Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 159–164
Tetrahedron

Sulfur-containing sesquiterpenes from *Thapsia villosa*

Juan J. Rubal,[a] Francisco M. Guerra,[a] F. Javier Moreno-Dorado,[a] M. Akssira,[b] F. Mellouki,[b] Antonio J. Pujadas,[c] Zacarías D. Jorge[a] and Guillermo M. Massanet[a,*]

[a]*Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain*
[b]*Faculté des Sciences et Techniques,Université Hassan II Mohammedia, BP 146 Mohammedia 20650, Morocco*
[c]*Departamento de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Apartado 3048, 14080 Córdoba, Spain*

Received 24 July 2003; revised 6 October 2003; accepted 28 October 2003

Abstract—Three new sesquiterpenoids bearing sulfurated ester groups have been isolated from the roots of *Thapsia villosa* L. Their structures have been elucidated by spectroscopic means. This is the first time that a methylthiopropionic acid ester is isolated from natural sources.

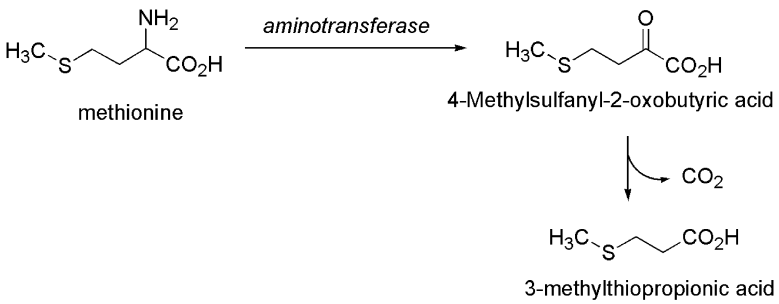
1. Introduction

The presence of sulfur atoms in groups other than sulfates and disulfide bridges in metabolites isolated from natural sources is infrequent. Only certain species belonging to the genus *Petasites* (Compositae),[1] are known to produce sulfurated sesquiterpenes, in higher plants. Sulfur is usually found in ester groups, which biogenetically comes from methionine through the action of an aminotransferase and further decarboxylation to yield 3-methylthiopropionic acid (Scheme 1).[2] Those sulfur-containing compounds isolated from *Petasites* have shown a broad range of activities such as inhibitors of testosterone secretion,[3] calcium channel blockers[4] or anti-inflammatory.[5]

Thapsia villosa L. (Apiaceae) is a perennial herb which grows in uncultured soils of the Western Mediterranean area. Traditionally, it has been used in the folk medicine in Catalonia against scabies.[6] Recently, it has been reported to possess ichthyotoxic activity.[7]

T. villosa displays an extremely variable morphology which often leads to misidentification of the material collected. From a taxonomic point of view, the species is divided into two groups differing in the number of chromosomes and the compounds that they produce. Previous studies of *T. villosa* have provided phenyl propanoids, germacranes, thapsigargin-related guaianolides, slovenolide-type guaianolides and a relatively small group of sesquiterpenes known as thapsanes.[8]

In this work, we report our results on the reinvestigation of the roots of *T. villosa* L. Along with the known phenylpropanoid helmanticine[8b] and the guaianolide thapsivillosine C,[8a] three new sesquiterpenoids bearing sulfur-containing ester groups have been isolated (Fig. 1).



Scheme 1. Formation of 3-methylthiopropionic acid from methionine.

Keywords: *Thapsia villosa*; Sulfur; Sesquiterpene; Methylthiopropanoate ester; Methylthiopropenoate ester.
* Corresponding author. Tel.: +34-956-016405; fax: +34-956-016288; e-mail address: francisco.guerra@uca.es

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.10.079

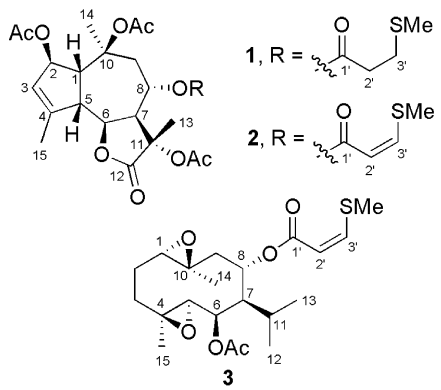


Figure 1. Novel sesquiterpenes from *T. villosa*.

2. Results and discussion

The dichloromethane extract of the roots was subjected to flash chromatography affording **1** (28 mg), **2** (7 mg) and **3** (250 mg).

The presence of a sulfur atom in compound **1** was detected by elemental analysis and confirmed by HREIMS in which a molecular ion [M^+] at *m/z* 526.1860 was in agreement with the molecular formula $C_{25}H_{34}O_{10}S$ (9 degrees of unsaturation). The IR spectrum showed the presence of carbonyl groups at 1791 and 1738 cm^{-1}, the former corresponding to a γ-lactone moiety.

In the ^1H NMR spectrum, some protons, later identified as those of the methylthiopropionate group showed very broad signals. Likewise, the intensity of the ^{13}C NMR signals of their corresponding carbon atoms was also very low. This problem was minimized by running the spectra at −50 °C.

The ^{13}C NMR spectrum displayed twenty five signals, five of which corresponding to carbonyl groups (δ_C 173.9, 170.7, 170.6, 170.1 and 169.9), two were olefinic carbons (δ_C 149.5 and 126.1), and five of them corresponding to carbon atoms bearing oxygenated functionalization (δ_C 79.7, 79.3, 77.8, 75.4 and 65.7).

The five carbonyl groups and the double bond, accounted for 6 degrees of unsaturation. If a γ-lactone ring was present, the compound should be bicyclic. Assuming a bicyclic fused structure, two likely possibilities were considered: a 6.6 or a 5.7 bicyclic compound. The latter possibility was finally confirmed by the different correlations found in the HMBC spectrum.

In the $^1H-^1H$ COSY spectrum, the lactone ring proton, H-6 (δ_H 4.83) was coupled to a C*H* proton (H-7, δ_H 3.60), which in turn was coupled to another C*H* proton (H-8, δ_H 5.80). The chemical shift of C-8 (δ_C 65.7), indicated the presence of an oxygenated group. H-8 was also coupled to two geminal protons at δ_H 2.60 and 1.96 which showed no additional coupling. Thus, the partial structure **A**, shown in Figure 2, was deduced.

A different coupling sequence was found starting from H-6 (partial structure **B**, Fig. 2): H-6 with H-5 (δ_H 3.10), H-5 with H-1 (δ_H 3.42), and H-1 with H-2 (δ_H 5.70). H-2 showed in the HSQC spectrum a cross coupling correlation with C-2 at δ_C 79.4, thus indicating the presence of an ester group at C-2. -2 was finally coupled with a vinylic methine (H-3). Its corresponding carbon, C-3 was correlated in the HMBC spectrum with H-15 (Fig. 2).

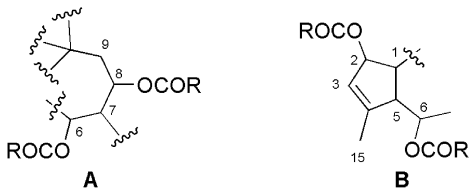


Figure 2. Partial structures found for **1**.

The ^1H NMR spectrum showed additionally the presence of three acetate groups at δ_H 2.10, 2.03, 2.02 which accounted for three of the carbonyl groups. The remaining two carbonyl groups were then assigned to the γ-lactone ring and an additional ester group.

The nature and location of the ester group was deduced as follows. The ^1H NMR spectrum showed two methylene groups coupled each other at δ_H 2.60 (*m*, 2H) and 2.80 (*m*, 2H), assigned to 2H-2′ and 2H-3′ respectively. C-3′ showed a long distance correlation with a $-S-CH_3$ group. The two H-2′ protons were coupled in the HMBC spectrum with C-1′ (δ_C 170.1), which showed additionally a coupling correlation with H-8 at δ_H 5.80. All these correlations were in agreement with a methylthiopropionate ester, $-O-CO-CH_2CH_2-S-CH_3$, located at C-8. His is the first time that this ester group has been reported as part of a natural product.

The HMBC spectrum allowed also to locate the remaining acetoxyl group at C-2 (δ_C 79.4), C-10 (δ_C 79.7) and C-11 (δ_C 77.8) (Fig. 3).

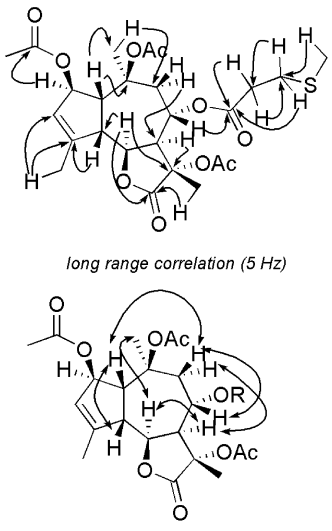


Figure 3. Long range correlations (up) and NOE effects (down) observed in **1** (*J*=5 Hz).

Finally, the relative stereochemistry of the different stereogenic centres was confirmed by NOE experiments and the coupling constant values. The structure of

compound **1** and the observed HMBC correlations are depicted in Figure 3.

Spectral data of compound **2** showed a close resemblance to those of compound **1**. The presence of a sulfur atom was again confirmed by elemental analysis. The HREIMS displayed a peak at *m/z* 464.1500 according to a molecular formula $C_{23}H_{28}O_8S$ and corresponding to $[M-HOAc]^+$ ion. The carbonyl absorptions in the IR spectrum were shown at 1791 and 1734 cm^{-1}. Finally, the ^{13}C NMR showed the presence of an additional double bond in comparison to **1** (δ_C 153.5 and 112.3).

The main difference in the ^1H NMR spectrum consisted on the presence of two mutually coupled doublet signals centered at δ 7.10 and 5.76 respectively. The HSQC spectrum allowed the identification of their corresponding carbons at δ_C 153.5 and 112.3, the former showing a correlation in the HMBC with the S–CH_3 group. These facts led to the identification of the side chain as –O–CO–CH=CH–SMe.[9] The value of the coupling constant $J_{2'-3'}$=10.3 Hz, suggested a *Z*-configuration of the double bond in the chain.

The remaining signals were similar to those in compound **1**. With all these data, the structure of compound **2** is proposed as depicted in Figure 1.

The molecular formula of germacrane **3** was determined by elemental analysis and confirmed by HREIMS (*m/z* 412.1905, $C_{21}H_{32}O_6S$, 6 degrees of unsaturation). The IR spectrum showed an absorption at 1740 cm^{-1}, corresponding to an ester group.

The ^{13}C NMR showed signals corresponding to the presence of two carbonyl groups (δ_C 170.4 and 165.6) and a double bond (δ_C 153.1 and 112.9), which accounted for three degrees of unsaturation. The molecule should be therefore a tricyclic compound.

The presence of six carbons bearing oxygenated groups (δ_C 73.2, 69.3, 66.6, 61.5, 58.8 and 58.7) was also shown in the spectrum. Taking into account that one carbonyl group was located as a part of an acetoxyl group and the other belonged to a different ester group, it left unassigned only two oxygen atoms bonded to four carbons.

From the analysis of the $^1H-^1H$ COSY spectrum, the following correlation sequence could be deduced: The proton H-5 (δ_H 3.16) was coupled to H-6 (δ_H 4.89), which was in turn coupled to H-7 (δ_H 1.60). H-7 was correlated to a methyne at δ_H 1.84 (H-11). Finally H-11 was coupled to two methyl groups at δ_H 1.13 (3 H-12, *J*=6.5 Hz) and 0.95 (3 H-13, *J*=6.5 Hz), respectively. This fact meant that H-11, H-12 and H-13 formed an isopropyl group bonded at C-7 (partial structure B, Fig. 4).

The HSQC spectrum allowed the identification of C-11 (δ_C 26.4), which was correlated in the HMBC spectrum to a C*H* at δ 5.66 (H-8). The chemical shift of C-8 (δ_C 69.3) implied the presence of an oxygenated function at C-8. Surprisingly, no correlation was found between H-7 and H-8 in the $^1H-^1H$ COSY, nor between H-7 and C-8 in the HMBC

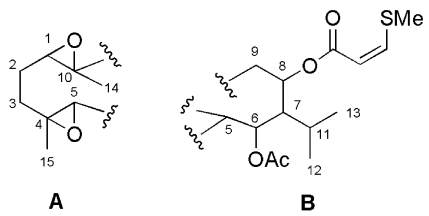


Figure 4. Partial structures found for **3**.

spectrum. Finally, two protons at δ_H 2.23 (H-9α) and 1.85 (H-9β) showed a correlation with C-8 in the HMBC spectrum and confirmed their identity in the HSQC spectrum by a coupling with C-9 (δ_C 42.5).

Another correlation set was also observed in the $^1H-^1H$ COSY spectrum. The proton at δ_H 3.08 (H-1) was coupled to two protons mutually coupled at δ_H 1.45 and 2.07, assigned to H-2β and H-2α. These two protons were coupled to H-3β (δ_H 1.26) and H-3α (2.17), whose corresponding carbon C-3 was shown at δ_C 36.5. -3 showed a correlation in the HMBC spectrum with H-15, which in turn exhibited two additional correlations in the HMBC spectrum with two oxygenated carbons, C-4 (58.8) and C-5 (66.6). This would place an epoxide ring between C-4 and C-5 (partial structure A, Fig. 4).

Similarly, the methyl group located at δ 1.45 (3 H-14) displayed correlations in the HMBC spectrum with carbons at δ_H 58.7 (C-10) and δ_H 61.5, which indicated the presence of a second epoxide ring between C-10 and C-1.

The nature of the ester group was deduced similarly as in **1**. The ^1H NMR showed the presence of an isolated ethylene group as two doublets mutually coupled at δ_H 5.80 (H-2′) and 7.05 (H-3′), whose corresponding carbon atoms were found in the HSQC spectrum at δ_C 112.9 (C-2′) and 153.2 (C-3′). C-3 was correlated with the –S–C*H*$_3$ protons at δ_H 2.38. -2′ showed a correlation with the carbonyl C-1′, which was in turn correlated with H-8 in the HMBC spectrum, confirming the presence of a –O–CO–CH=CH–SCH_3 group located at C-8. The *Z* configuration of the double bond was inferred from the value of the coupling constant (*J*=10.0 Hz).

Finally, the relative configuration of the germacrane was deduced from a NOE study of the molecule. The main effects observed are depicted in Figure 5.

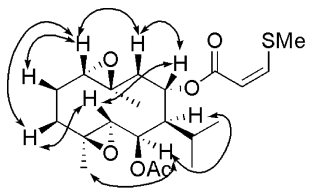


Figure 5. NOE effects observed in **3**

In summary, three new metabolites have been isolated from *Thapsia villosa*. The main novelty of these compounds is the presence of methylthiopropionate or methylthiopropenoate esters. To our knowledge, it is the first time that a methylthiopropionate group is reported as part of a natural

Table 1. ^{1}H and ^{13}C NMR data for compounds **1–3**

Guaianolide 1					Guaianolide 2					Germacrane 3				
H	δ_H	Mult	*J* (Hz)	δ_C	H	δ_H	Mult	*J* (Hz)	δ_C	H	δ_H	Mult	*J* (Hz)	δ_C
1	3.42	dd	$J_{1,5}$=7.8 Hz, $J_{1,2}$=2.1 Hz	50.1	1	3.33	dd	$J_{1,2}$=2.2 Hz, $J_{1,5}$=8 Hz	51.9	1	3.08	d	10.4	61.5
2	5.70	m	–	79.4	2	5.77	m	–	79.6	2α	1.45	m	–	23.8
3	5.60	m	–	126.1	3	5.63	m	–	126.6	2β	2.07	dt	$J_{3\alpha,2\alpha}$=14.6 Hz, $J_{2\alpha-3\alpha}$=3.4 Hz	
4	–	–	–	149.6	4	–	–	–	149.5	3-α	2.17	dt	$J_{3\alpha,2\beta}$=13.2 Hz, $J_{3\alpha-2\alpha}$=3.4 Hz	36.6
5	3.10	m	–	49.6	5	3.1	m	–	50.1	3-β	1.26	m	–	
6	4.83	dd	$J_{6,5}$=11.7 Hz, $J_{6,7}$=9.6 Hz	75.4	6	4.83	dd	$J_{6,5}$=11.9 Hz, $J_{6,7}$=9.7 Hz	76.1	4	–	–	–	58.8
7	3.60	dd	$J_{7,6}$=9.6 Hz, $J_{7,8}$=11.0 Hz	48.3	7	3.68	dd	$J_{7,6}$=9.9 Hz, $J_{7,8}$=11.0 Hz	48.3	5	3.16	d	J_{5-6}=6.8 Hz	66.6
8	5.80	td	$J_{8,7}$=$J_{8,9\alpha}$=11.0 $J_{8,9\beta}$=2.7 Hz	65.7	8	5.73	td	$J_{8,7}$=$J_{8,9\alpha}$=11.2 Hz, $J_{8,9\beta}$=2.8 Hz	65.7	6	4.89	dd	$J_{6,5}$=6.8 Hz, $J_{6,7}$=1.0 Hz	73.2
9α	1.96	dd	$J_{9\alpha,9\beta}$=13.5 $J_{9\alpha,8}$=11.2	44.5	9α	2.14	dd	$J_{9\alpha,9\beta}$=15.4 Hz, $J_{9\alpha,8}$=11.2	44.9	7	1.6	d	$J_{7,11}$=8.7 Hz	48.5
9β	2.60	dd	$J_{9\beta,9\alpha}$=13.5 Hz, $J_{9\beta,8}$=2.7 Hz		9β	2.62	dd	$J_{9\beta,9\alpha}$=15.4 Hz, $J_{9\beta,8}$=2,8 Hz		8	5.66	dd	$J_{8\text{-}9\alpha}$=12.2 Hz, $J_{8\text{-}9\beta}$=5.8 Hz	69.3
10	–	–	–	79.7	10	–	–	–	80.6	9-α	2.23	t	$J_{9\alpha\text{-}8}$=12.2 Hz	
11	–	–	–	77.8	11	–	–	–	78.1	9-β	1.85	dd	$J_{9\beta\text{-}8}$=5.8 Hz, $J_{9\beta-9\alpha}$=13,7 Hz	42.5
12	–	–	–	173.9	12	–	–	–	173.7	10	–	–	–	58.7
13	1.60	s	–	20.3	13	1.62	s	–	20.6	11	1.84	m	–	26.5
14	1.24	s	–	26.9	14	1.38	s	–	26.4	12	1.13	d	J_{12-13}=6.5 Hz	23.2
15	1.90	d	$J_{15,3}$=1.0 Hz	17.3	15	1.95	d	$J_{15,3}$=1.1 Hz	17.4	13	0.95	d	J_{13-12}=6.5 Hz	21.5
1′	–	–	–	170.1	1′	–	–	–	164.8	14	1.45	s	–	22.4
2′	2.60	m	–	34.1	2′	5.76	d	10.3	112.3	15	1.26	s	–	17.2
3′	2.80	m	–	28.34	3′	7.1	d	10.3	153.5	1′	–	–	–	165.6
$-SCH_3$	2.13	s	–	15.3	$-SCH_3$	2.42	s	–	19.3	2′	5.80	d	$J_{2',3'}$=10.0 Hz	112.9
(*C-2*)$-OCOCH_3$	–	–	–	170.7	(*C-2*)$-OCOCH_3$	–	–	–	170.2	3′	7.05	d	$J_{3',2'}$=10.0 Hz	153.1
(*C-2*)$-OCOCH_3$	2.03	s	–	21.0	(*C-2*)$-OCOCH_3$	2.03	s	–	21.2	$-SCH_3$	2.38	s	–	19.6
(*C-10*)$-OCOCH_3$	–	–	–	170.7	(*C-10*)$-OCOCH_3$	–	–	–	170.4	$-OCOCH_3$	–	–	–	170.4
(*C-10*)$-OCOCH_3$	2.02	s	–	21.2	(*C-10*)$-OCOCH_3$	2.03	s	–	20.9	$-OCOCH_3$	1.92	s	–	21.0
(*C-11*)$-OCOCH_3$	–	–	–	170.0	(*C-11*)$-OCOCH_3$	–	–	–	169.9					
(*C-11*)$-OCOCH_3$	2.10	s	–	22.3	(*C-11*)$-OCOCH_3$	2.05	s	–	22.3					

product. Methylthiopropionate esters seems to come from the methylmethionine (MMT) and can be considered as precursors of DMSP (dimethylsulfonium propionate, its thiomethylated derivative). High levels of DMSP in the chloroplasts are related to the control of the saline levels in plants, serving as osmolites.[10] Enzymatic cleavage of DMSP in marine algae has been also reported, to lead to breakdown products that act as scavengers of hydroxyl radicals, thus serving as an antioxidant system.[11] It is noteworthy the fact that the enzymatic pool of *T. villosa* is able to place sulfurated esters on different sesquiterpenoid scaffolds. However, the role of the sulfur atoms in this species remains to be disclosed.

3. Experimental

3.1. General

Melting points are uncorrected and were measured in a Reichert-Jung apparatus. NMR spectra were recorded on a Varian Gemini 300, a Varian Inova 400 or in a Varian Inova 600. H chemical shifts were referenced to the residual $CHCl_3$ signal at δ 7.26 ppm. ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at δ 77.0 ppm. HMBC, HSQC and COSY spectra were recorded with standard Varian pulse gradient sequences. IR spectra were recorded in a Mattson Genesis Series FTIR, using NaCl plates; data are reported in cm^{-1}. Mass spectra were obtained in a Voyager GC–MS or in a VG Autospec-Q. Visualization of the TLC was performed by fluorescence quenching, aqueous ceric ammonium molybdate, anisaldehyde stains or H_2SO_4–H_2O–AcOH (1:4:20).

3.2. Biological material

Specimens of *T. villosa* var *villosa* were collected in Sierra de San Cristóbal, El Puerto de Santa María, Cádiz, in May, 2002. A voucher specimen has been deposited at the Departamento de Ciencias y Recursos Agrícolas y Forestales, University of Córdoba collection (voucher # COA-31092).

3.3. Extraction and isolation

100 g of dried roots were extracted with CH_2Cl_2 in a Soxhlet apparatus for 6 h and concentrated to give a clear yellow oily residue (13.5 g). The extract was subjected to flash chromatography on silica gel. The fraction eluted with hexane–EtOAc (80:20) (4 g) was further chromatographed to give compounds **1** (28 mg) and **3** (250 mg). The fraction eluted with hexane–EtOAc (60:40) yielded **2** (7 mg).

3.3.1. Compound 1. Colorless oil; $[\alpha]_D^{25}=-40$ (*c* 0.13, $CHCl_3$); IR ν_{max} (film) cm^{-1} 2924, 1791, 1738, 1437, 1371, 1241, 1019, 757; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 466 $[M-HOAc]^+$ (1), 244 (16), 226 $[M-3HOAc-C_4H_8O_2S]^+$ (100), 173 (42); HREIMS 526.1860 (calcd for $C_{25}H_{34}O_{10}S$, 526.1873). $C_{25}H_{34}O_{10}S$: calcd. C 57.02, H 6.51, S 6.09; found C 57.35, H 6.64, S 6.21.

3.3.2. Compound 2. Amorphous white powder; $[\alpha]_D^{25}=-24.5$ (*c* 0.25, $CHCl_3$); IR ν_{max} (film) cm^{-1} 2921, 1790, 1733, 1566, 1371, 1240, 1155, 1019, 797; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 464 $[M-HOAc]^+$ (2), 422 $[M-C_4H_6O_3]^+$ (5), 226 $[M-3HOAc-C_4H_6O_2S]^+$ (54), 101 $[C_4H_5OS]^+$ (100); HREIMS 464.1500 $[M-HOAc]^+$ (calcd for $C_{23}H_{28}O_8S$, 464.1505). $C_{25}H_{32}O_{10}S$: calcd. C 57.24, H 6.15, S 6.11; found C 57.11, H 6.17, S 6.31.

3.3.3. Compound 3. Amorphous white powder; $[\alpha]_D^{25}=-18.3$ (*c* 0.25, $CHCl_3$); IR ν_{max} (film) cm^{-1} 2960, 1740, 1698, 1558, 1387, 1235, 1161, 992, 796; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 412 $[M]^+$ (1), 235 $[M-HOAc-C_4H_5O_2S]^+$ (1), 195 $[M-HOAc-C_3H_7-C_4H_6O_2S]^+$ (4), 193 (2), 163 (4), 149 (5), 101 $[C_4H_5OS]^+$ (100); HREIMS 412.1905 (calcd. for $C_{21}H_{32}O_6S$, 412.1920). $C_{21}H_{32}O_6S$: calcd C 61.14, H 7.82, S 7.77; found C 60.81, H 7.85, S 7.97.

Acknowledgements

We are grateful to the Spanish Ministerio de Ciencia y Tecnología (grant BQU2001-3076) and Consejería de la Presidencia de la Junta de Andalucía (grant A36/03) for the financial support.

References and notes

1. Tori, M.; Kawahara, M.; Sono, M. *Phytochemistry* **1998**, *47*, 401.
2. Iciek, M.; Wlodek, L.; Pol, *J. Pharmacol.* **2001**, *53*, 215.
3. Lin, H.; Chien, C. H.; Lin, Y. L.; Chen, C. F.; Wang, P. S. *Chin. J. Physiol* **2000**, *43*, 99.
4. Wang, G.; Wu, X.; Lin, Y.; Ren, J.; Shum, A. Y.; Wu, Y.; Chen, C. *Eur. J. Pharm.* **2002**, *445*, 239.
5. Thomet, O. A. R.; Wiesmann, U. N.; Schapowal, A.; Bizer, C.; Simon, H. *Biochem. Pharm.* **2001**, *61*, 1041.
6. Pujadas, A.; Roselló, J. A. In *Flora Iberica*; Nieto Feliner, G., Jury, S. L., Herrero, A. Eds. (Castroviejo, S., Series Ed.); Real Jardín Botánico, CSIC, Madrid, 2003, Vol. 10, pp 401–410.
7. Álvarez Arias, B. T. *J. Ethnopharm.* **2000**, *73*, 505.
8. (a) For a complete review of sesquiterpenes from *Thapsia genus* see: Christensen, S. B.; Andersen, A.; Smitt, W. U. *Prog. Chem. Org. Nat. Prod.* **1997**, *71*, 130. Sesquiterpenoids from Thapsia Species and Medicinal Chemistry of the Thapsigargins. (b) De Pascual Teresa, J.; De Pascual, M.; Arias, A.; Hernández, J. M.; Morán, J. R.; Grande, M. *Phytochemistry* **1985**, *24*, 1773. (c) De Pascual Teresa, J.; Morán, J. R.; Hernández, J. M.; Grande, M. *Phytochemistry* **1985**, *24*, 1779. (d) De Pascual Teresa, J.; Morán, J. R.; Hernández, J. M.; Grande, M. *Phytochemistry* **1985**, *24*, 2071. (e) Smitt, U. W.; Cornett, C.; Andersen, A.; Christensen, S. B. *J. Nat. Prod.* **1990**, *53*, 1479.
9. (a) The 3-methylthiopropenoate ester group has been previously found in: (a) the ascidian *Aplidium uouo*, see: McCoy, M. C.; Faulkner, D. J. *J. Nat. Prod.* **2001**, *64*, 1087. (b) Higher plants, *Petasites formosanus*, see: Wu, T. S.; Kao, M. S.; Wu, P. L.; Shi, L. S.; Liou, M. J.; Li, C. Y. *Chem. Pharm. Bull.* **1999**, *47*, 375. (c) *Petasites formosanus* var. kitamura, see: Lin, Y. L.; Ou, J. C.; Chrn, C. F.; Kuo, Y. H. *Chem. Pharm. Bull.* **1998**, *46*, 1807. (d) *Etasites japonicus* see: Tori, M.; Kawahara, M.; Sono, M. *Tetrahedron Lett.* **1997**, *38*, 1965.

(e) *Petasites japonicus*, see: Tori, M.; Kawahara, M.; Sono, M. *Phytochemistry* **1998**, 401. (f) Liverworts, *Balantiopsis rosea*, see: Asakawa, Y.; Takikawa, K.; Tori, M.; Campbell, E. O. *Phytochemistry* **1986**, *25*, 2543. (g) *Isotachis japonica*, see: Asakawa, Y.; Toyota, M.; Harrison, L. V. *Phytochemistry* **1984**, *24*, 1505.

10. Trossat, C.; Rathinasabapathi, B.; Weretilnyk, E. A.; Shen, T. L.; Huang, Z. H.; Gage, D. A.; Hanson, A. D. *Plant Physiol.* **1998**, *116*, 165.
11. Sunda, W.; Kleber, D. J.; Kiene, R. P.; Huntsman, S. *Nature* **2002**, *418*, 317.