins, 1650 Orleans Street, Baltimore, MD 21231-1000, USA*

Received 14 July 2006; received in revised form 3 October 2006

Abstract

Four phenylpropanoids and a thapsigargin analogue have been isolated from the fruits of *Thapsia garganica*. A spectroscopic method for elucidating the relative stereochemistry at the two pairs of stereogenic centers in the phenylpropanoids has been developed. The phenylpropanoids were found to be potent cytotoxins.

Keywords: *Thapsia garganica*; Apiaceae; Phenylpropanoids; 2,3-Dihydroxy-2-methylbutanoic acid; Relative configuration; Cytotoxicity; Thapsigargin

1. Introduction

A number of new either biosynthetically or pharmacological very interesting compounds have been isolated from plants belonging to the genus *Thapsia*. Most famous is thapsigargin (**1**, Fig. 1) isolated from *T. garganica* L., which has become an indispensable tool for the study of the sarco/endoplasmic calcium pump (SERCA) (Olesen et al., 2004; Treiman et al., 1998) and might offer a new type of chemotherapeutics for treatment of prostate cancer (Denmeade and Isaacs, 2005; Denmeade et al., 2003; Janssen et al., 2006).

Esters of 1,2-dihydroxylated phenylpropanoids have been found in plants belonging to the Apiaceae (Barrero et al., 1991; Gonzales et al., 1993; Gonzalez et al., 1988; Holub et al., 1968; Miski and Jakupovic, 1990a,b; Miski and Mabry, 1986; Pascual Teresa et al., 1985b; Saouf et al., 2006; Teresa et al., 1985). In addition 1,2-dihydroxylated phenylpropanoids are building blocks in neolignans (Jeong et al., 2005; Konya et al., 2004; Lee and Ley, 2003; Zhang et al., 2001). The elucidation of the relative configuration of these compounds is facilitated by the difference in the size of the coupling constant of the coupling between H-1 and H-2 in the *erythro*-isomer ($J_{1,2} = 4.8 \pm 0.4$ Hz) and the *threo*-isomer ($J_{1,2} = 7.5 \pm 0.5$ Hz) (Konya et al., 2004; Pascual Teresa et al., 1985a; Pascual Teresa et al., 1985b). The *erythro* as well as the *threo*-isomer of the 1,2-dihydroxyphenylpropanoid skeleton, 1-(3,4-methylendioxy-5-methoxyphenyl)-propan-1,2-diol (**1**), have been found incorporated in natural products isolated from Apiaceae (Barrero et al., 1991). Concerning the *erythro*-isomer both antipodes have been found to be naturally occurring (Pascual Teresa et al., 1985b; Saouf et al., 2006) whereas the *threo*-isomers are poorly characterized. Many 1,2-dihydroxyphenylpropanoids isolated from umbelliferous plants are esterfied with 2,3-dihydroxy-2-methylbutanoic acid (Miski and Jakupovic, 1990a,b; Pascual Teresa et al., 1985b; Teresa et al., 1985), which also has been found incorporated into pyrrozolidine alkaloids

* Corresponding author. Tel.: +45 3530 6253; fax: +45 3530 6041.
E-mail address: sbc@dfuni.dk (S. Brøgger Christensen).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.10.005

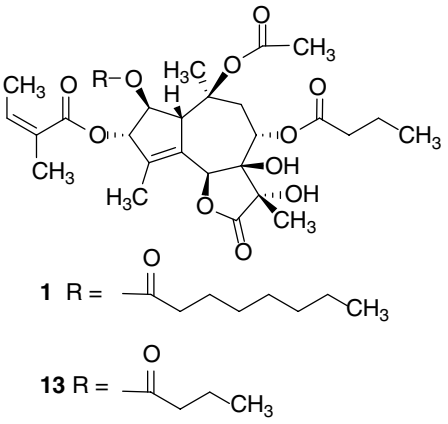


Fig. 1. Structure of thapsigargin (**1**) and thapsivillosin L (**13**).

(Jennett-Siems et al., 1993). The *erythro*- and *threo*-isomers of 2,3-dihydroxy-2-methylbutanoate residues are claimed to be distinguishable by the δ-values of the two pairs of methyl groups (Jennett-Siems et al., 1993; Pinar et al., 1982) but no comparison of spectra of authentic samples of both isomers has been reported and only spectra recorded in different solvents have been compared.

2. Results and discussion

2.1. Structure elucidation

During our work with isolation of 40 g amounts of thapsigargin from fruits of *Thapsia garganica* L. we isolated a series of compounds (**3A**–**6A**, Fig. 2), the NMR spectra of which, clearly proved, that they all were esters of 1-methyl-2-(3,4-methylendioxy-5-methoxyphenyl)-2-hydroxyethyl 2,3-dihydroxy-2-methylbutanoate. Except for signals originating in one of the acyl groups the NMR spectra were super imposable. The identity of the varying acyl group as angeloyl (**3A**), octanoyl (**4A**), hexanoyl (**5A**), and butanoyl (**6A**), respectively, appeared from the characteristic patterns in the NMR spectra (Table 1) and from the mass spectra. All signals could be assigned by COSY, HMQC, and HMBC correlations and the acyl groups were located through long distance couplings as revealed in HMBC correlations.

The *erythro*-configuration of the phenylpropanoid residue was revealed by coupling constants between H-1 and H-2 of 4.8–5.1 Hz. Basic hydrolysis afforded the dihydroxyphenylpropan, the optical rotation of which revealed, that the stereocenters in the phenylpropanoid residue possess the absolute configuration 1*S*,2*R* (**2A**), as is the case for phenylpropanoids isomers isolated from *T. transtagana* (Saouf et al., 2006). This contrasts the absolute configuration of the phenylpropanoid residue of helmanthicin, isolated from *T. villosa*, which was found to possess the absolute configuration 1*R*,2*S* (**3B**) (Pascual Teresa et al., 1985b). Concerning the relative configuration of the two

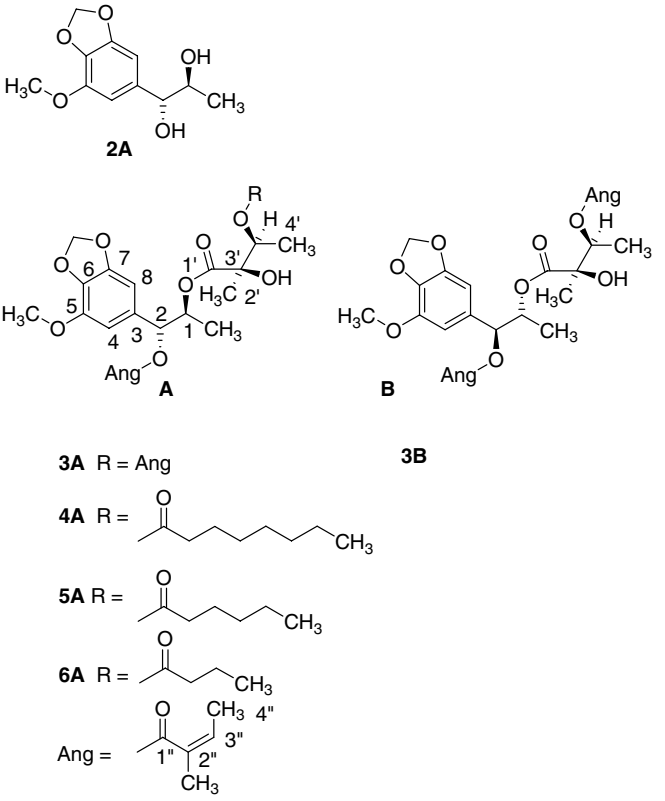
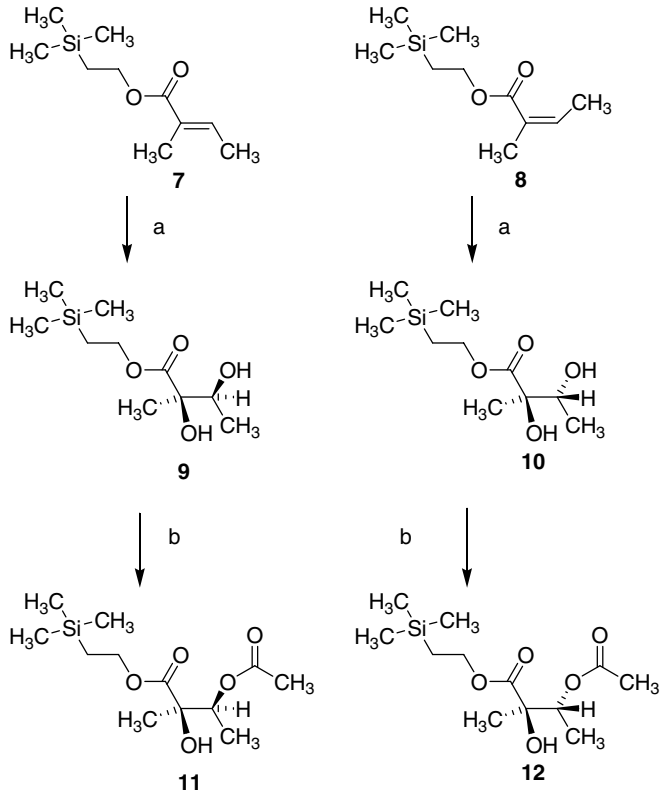


Fig. 2. Structure of phenylpropanoids isolated from plants belonging to the genus *Thapsia*.

stereocenters in the 2,3-dihydroxy-2-methylbutanoyl residue a difference in the chemical shifts ($\Delta\delta$ value) between the signals of the two methyl groups of the 2,3-dihydroxy-2-methylbutanoate residue of 0.05 ppm was observed. In the series of ipanguline alkaloids, in which 2,3-dihydroxy-2-methylbutanoate possess the *erythro*-configuration the $\Delta\delta$ value, recorded in deuterochloroform, is 0.25 ppm, whereas the value in the *threo*-series, the isoipanguline alkaloids, is 0.08 ppm. In the report on ipanguline alkaloids a general applicability of the rule was stated, because the $\Delta\delta$ value of *threo*-2,3-dihydroxy-2-methylbutanoic acid recorded in deuteromethanol was 0.12 ppm (Jennett-Siems et al., 1993). To verify the general applicability of the use of the $\Delta\delta$ value for distinguishing the *erythro*- and the *threo*-forms we decided to prepare authentic samples of both isomers.

The trimethylsilylethyl ester of tiglig (**7**) and angelic acid (**8**) was oxidized with AD-mix-α (Kolb et al., 1994) to give the *threo*- (**9**) and *erythro*-isomer (**10**), respectively, which were acetylated to give **11** and **12**, respectively (Scheme 1).

Comparison of the ^{1}H NMR spectra reveals that the $\Delta\delta$ value of the 2,3-dihydroxy-2-methylbutanoate residue in the *threo*-isomer (**9**) is 0.09 ppm whereas the value is 0.20 ppm in the *erythro*-isomer (**10**) if the spectra were recorded in deuterochloroform. Acylation of the secondary hydroxyl group to give **11** and **12**, respectively, did not change the $\Delta\delta$ value significantly, confirming that the $\Delta\delta$ value can be used for determining the relative configuration



Scheme 1. (a) AD-mix-α, 60–77 %; (b) acetic anhydride, DMAP, 63–72%.

of the dihydroxyacid derivatives as well as of monoacylated derivatives if attention is paid to the solvent used. The $\Delta\delta$ value of the *p*-phenylphenacyl ester of *threo*-2,3-dihydroxy-2-methylbutanoic acid recorded in pyridine-d_5 is 0.21 (see Section 3.11). This observation also enables assignment of the relative configuration of the two 2,3-dihydroxy-2-methylbutanoate residues in two phenylpropanoids isolated from *T. transtagana*, since $\Delta\delta$ values of 0.02 and 0.06 ppm confirm a *threo* configuration. No attention was paid to the relative configuration of these two centers in the article concerning phenylpropanoids isolated from *T. transtagana* (Saouf et al., 2006).

The unexpected appearance of the signals originating in the α-protons of the fatty acids could be explained by assuming that the stereocenters make the two α-protons nonequivalent and the two β-protons nonequivalent but isochronous. A good simulation of the spectrum was obtained using gNMR by assuming a coupling constant of |14.5| Hz for the coupling between the two α protons and coupling constants for the coupling between the α-protons to the β-protons of 7.6, 7.7, 8.3 and 8.4 Hz and coupling constants for the coupling between the two β-protons to the methyl group of 7.3 and 7.4 Hz (Fig. 3). No simulation was performed for the hexanoate part of **4A** and for the octanoate part of **3A**, but similar appearance of the patterns of the signals suggest similar coupling constants.

The absolute configuration of the two stereocenters of the 2,3-dihydroxy-2-methylbutanoate residue was determined by measuring the optical rotation of the *p*-phenylphenacylester and found to be 2*R*,3*S*, which is identical to that of the corresponding stereocenters of helmanthicin (**3B**) (Nielsen and Lemmich, 1965). In conclusion the absolute configurations of the four phenylpropanoids isolated from the fruits of *T. garganica* were determined to be **3A**, **4A**, **5A**, and **6A**, respectively. In order to distinguish these phenylpropanoids from those with the relative stereochemistry found in helmanthicin we suggest the names neohelmanthicin A (**3A**), neohelmanthicin B (**4A**), neohelmanthicin C (**5A**), and neohelmanthicin D (**5A**).

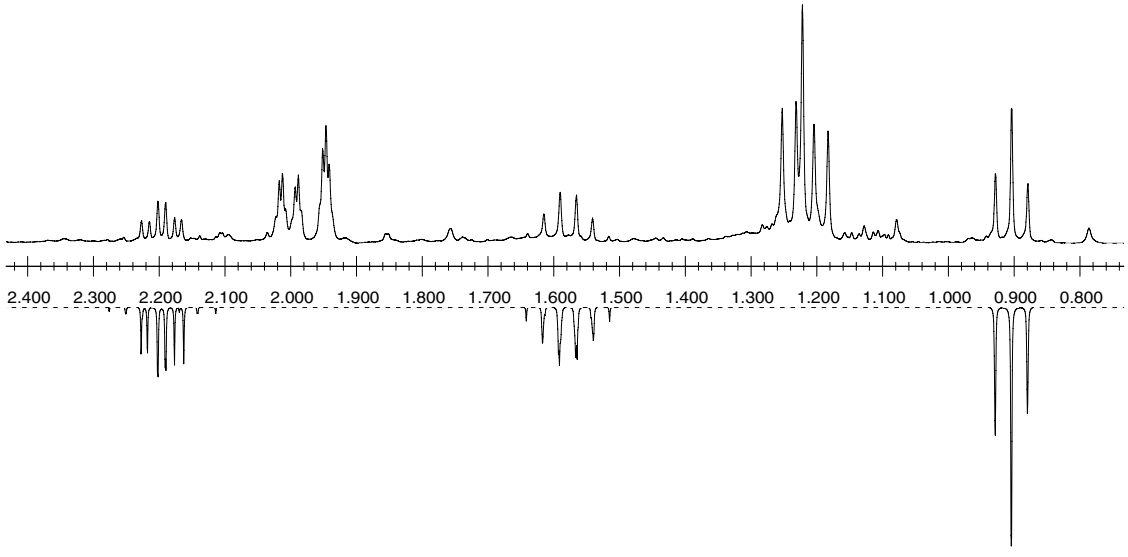


Fig. 3. Recorded (upper spectrum) and simulated (lower spectrum) of the butanoate region (0.8–2.4 ppm) of the spectrum of **6A**. The simulation was performed assuming that the coupling constant for the coupling between the two α-protons is |14.5| Hz, the coupling constant for the coupling between the two α- and the two β-protons is 7.6, 7.7, 8.3 and 8.4 Hz, and the coupling constants for the coupling between the two β-protons and the methyl group is 7.3 and 7.4 Hz.

In addition to the phenylpropanoids a new thapsigargin analogue (**13**, Fig. 1) was isolated from the seeds. Inspection of the ^{1}H and ^{13}C NMR spectra revealed that the signals originating in the octanoyl group of thapsigargin (**1**) were missing and replaced with signals originating in a butanoyl residue. Based on this observation we suggest the structure **13** and since the latest isolated analogue of thapsigargin was named thapsivillosin K we suggest the name thapsivillosin L for this new natural product.

An interesting observation is that the phenylpropanoids isolated from *T. garganica* is esterified with the same acids as the thapsigargins. The fatty acids, butanoic, hexanoic and octanoic acid are not usually found esterified with terpenoids or phenylpropanoids.

2.2. Biological activity

The cytotoxicity of the naturally occuring phenylpropanoids **3A**, **4A**, **5A**, and **6A** and of the diol **2A** towards leukemia cell line EL_4, carcinoma cell line S180 and breast cancer cell line MCF_7 was compared with thapsigargin, which is known as a potent cytotoxin (Denmeade et al., 2003; Furuya et al., 1994; Tombal et al., 2000, 2002) (Table 2). Even though the compounds are considerably less cytotoxic than thapsigargin, they still possess significant activities. Especially the activity against the slow growing breast cancer cell line, which for the natural occurring phenylpropanoids is less than ten times smaller than the activity of Tg, is interesting.

Compound **13** was equipotent with **1** as in inhibitor of SERCA but 13 times less potent as an inhibitor of the growth of humane prostatic cancer TSU cells.

Table 2
Cytotoxicity of the dioxygenated phenylpropanoids **2A** to **6A**

Compound	EL_4	S180	MCF_7
	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
1	0.10 ± 0.37	0.03 ± 0.03	2.3 ± 0.7
3A	0.13 ± 0.01	7 ± 2	23 ± 5
4A	10 ± 3	5 ± 1	12 ± 2
5A	7 ± 3	2.8 ± 0.6	13 ± 3
6A	16 ± 5	18 ± 7	19 ± 5
2A	8 ± 7	5.6 ± 0.2	35 ± 7

Table 1
^{1}H (300 MHz, $CDCl_3$) and ^{13}C NMR data (75 MHz, $CDCl_3$) of the compounds **2A**–**6A**

	2A		3A		4A		5A		6A	
	δ_H (*mult*, *J*)	δ_C	δ_H (*mult*, *J*)	δ_C	δ_H (*mult*, *J*)	δ_C	δ_H (*mult*, *J*)	δ_C	δ_H (*mult*, *J*)	δ_C
1	4.55 (*d*, 4.5)	73.0	5.21 (*dq*, 6.6, 5.1)	73.5	5.21 (*dq*, 6.3, 4.8)	73.5	5.21 (*dq*, 6.6, 5.1)	73.6	5.21 (*dq*, 6.6, 5.1)	73.6
2	3.95 (*dq*, 6.6, 4.5)	77.5	5.83 (*d*, 5.1)	75.2	5.86 (*d*, 4.8)	75.2	5.83 (*d*, 5.1)	75.2	5.86 (*d*, 4.8)	75.2
3		134.6		130.4		130.7		130.7		130.6
4[a]	6.55 (*s*)	100.5	6.52 (*d*, 1.5)	100.8	6.53 (*d*, 1.5)	100.9	6.53 (*d*, 1.5)	100.9	6.52 (*d*, 1.5)	100.9
5		143.0		142.9		143.1		143.1		143.1
6		135.0		134.8		134.9		134.9		134.9
7		148.5		148.5		148.7		148.7		148.7
8[a]	6.55 (*s*)	105.8	6.51 (*d*, 1.5)	106.7	6.51 (*d*, 1.5)	106.8	6.51 (*d*, 1.5)	106.8	6.51 (*d*, 1.5)	106.8
Me-1	1.02 (*d*, 6.6)	17.5	1.19 (*d*, 6.3)	14.9	1.19 (*d*, 6.3)	14.9	1.19 (*d*, 5.1)	14.9	1.19 (*d*, 6.3)	14.9
MeO	3.90 (*s*)	59.0	3.89 (*s*)	56.5	3.88 (*s*)	56.5	3.89 (*s*)	56.6	3.89 (*s*)	56.6
OCH_2O	5.95 (*s*)	101.5	5.95 (*bs*)	101.4	5.96 (*d*, 1.5)	101.4	5.96 (*d*, 1.5)	101.4	5.95 (*bs*)	101.4
					5.95 (*d*, 1.5)		5.95 (*d*, 1.5)			
1′				173.9		174.0		174.1		174.0
2′				75.9		75.8		75.8		75.9
3′			5.11 (*q*, 6.6)	73.6	5.04 (*q*, 6.6)	73.6	5.04 (*q*, 6.6)	73.5	5.11 (*q*, 6.3)	73.5
4′			1.29 (*d*, 6.6)	13.6	1.21 (*d*, 6.6)	13.2	1.24 (*d*, 6.6)	13.2	1.24 (*d*, 6.6)	13.1
Me-2′			1.24 (*s*)	21.5	1.25 (*s*)	21.5	1.22 (*s*)	21.5	1.22 (*s*)	21.5
1″				165.7		165.9		165.9		165.9
2″				126.9		126.9		126.9		126.9
3″			6.15 (*qq*, 7.2, 1.5)	138.7	6.14 (*qq*, 7.2, 1.5)	139.6	6.12 (*qq*, 7.5, 1.5)	139.6	6.15 (*qq*, 7.2, 1.5)	139.6
4″			2.00 (*dq*, 7.2, 1.5)	15.8	2.00 (*dq*, 7.5, 1.5)	15.9	2.00 (*dq*, 7.2, 1.5)	15.9	1.99 (*dq*, 7.2, 1.5)	15.9
Me-2″			1.94 (*q*, 1.5)	20.5	1.95 (*q*, 1.5)	20.6	1.94 (*q*, 1.5)	20.6	1.95 (*q*, 1.5)	20.6

The coupling constant (*J*) is given in Hz.

The remaining signals were found at: Angelicate: δ_H 6.05 (*qq*, $J = 7.2$ and 1.5 Hz, H-3), 1.95 (*dq*, $J = 7.2$ and 1.5 Hz, H-4), 1.80 (*q*, $J = 1.5$, Me-2); δ_C 165.7 (C-1), 126.7 (C-2), 138.7 (C-3), 15.9 (C-4), 20.6 (Me-2). Butanoate: δ_H2.21 (*m*, $J = 14.5$, 7.7 and 7.6 Hz, H-2), 2.18 (*m*, $J = 14.5$, 8.4 and 8.3, H-2′), 1.58 (*m*, $J = 8.4$, 7.7 and 7.3 Hz, H-3), 1.58 (*m*, $J = 8.3$, 7.6 and 7.4 Hz, H-3′), 0.90 (*t*, $J = 7.4$ and 7.3 Hz, H-4); δ_C 172.0, (C-1), 36.0 (C-2), 18.3 (C-3), 13.6 (C-4); Hexanoate: δ_H 2.23 (*m*, $J = 14.5$, 7.7, and 7.6 Hz, H-2), 2.18 (*m*, $J = 14.5$, 8.4 and 8.3 Hz, H-2′), 1.55 (*m*, $J = 8.4$, 8.3, 7.7 and 7.6 Hz, H-3), 1,27 (*m*, H-4 and H-5), 0.87 (*t*, $J = 6.9$ Hz, H-6), δ_C: 172.1 (C-1), 34.1 (C-2), 24.5 (C-3), 31.1 (C-4), 22.2 (C-5), 13.9 (C-6); Octanoate δ_H 2.22 (*m*, $J = 14.5$, 7.7 and 7.6 Hz, H-2), 2.18 (*m*, $J = 14.5$, 8.4 and 8.3 Hz, H-2′), 1.55 (*m*, $J = 8.4$, 8.3, 7.7 and 7.6 Hz, H-3), 1,23 (*m*, H-4, H-5, H-6 and H-7), 0.88 (*t*, $J = 6.9$ Hz, H-6), δ_C: 172.1 (C-1), 34.2 (C-2), 24.8 (C-3), 29.0 (C-4), 28.8 (C-5), 31.6 (C-6), 22.5 (C-7), 14.0 (C-8).

The following abbreviations have been used: *s* singlet, *d* doublet, *q* quartet, *b* broad, *m* multiplet.

[a] The signals originating in H-4 and H-8 or C-4 and C-8 might be interchanged.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Varian Mercury spectrometer or Varian Gemini 2000 spectrometer using tetramethylsilane as internal standard. Splitting patterns are described as singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*), broad (*b*) and multiplet (*m*). The standard programs supplied by Varian for 2D NMR spectra were used. Optical rotation was measured on a Perkin–Elmer 241 polarimeter using a 1 dm cuvette. Normal phase column chromatography was performed on silica gel 60 (Merck 107734) and reversed phase chromatography on LiChroprep RP-18 (Merck 113900). For HPLC a Waters 6000A pump and a Shimadzu SPD 6A detector was used chromatography performed over a Luna C18 150 × 21 mm, 5 μm, column. Mass spectra were recorded on a JEOL AX505 W instrument using the FAB-liquid technique (HEDS). The NMR simulation was performed using gNMR 5.0.4, Adept Scientific.

3.2. Plant material

Fruits of *Thapsia garganica* L. were collected on the island of Ibiza, Spain, in July 2003. A voucher specimen (DFHSBC1) is deposited at The Herbarium at the University of Copenhagen. The same plant material was used for isolation of tethered fats (Liu et al., 2004).

3.3. Isolation

The fruits (1 kg) were blended with 5 L of ethanol and the mixture left overnight. The mixture is filtered and the filtrate concentrated *in vacuo* to give 46 g of a brown gum. The residue was suspended in water (400 ml) and the suspension washed three times each time with 400 ml of toluene. The organic phase is concentrated to give approximately 40 g of an oily dark residue.

The residue was chromatographed over silica gel 60 (800 g, Merck 07734, 0.063–0.20 mm) using the following eluents toluene–ethyl acetate–acetic acid (8:2:0.05, 5.6 l), toluene–ethyl acetate–acetic acid (7:3:0.05, 5.6 l), toluene–ethyl acetate–acetic acid (6:4:0.05, 2 l), toluene–ethyl acetate–acetic acid (1:1:0.05, 2 l) and dichloroethane–ethyl acetate–acetic acid (4:6:0.05, 2 l). The fractions eluting between 7.6 and 15.6 l of eluent were combined and concentrated to give 21.8 g of a gum. The residue was rechromatographed over LiChroprep RP-18 using methanol–water–acetic acid (7:3:0.1, 2.4 l), methanol–water–acetic acid (8:2:0.1, 2.6 l) and methanol–water–acetic acid (9:1:0.1, 1.2 l). The fractions eluting between 2.6 and 3 l of eluent were combined and concentrated to give 2.6 g of a gum. An amount of 500 mg of the fraction was purified by HPLC using methanol–water–acetic acid 7:3:0.1 as en eluent to give 20 mg of **13** eluting between 100 and 120 ml, 30 mg of **6A** eluting between 130 and 135 ml, 20 mg of **3A** eluting between 135 and 145 ml, 10 mg of **5A** eluting between 180 and 200 ml and 30 mg of **13** eluting between 250 and 300 ml.

3.4. Neohelmanthicin A (*3A*)

Colorless gum. $[\alpha]_D^{25}$ −25 ($CHCl_3$, *c* 0.37). HRFABMS *m/z* 529.2028 $[M+Na]^+$ calcd for $C_{26}H_{34}NaO_{10}$, 529.2050. The ^1H and ^{13}C NMR spectra are reported in Table 1.

3.5. Neohelmanthicin B (*4A*)

Colorless gum. $[\alpha]_D^{25}$ −21 ($CHCl_3$, *c* 0.096). HRFABMS *m/z* 573.2645 $[M+Na]^+$ calcd for $C_{29}H_{42}NaO_{10}$, 573.2676. The ^1H and ^{13}C NMR spectra are reported in Table 1.

3.6. Neohelmanthicin B (*5A*)

Colorless gum. $[\alpha]_D^{25}$ −32 ($CHCl_3$, *c* 0.23). HRFABMS *m/z* 545.245 $[M+Na]^+$ calcd for $C_{27}H_{38}NaO_{10}$, 545.2363. The ^1H and ^{13}C NMR spectra are reported in Table 1.

3.7. Neohelmanthicin C (*6*)

Colorless gum. $[\alpha]_D^{25}$ −33 ($CHCl_3$, *c* 0.5). HRFABMS *m/z* 494.2190 $[M]^+$ calcd for $C_{25}H_{34}NaO_{10}$, 494.2152. The ^1H and ^{13}C NMR spectra are reported in Table 1.

3.8. Thapsivillosin L (*13*)

Colorless gum. $[\alpha]_D^{25}$ −26 ($CHCl_3$, *c* 1.15). HRMS *m/z* 617.2596 $[M+Na]^+$ calcd for $C_{30}H_{42}NaO_{12}$, 617.2574. ^1H NMR (300 MHz, $CDCl_3$) δ guaianolide 4.34 (1H, *bs*, H-1), 5.6–5.7 (3H, *m*, H-3, H-6 and H-8), 5.46 (1H, *t*, $J = 3$ Hz, H-2), 3.10 (1H, *dd*, $J = 15$ and 3 Hz, H-9), 2.3 (1H, overlapped by other signals, H-9′), 1.56 (3H, *s*, H-13), 1.39 (3H, *s*, H-14), 1.86 (3H, *bs*, H-15), angeloate 6.09 (1H, *qq*, $J = 5.7$ and 1.5 Hz, H-3), 2.01 (3H, *dq*, $J = 5.7$ and 1.5 Hz, H-4), 1.91 (3H, *q*, $J = 1.5$ Hz, Me-2), butanoate 2.4–2.2 (4H, *m*, H-2), 1.7–1.6 (4H, *m*, H-3), 0.95 (3H, *t*, $J = 7.5$ Hz, H-4), acetate 1.89 (3H, *s*, H2). ^{13}C NMR (75 MHz, $CDCl_3$)s δ guaianolide 57.1 (C-1), 77.5 (C-2), 84.6 (C-3), 130.1 (C-4), 141.0 (C-5), 76.6 (C-6), 78.2 (C-7 or C-11) 65.9 (C-8), 38.1 (C-9), 84.6 (C-10), 78.3 (C-11 or C-7), 175.3 (C-12), 16.0 (C-13), 22.5 (C-14), 12.9 (C-15), acetate 170.6 (C-1), 20.5 (C-2), angeloate 166.6 (C-1), 127.8 (C-2), 138.3 (C-3), 15.8 (C-4), 20.6 (Me-2), butanoate 171.9, 172.2 (C-1), 36.4, 35.9 (C-2), 18.2, 17.9 (C-3), 13.7, 13.6 (C-4).

3.9. Hydrolysis of *3A*

A solution of **3A** (200 mg, 0.62 mmol) and sodium hydroxide (150 mg, 3.8 mmol) in methanol (5 ml) and water (2 ml) was stirred for 4 h at room temperature. The reaction mixture was concentrated in vacuo to remove the methanol and the residue diluted to 5 ml with

water. The solution was extracted three times with diethyl ether (10 ml), the combined organic phases dried with magnesium sulfate and concentrated to give **2A** (85 mg, 60%) as colorless oil. The aqueous layer was neutralized with hydrochloric acid (pH 7.5) and added ethanol (5 ml), *p*-phenylphenacyl bromide (200 mg) and refluxed for 40 min. The reaction mixture was concentrated and the residue extracted with dichloromethane. The dicholoromethane extract was purified by column chromatography over Lichroprep using methanol–water (4:1) added 1% of acetic acid as an eluent to give *p*-phenylphenacyl 2,3-dihydroxy-2-methylbutanoate (40 mg, 20%) as a colorless gum.

3.10. Compound *2A*

Gum. $[\alpha]_D^{25}$ −23 ($CHCl_3$, *c* 0.62). The ^1H and ^{13}C NMR data are reported in Table 1. The optical antipode has $[\alpha]_D^{25}$ +23 ($CHCl_3$, *c* 0.6) (Pascual Teresa et al., 1985b).

3.11. Phenylphenacyl 2,3-dihydroxy-2-methylbutanoate

Gum. $[\alpha]_D^{25}$ −5.8 ($CHCl_3$, *c* 0.62), $[\alpha]_{365}^{25}$ −95 ($CHCl_3$, *c* 0.62) lit. $[\alpha]_{365}^{25}$ −97 ($CHCl_3$, *c* 0.27) (Pascual Teresa et al., 1985b), ^1H NMR (300 MHz, pyridine-d_5) δ 8.10 (2H, *m*, H-*o*), 7.70 (2H, *m*, H-*m*), 7.67 (2H, *m*, H-*o*′), 7.5–7.4 (3H, *m*, H-*m*′ and H-*p*′), 5.83 (1H, *d*, $J = 14.1$ Hz, H-α), 5.74 (1H, *d*, $J = 14.1$ Hz, H-α′), 4.59 (1H, *q*, $J = 6.3$ Hz, H-3), 1.81 (3H, *s*, Me-2), 1.60 (3H, *d*, $J = 6.3$ Hz, H-4).

3.12. threo-Trimethylsilylethyl 2,3-dihydroxy-2-methylbutanoate (*9*)

A solution of tiglic acid (0.3 g, 3 mmol), 2-trimethylsilyethanol (0.35 g, 3 mmol), dicyclocarbodiimide (0.62 g, 3 mmol) and 4-dimethylaminopyridine (100 mg, DMAP) was stirred at room temperature for 18 h. The mixture was filtered and the filtrate concentrated. The residue was purified by column chromatography over silica gel using toluene–ethyl acetate (100:1) added 0.5% acetic acid as an eluent to give **7** as colorless oil (400 mg, 67%). To a solution of AD-mix-α (1.4 g) in 10 ml of *tert*-butyl alcohol–water (1:1) was added **7** (200 mg, 0.99 mmol) and the mixture was stirred at room temperature overnight. To the reaction mixture was added sodium sulfite (1.6 g) and the mixture was stirred for additional 30 min. The mixture was extracted three times with ethyl acetate (20 ml) and the combined organic phases were concentrated in vacuo. The residue was purified by column chromatography over silica gel using toluene–ethyl acetate (5:1) as an eluent to give **9** (180 mg, 77.2%) as colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 4.27 (2H, *m*, CH_2O), 3.91 (1H, *q*, $J = 6.3$ Hz, H-3), 1.29 (3H, *s*, Me-2), 1.20 (1H, *d*, $J = 6.3$ Hz, H-4), 1.04 (2H, *m*, CH_2Si) 0.04 (9H, *s*, $(CH_3)_3Si$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.6 (C-1), 77.4 (C-2), 71.9 (C-3), 65.1 (CH_2O), 17.1 (C-4), 22.1 (Me-2), 17.8 (CH_2Si), 1.1 (CH_3Si).

3.13. threo-Trimethylsilylethyl 3-acetoxy2-hydroxy-2-methylbutanoate (*11*)

To a solution of **9** (70 mg, 0.3 mmol) in dichloromethane (2 ml) was added DMAP (5 mg) and acetic anhydride (64 mg, 0.63 mmol). The reaction mixture was stirred at room temperature for 2 h. he mixture was filtered and the residue concentrated. The residue was purified by column chromatography over silica gel using toluene–ethyl acetate (10:1) as an eluent to give **11** (60 mg, 72%) as colorless oil. ^1H NMR (300 MHz, $CDCl_3$) 5.10 (1H, *q*, $J = 6.0$ Hz, H-3), δ 4.22 (2H, *m*, CH_2O), 1.99 (3H, *s*, CH_3CO) 1.33 (3H, *s*, Me-2), 1.27 (1H, *d*, $J = 6.0$ Hz, H-4), 1.00 (2H, *m*, CH_2Si) 0.04 (9H, *s*, $(CH_3)_3Si$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6 (C-1), 170.1 (CH_3CO), 76.3 (C-2), 74.5 (C-3), 65.0 (CH_2O), 22.1 (Me-2), 21.4 (CH_3CO), 17.8 (CH_2Si), 13.6 (C-4), −1.2 (CH_3Si).

3.14. erythro-Trimethylsilylethyl 2,3-dihydroxy-2-methylbutanoate (*10*)

To a stirred solution of angelic acid (800 mg, 8 mmol) and triethylamine (0.8 g, 8 mmol) in toluene (8 ml) was added 2,4.6-trichlorobenzoyl chloride (2 g, 8 mmol) and the mixture was stirred for 2 h at room temperature, whereupon a solution of 2-trimethylsilylethanol (236 mg, 2 mmol) in toluene (2 ml) was added and the mixture stirred for additional 18 h at 75 °C. The reaction mixture was quenched with a half saturated solution of sodium hydrogen carbonate (20 ml). The aqueous phase was extracted three times with diethyl ether (30 ml). The combined organic phases were dried (sodium sulfate), concentrated and the residue purified over silica gel using toluene–ethyl acetate (100:1) to give **8** (153 mg, 40%) as colorless oil. Compound **10** was prepared from **8** as described for preparation of **9** from **7** using 100 mg (0.5 mmol) of **8** as starting material and 70 mg of AD-mix-α to give 70 mg (60 %) of **10.** ^1H NMR (300 MHz, $CDCl_3$) 4.27 (2H, *m*, CH_2O), δ 3.79 (1H, *q*, $J = 6.6$ Hz, H-3), 1.42 (3H, *s*, Me-2), 1.14 (1H, *d*, $J = 6.6$ Hz, H-4), 1.03 (2H, *m*, CH_2Si) 0.04 (9H, *s*, $(CH_3)_3Si$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9 (C-1), 77.3 (C-2), 72.5 (C-3), 65.0 (CH_2O), 22.6 (Me-2), 18.0 (CH_2Si), 17.8 (C-4), −1.2 (CH_3Si).

3.15. erythro-Trimethylsilylethyl 3-acetoxy-2-hydroxy-2-methylbutanoate (*12*)

Compound **12** was prepared from **10** as described for preparation of **11** from **9** using 70 mg (0.3 mmol) of 10 as starting material to give 52 mg (63 %) of **12**. ^1H NMR (300 MHz, $CDCl_3$) δ 5.10 (1H, *q*, $J = 6.6$ Hz, H-3), 4.28 (2H, *m*, CH_2O), 2.09 (3H, *s*, CH_3CO), 1.38 (3H, *s*, Me-2), 1.18 (1H, *d*, $J = 6.6$ Hz, H-4), 1.03 (2H, *m*, CH_2Si) 0.06 (9H, *s*, $(CH_3)_3Si$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.3 (C-1), 170.7 (CH_3CO), 76.2 (C-2), 73.9 (C-3), 65.3 (CH_2O), 22.6 (Me-2), 21.4 (CH_3CO), 17.8 (CH_2Si), 15.1 (C4), −1.2 (CH_3Si).

3.16. Assay for cytotoxicity

The three different cancer cell lines used in this study, EL_4 (murine lymphoma), S180 (murine sarcoma) and MCF7 (breast cancer) were purchased from National Cancer Institute. A standard high-flux anticancer-drug screening method was employed in this study (Skehan et al., 1990). Briefly, cancer cells were incubated with the test compound in different concentrations at 37 °C for 48 h. Cultures were fixed with trichloroacetic acid, then stained with sulforhodamine B and read at 490 nm by ELISA reader. The apoptotic activity of **13** towards TSU cells was expressed as the concentration capable of inducing 50% loss of clonogenic survival of TSU-Pr1 human protatic cancer cells compared to untreated cells as previous described (Jakobsen et al., 2001). All experiments were performed at least two times in triplicates.

3.17. Assay for SERCA activity

Isolation of sarcoplasmic reticulum (SR) vesicles containing Ca^{2+}-ATPases from rabbit muscle was done as previously described (Jakobsen et al., 2001). The SR vesicles were stored at −80 °C prior to use. The Ca^{2+}-ATPase inhibitory potencies of **13** was determined as previous described with a coupled enzyme assay (Seidler et al., 1989; Varga et al., 1986). The activity **13** and control (**1**) was determined in triplicate. Plotting the ATPase activity as a function of inhibitor concentration the IC_{50} value was estimated by fitting using the program Origin version 5.0 from Microcal™ Origin™, Microcal Software Inc.

Acknowledgement

This work was supported by the Danish Cancer Society, Carlsbergfondet and Apotekerfonden af 1991.

References

Barrero, A.F., Mar Herrador, M., Arteaga, P., 1991. Sesquiterpenes and phenylpropanoids from *Seseli vayredanum*. Phytochemistry 31, 203–207.

Denmeade, S., Isaacs, J.T., 2005. The SERCA pump as a therapeutic target. Cancer Biol. Ther. 4, e69–e77.

Denmeade, S.R., Jakobsen, C.M., Janssen, S., Khan, S.R., Garrett, E.S., Lilja, H., Christensen, S.B., Isaacs, J.T., 2003. Prostate-specific antigen-activated thapsigargin prodrug as targeted therapy for prostate cancer. J. Natl. Cancer Inst. 95, 990–1000.

Furuya, Y., Lundmo, P., Short, A.D., Gill, D.L., Isaacs, J.T., 1994. The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin. Cancer Res. 54, 6167–6175.

Gonzales, A.G., Diaz, J.G., Lopez, L.A., Valencia, L.E., Paz, P.P.D., Barrea, J.B., 1993. Sesquiterpene estersa nd sesquiterpene coumarin ethers from *Ferula linkii*-TF. Phytochemistry 33, 863–866.

Gonzalez, A.G., Diaz, J.G., Arancibia, L., Bermejo, J., 1988. Phenylpropanoid and stilbene compounds from *Ferula latipinna*. Planta Med. 54, 184–185.

Holub, M., De Groote, R., Herout, V., Sorm, F., 1968. Plant substances XXVIII. Oxygen containing components of light petroleum extract of *Laser trilobum* (L.) Borkh. root. Structure of laserine. Collect. Czech. Chem. Commun. 33, 2911–2917.

Jakobsen, C.M., Denmeade, S.R., Isaacs, J.T., Gady, A., Olsen, C.E., Christensen, S.B., 2001. Design, synthesis, and pharmacological evaluation of thapsigargin analogues for targeting apoptosis to prostatic cancer cells. J. Med. Chem. 44, 4696–4703.

Janssen, S., Rosen, D.M., Ricklis, R.M., Dionne, C.A., Lilja, H., Christensen, S.B., Isaacs, J.T., Denmeade, S.R., 2006. Pharmacokinetics, biodistribution, and antitumor efficacy of a human glandular kallikrein 2 (hK2)-activated thapsigargin prodrug. Prostate 66, 358–368.

Jennett-Siems, K., Kaloga, M., Eich, E., 1993. Ipangulines, the first pyrrolizidine alkaloids from the Convolvulaceae. Phytochemistry 34, 437–440.

Jeong, T.S., Kim, K.S., Yu, H., Kim, M.J., Cho, K.H., Choi, Y.K., Kim, H.C., Park, H.Y., Lee, W.S., 2005. Saucerneol B derivatives as human acyl-CoA: cholesterol acyltransferase inhibitors. Bioorg. Med. Chem. Lett. 15, 385–388.

Kolb, H.C., van Nieuwenhze, M.S., Sharpless, K.B., 1994. Catalytic asymmetric dihydroxylation. Chem. Rev. 94, 2483–2547.

Konya, K., Kiss-Szikszai, A., Kurtan, T., Antus, S., 2004. Enantiomeric separation of racemic neolignans on chiralcel OD and determination of their absolute configuration with online circular dichroism. J. Chromatogr. Sci. 42, 478–483.

Lee, A.L., Ley, S.V., 2003. The synthesis of the anti-malarial natural product polysphorin and analogues using polymer-supported reagents and scavengers. Org. Biomol. Chem. 1, 3957–3966.

Liu, H., Olsen, C.E., Christensen, S.B., 2004. Tethered lipids from *Tahpsia garganica*. J. Nat. Prod. 67, 1339–1340.

Miski, M., Jakupovic, J., 1990a. Cyclic farnesyl-coumarin and farnesylchromone derivatives from *Ferula communis* subsp. *communis*. Phytochemistry 29, 1995–1998.

Miski, M., Jakupovic, J., 1990b. Daucane esters from *Ferula rigidula*. Phytochemistry 29, 173–178.

Miski, M., Mabry, T.J., 1986. New daucane esters from *Ferula tingitana*. J. Nat. Prod. 49, 657–660.

Nielsen, B.E., Lemmich, J., 1965. Constituents of umbelliferous plants VI. The structure of peulustrin, a new coumarin from *Peucedanum palustre* L. Acta Chem. Scand. 19, 601–604.

Olesen, C., Sorensen, T.L.M., Nielsen, R.C., Moller, J.V., Nissen, P., 2004. Dephosphorylation of the calcium pump coupled to counterion occlusion. Science 306, 2251–2255.

Pascual Teresa, J., Arias, A., Hernandez, J.M., Grande, M., 1985a. Synthesis of (+-)-deacylhelmanticine, erythro- and threo-1-[3,4-(methylenedioxy)-5-methoxyphenyl]-1,2-propanediols. Anal. Quim. Ser. C: Quim. Org. Bioquim. 81, 153–156.

Pascual Teresa, J., De Pascual, M., Arias, A., Hernandez, J.M., Moran, J.R., Grande, M., 1985b. Helmanticine, a phenylpropanoid from *Thapsia villosa*. Phytochemistry 24, 1773–1778.

Pinar, M., Rico, M., Rodriguez, B., 1982. Laserine oxide, an epoxide from *Guillonea scabra*. Phytochemistry 21, 735–737.

Saouf, A., Guerra, F.M., Rubal, J.J., Jorge, Z.D., Akssira, M., Mellouki, F., Moreno-Dorado, F.J., Massanet, G.M., 2006. Phenylpropanoids from *Thapsia transtagana*. Phytochemistry 67, 800–804.

Seidler, N.W., Jona, I., Vegh, M., Martonosi, A., 1989. Cyclopiazonic acid is a specific inhibitor of the Ca-2+-ATPase of sarcoplasmic-reticulum. J. Biol. Chem. 264, 17816–17823.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., Mcmahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107–1112.

Teresa, J.D.P., Morán, J.R., Hernandez, J.M., Grande, M., 1985. Phenylpropanoids and other derivatives from *Thapsia villosa*. Phytochemistry 24, 2071–2074.

Tombal, B., Denmeade, S.R., Gillis, J.M., Isaacs, J.T., 2002. A supramicromolar elevation of intracellular free calcium ([Ca(2+)](i)) is consistently required to induce the execution phase of apoptosis. Cell Death. Differ. 9, 561–573.

Tombal, B., Weeraratna, A.T., Denmeade, S.R., Isaacs, J.T., 2000. Thapsigargin induces a calmodulin/calcineurin-dependent apoptotic cascade responsible for the death of prostatic cancer cells. Prostate 43, 303–317.

Treiman, M., Caspersen, C., Christensen, S.B., 1998. A tool coming of age – thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases. Trends. Pharmacol. Sci. 19, 131–135.

Varga, S., Mullner, N., Pikula, S., Papp, S., Varga, K., Martonosi, A., 1986. Pressure effects on sarcoplasmic-reticulum. J. Biol. Chem. 261, 3943–3956.

Zhang, H.J., Tamez, P.A., Vu, D.H., Ghee, T.T., Nguyen, V.H., Le, T.X., Le, M.H., Nguyen, M.C., Do, T.T., Soejarto, D.D., Fong, H.H., Pezzuto, J.M., 2001. Antimalarial compounds from *Rhaphidophora decursiva*. J. Nat. Prod. 64, 772–777.