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Phenylpropanoids from *Thapsia transtagana*

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Abstract

Five phenylpropanoids have been isolated from the roots of *Thapsia transtagana*. Their structures have been elucidated by spectroscopic means.

Keywords: Phenylpropanoids; *Thapsia transtagana*; Umbelliferae

1. Introduction

Thapsia is a complex genus not only from the taxonomical point of view, but also from the nature of the metabolites isolated and the distribution in its species (Christensen et al., 1997). Although Tutin described in 1968 in *Flora Europaea* only three species (Tutin, 1968), new species have later been characterized or differentiated (Pujadas-Salvá and Plaza-Arregui, 2003). *Thapsia transtagana* Brot. (Apiaceae, tribe Laserpitieae) is a perennial herb of 140–180 cm of height and robust stem, distributed along the Western Mediterranean area (Pujadas and Roselló, 2003). Although initially *T. transtagana* Brot. was classified as synonymous of *T.* garganica, Christensen et al. proposed their consideration as different species (Smitt et al., 1995). Previous studies of *T. transtagana* have provided thapsigargin-related guaianolides (Rasmussen et al., 1981; Avato et al., 1993), and we recently have reported the isolation of a new class of compounds that have been generically named as transtaganolides (Saouf et al., 2005).

In our search for new natural products in umbefelliferous species of both sides of the Gibraltar Strait, we have examined the roots of *T. transtagana* collected in Bouznika (Morocco). In addition to thapsitranstagin, we have isolated five new metabolites with a phenylpropanoid structure.

2. Results and discussion

The roots of *T. transtagana* were extracted with dichloromethane in a Soxhlet apparatus yielding an oily residue that was purified by column chromatography using increasing polarities of EtOAc/hexanes mixtures affording compounds **1** (90 mg), **3** (30 mg), **4** (60 mg), **5** (12 mg) and **6** (7 mg) (Fig. 1).

Compound **1** showed the molecular ion in its HREIMS spectrum at m/z 350.1365 corresponding to a molecular formula of $C_{18}H_{22}O_7$, which accounted for eight degrees of unsaturation. This unsaturation degree was in agreement with the observation in the NMR and IR spectra of the signals corresponding to an aromatic ring, an additional ring formed by methylenedioxy group, two carbonyl groups belonging to two ester groups and two vinylic carbons.

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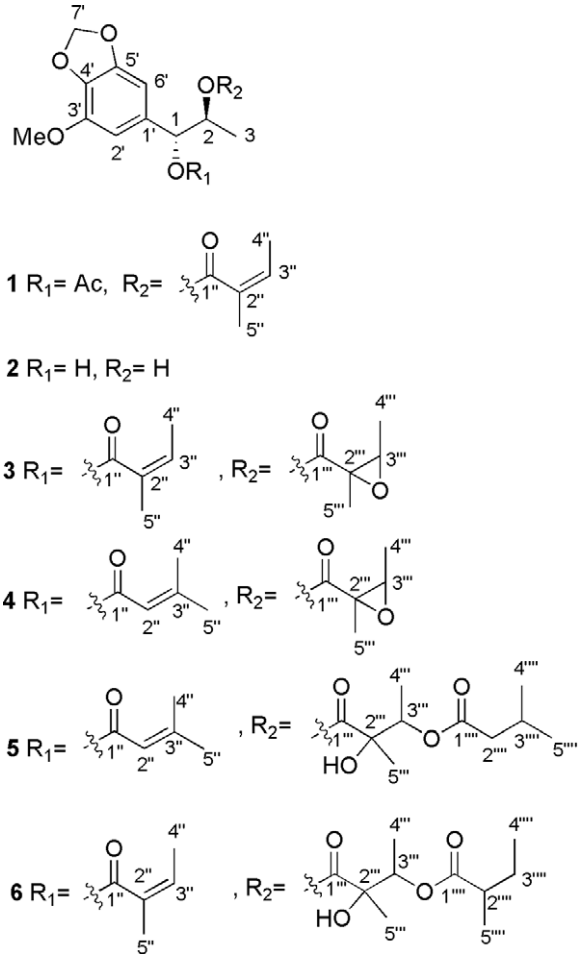

Fig. 1. Phenylpropanoids isolated from *T. transtagana*.

The presence of the aromatic ring and an additional C3 spin system suggested a phenylpropanoid structure. This kind of metabolite is common in Umbelliferae plants and has been found in species of this family such as *Thapsia villosa* De Pascual Teresa et al., 1985a,b, *Guillonea scabra* (Pinar et al., 1982), *Seseli vayredanum* (Barrero et al., 1992) and *Ferula latipinna* (González et al., 1988). This type of metabolites have been reported to present a wide range of biological activities (as an example see Pan et al., 2003).

The ^{1}H NMR spectrum of **1** showed the presence of only two aromatic protons, thus indicating a tetrasubstituted ring. These two protons were assigned to H-2′ (δ_H 6.47, *d*, 3.0 Hz) and H-6′ (δ_H 6.49, *d*, 3.0 Hz). Additionally, the aromatic ring presented a methoxy group (δ_H 3.80) located at C-3′ as deduced from the NOE with H-2′, and a 2H singlet at δ_H 5.86, assigned to a methylenedioxy group.

The aromatic ring was linked to a propyl group, whose existence was confirmed by DQF-COSY experiments. The H-1 proton of the C-3 unit (δ_H 5.76) showed in the DQF-COSY spectrum a cross coupling with H-2 at δ_H 5.17, which in turn was correlated with a methyl group, 3H-3, at δ_H 1.16. The deshielding shift experienced by H-1 and H-2 suggested that two oxygen atoms were located at C-1 and C-2, respectively. The HMBC spectrum showed a correlation between H-1 and a carbonyl group at δ_C 169.4, which in turn showed a correlation with the three protons of a methyl group at δ_H 2.10, placing an acetate group at C-1.

An additional ester group was located at C-2, as could be inferred from the ^{1}H NMR downfield shift value of H-2 and its correlation in the HMBC spectrum with C-1″ (δ_C 166.7). The presence of a vinylic proton as a quartet of quartets at δ_H 5.95 (H-3″) and two methyl groups at δ_H 1.75 (*dq*, 3H-4″) and δ_H 1.85 (*dq*, 3H-5″) confirmed the nature of the ester as an angelate group.

The following task was the assignment of the relative and absolute configuration of C-1 and C-2. For this purpose, a small sample of **1** was saponified by treatment with methanolic KOH, giving rise to diol **2**. The inspection of the coupling constant between protons H-1 and H-2 ($J_{1,2} = 4.5$ Hz), suggested an *erythro* configuration of the diol system as in the case of deacylhelmanticine.[1] Nevertheless, the sign of the optical rotation is opposite to that of deacylhelmanticine, indicating an absolute configuration 1*R*,2*S* for diol **2** and for compound **1** (see Section 3).

The molecular formula of **3** was determined to be $C_{21}H_{26}O_8$ by HREIMS. The substitution pattern of the aromatic ring was similar to that of **1**. The presence of a vinyl proton at δ_H 6.06 as a quartet of quartets indicated again the presence of an angelate ester group, now located at C-1 on the basis of the long-range correlations with the carbonyl group at δ_C 166.1 (C-1″).

A second ester group was located at C-2 as deduced from the HMBC spectrum correlation between H-2 and C-1‴. The chemical shifts of C-2‴ and C-3‴ (δ_C 59.5 and 59.6, respectively) indicated the presence of an epoxide ring, confirming the nature of this ester as an epoxyangelate.

The molecular formula $C_{21}H_{26}O_8$ determined for **4** by HREIMS and the similarities in its ^{1}H and ^{13}C NMR spectra suggested that it might be an isomer of **3**. In addition to the signals belonging to the C_6–C_3 unit and the epoxyangelate ester, the signals corresponding to a senecioate ester at C-1 were observed, instead the angeloyl moiety present in **3.**

Compound **5** showed a molecular ion at *m*/*z* 508.2308 which accounted for a $C_{26}H_{36}O_{10}$ and nine degrees of unsaturation. An inspection of the ^{1}H NMR showed a structure closely related to that of the former compounds. The differences in the NMR spectra of **5** and above described compounds **1**–**4**, clearly revealed that **5** differed only in the ester moiety.

A first ester group was located at C-2 as deduced from the HMBC spectrum from the correlation between H-2 and C-1‴ (δ_C 174.0). The downfield shift value of C-2‴ (δ_C 75.9) indicated the presence of a hydroxyl group at this carbon. C-3‴ was identified in the ^{13}C NMR at δ_C 73.9, sug-

[1] An *erythro* arrangement displays a $J_{1,2} = 4.5$ Hz, whilst a *threo* configuration presents a $J_{1,2} = 7.5$ Hz (see De Pascual Teresa et al., 1985a,b). The value of the $J_{1,2}$ in compounds **3**–**6** suggests an *erythro* configuration for all of them (vide Table 1).

gesting the presence of an additional oxygenated function. This assumption was confirmed by the presence of different correlations in the HMBC spectrum, setting a second ester group attached to the 2-hydroxy-2-methylbutanoyloxy moiety through C-3‴. On the basis of the correlations found in the HMBC spectrum and the DQF-COSY, this additional group was identified as a 3-methylbutanoyl ester.

The NMR data of this ester indicated that the two oxygen atoms at C-2‴ and C-3‴ presents a *syn* arrangement, yet the absolute configuration could not be determined. The remaining signals were identified as a senecioate ester located at C-1 as could be deduced from the HMBC spectrum by a cross coupling between H-1 and a third carbonyl group at δ_C 165.0 assigned as C-1″.

Compound **6** was obtained as an oil with the same molecular formula that compound **5**, suggesting an isomeric structure. The NMR data again were similar to those of **5**, only the senecioate signals being replaced by those of an angelate residue. Additionally, the ester group located at C-2 resulted to be a 3-(2-methylbutanoyloxy)-2-hydroxy-2-methylbutanoic acid derivative. The remaining ester group was easily identified as an angelate group located at C-1 as deduced by the HMBC spectrum correlations.

In summary, although phenylpropanoids are widely found in umbelliferous plants, the combination of the C_6–C_3 core with these ester groups in compounds **1** and **3–6** has never been reported. In fact, to the best of our knowledge the 2-hydroxy-2-methyl-3-(3-methylbutanoyloxy) butanoyl ester present in compound **5** has never been described as part of a natural product. The 2-hydroxy-2-methyl-3-(2-methylbutanoyloxy) butanoyl ester present in **6** has previously found only in some pyrrolizidine alkaloids from *Ipomoea hederifolia* (Jenett-Siems et al., 1993).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Perkin Elmer Model 341 digital polarimeter. IR spectra were measured in a Perkin Elmer Spectrum BX spectrophotometer. ^{1}H (1D, DQF-COSY, 1D-NOESY, 2D-NOESY) and ^{13}C (1D, gHSQC, HMBC) NMR spectra were recorded in a Varian Inova 400 spectrometer. Mass spectra were recorded at the Mass Spectra Facilities of Universidad de Alicante (Spain). Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on plates precoated with silica gel F_{254} (Merck, Germany).

3.2. Plant material

Specimens of *T. transtagana* were collected near Bouznika (Morocco) and a voucher of the plant is deposited in the Departamento de Ciencias y Recursos Agrícolas y Forestales of the University of Córdoba.

3.3. Isolation

The roots of *T. transtagana* (900 g) were grounded and extracted with dichloromethane in a Soxhlet apparatus, yielding 13 g of an oily residue, which was purified by column chromatography with increasing polarities of EtOAc/hexanes mixtures. The 1:3 and 1:4 EtOAc/hexanes-eluted fractions yielded after further purification by column chromatography compounds **1** (90 mg), **3** (30 mg), **4** (60 mg), **5** (12 mg) and **6** (7 mg).

3.4. Compound *1*

Oil. $[\alpha]_D^{25}$ −63.7 °C ($c = 0.14$, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 2972, 1723, 1635, 1511, 1197. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1. EIMS 70 eV, *m/z* (rel. int.): 306 $[M - CO_2]^+$ (3), 291 $[M - OAc]^+$ (2), 250 $[M - HOAng]^+$ (31), 208 (72) and 180 (100). HREIMS *m/z* 350.1365 calcd. for $C_{18}H_{22}O_7$ 350.1366.

3.5. Compound *3*

Oil. $[\alpha]_D^{25}$ −23.1 °C ($c = 0.13$, $CHCl_3$). IR $\nu_{max}CHCl_3$ cm^{-1}: 2943, 1747, 1633, 1512, 1453, 1379, 1137. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1. EIMS 70 eV, *m/z* (rel. int.): 290 $[M - HEpoxAng]^+$ (11), 208 (5), 180 (3), 82 (100). HREIMS *m/z* 406.1627 calcd. for $C_{21}H_{26}O_8$ 406.1628.

3.6. Compound *4*

Oil. $[\alpha]_D^{25}$ −22.5 °C ($c = 0.20$, $CHCl_3$). IR $\nu_{max}CHCl_3$ cm^{-1}: 2938, 1724, 1636, 1512, 1451, 1379, 1137. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1. EIMS 70 eV, *m/z* (rel. int.): 406 $[M]^+$ (10), 308 $[M - HOSen]^+$ (73), 290 $[M - HEpoxAng]^+$ (23), 208 (7), 180 (3), 83 (100). HREIMS *m/z* 406.1627 calcd. for $C_{21}H_{26}O_8$ 406.1628.

3.7. Compound *5*

Oil. $[\alpha]_D^{25}$ −15.5°C ($c = 0.40$, $CHCl_3$). IR $\nu_{max}CHCl_3$ cm^{-1}: 3450, 2961, 1734, 1636, 1379, 1197. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1. EIMS 70 eV, *m/z* (rel. int.): 508 $[M]^+$ (10), 409 $[M - HOSen]^+$ (78), 290 (38), 192 (38), 173 (41). HREIMS *m/z* 508.2308 calcd. for $C_{26}H_{36}O_{10}$ 508.2308.

3.8. Compound *6*

Oil. $[\alpha]_D^{25}$ −23.1°C ($c = 1.3$, $CHCl_3$). IR $\nu_{max}CHCl_3$ cm^{-1}: 3448, 2963, 1734, 1636, 1381, 1197. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1. EIMS 70 eV, *m/z* (rel. int.): 508 $[M]^+$ (14), 409 $[M - HOAng]^+$ (95), 290 (45), 192

Table 1
^{1}H and ^{13}C NMR data of compounds **1**, **3**–**6**

	1		**3**		**4**		**5**		**6**	
	δ_H (int, mult. *J*)	δ_C	δ_H (int, mult. *J*)	δ_C	δ_H (int, mult. *J*)	δ_C	δ_H (int, mult. *J*)	δ_C	δ_H (int, mult. *J*)	δ_C
1	5.76 (1H, *d*, 4.6)	75.8	5.80 (1H, *d*, 4.8)	75.7	5.75 (1H, *d*, 5.1)	75.2	5.81 (1H, *d*, 5.2)	74.4	5.91 (1H, *d*, 4.8)	75.3
2	5.17 (1H, *qd*, 6.5, 4.6)	71.0	5.20 (1H, *qd*, 6.5, 4.8)	72.7	5.28 (1H, *qd*, 6.2, 5.1)	73.0	5.19 (1H, *qd*, 6.4, 5.2)	73.8	5.22 (1H, *qd*, 6.4, 4.8)	73.8
3	1.16 (3H, *d*, 6.6)	14.8	1.20 (3H, *d*, 6.5)	15.3	1.26 (3H, *d*, 6.2)	15.6	1.19 (3H, *d*, 6.4)	14.9	1.15 (3H, *d*, 6.4)	16.5
1′		131.0		130.9		131.3		131.3		130.9
2′	6.47 (1H, *d*, 3.0)	106.8	6.51 (1H, *s*)	107.0	6.59 (1H, *s*)	107.1	6.52 (1H, *s*)	106.9	6.54 (1H, *s*)	106.9
3′		143.1		143.2		143.4		148.9		149.0
4′		134.8		134.9		135.1		135.1		136.0
5′		148.0		148.0		148.8		143.4		143.4
6′	6.49 (1H, *d*, 3.0)	101.0	6.52 (1H, *s*)	101.0	6.59 (1H, *s*)	101.5	6.55 (1H, *s*)	101.1	6.55 (1H, *s*)	101.6
7′	5.86 (2H, *s*)	101.2	5.95 (2H, *s*)	101.4	5.96 (2H, *s*)	101.5	5.95 (2H, *s*)	101.5	5.97 (2H, *s*)	101.1
1″		166.7		166.1		165.0		165.0		166.2
2″		127.5		127.0	5.71 (1H, *qq*, 1.1, 1.1)	115.3	5.75 (1H, *dq*, 1.1, 1.1)	115.5		127.1
3″	5.95 (1, *qq*, 7.2, 1.4)	137.8	6.06 (1H, *qq*, 7.2, 1.4)	139.2		158.7		158.8	6.16 (1H, *qq*, 7.4, 1.5)	139.6
4″	1.75 (3H, *dq*, 7.2, 1.4)	20.2	1.80 (3H, *dq*, 7.2, 1.4)	20.3	1.92 (3H, *d*, 1.1)	27.5	1.93 (3H, *d*, 1.2)	27.5	1.92 (3H, *dq*, 7.4, 1.5)	20.1
5″	1.85 (3H, *dq*, 1.4, 1.4)	15.3	1.93 (3H, *dq*, 1.4, 1.4)	15.6	2.16 (3H, *d*, 1.1)	20.3	2.16 (3H, *d*, 1.2)	20.5	1.98 (3H, *dq*, 1.5, 1.5)	15.4
1‴				169.9				174.0		175.2
2‴				59.5				75.9		73.8
3‴			2.94 (1H, *q*)	59.6			5.06 (1H, *q*, 6.4)	73.9	5.05 (1H, *q*, 6.4)	73.5
4‴			1.10 (3H, *d*, 5.5)	13.2			1.22 (3H, *d*, 6.4)	21.5	1.24 (3H, *d*, 6.4)	20.6
5‴			1.40 (3H, *s*)	18.8			1.24 (3H, *s*)	13.2	1.30 (3H, *s*)	13.2
1⁗								171.8		175.2
2⁗							2.10 (2H, *m*)	43.2	2.29 (1H, *m*)	41.2
3⁗							2.16 (1H, *m*)	25.5	1.60 (2H, *m*)	26.4
4⁗							0.90 (3H, *d*, 6.4)	22.3	1. 23 (3H, *d*, 7.4)	14.9
5⁗							0.92 (3H, *d*, 6.4)	22.3	0.87 (3H, *t*, 7.4)	11.6
OMe	3.80 (3H, *s*)	56.3	3.80 (3H, *s*)	56.4	3.90 (3H, *s*)	56.6	3.90 (3H, *s*)	56.6	3.91 (3H, *s*)	56.7
O*C*OCH$_3$		169.4								
OCO*CH*$_3$	2.10	20.7								

(49), 173 (58) 83 (100). HREIMS m/z 508.2308 calcd. for $C_{26}H_{36}O_{10}$ 508.2308.

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