ORGANIC LETTERS

2005
Vol. 7, No. 5
881−884

Transtaganolides A–D: Novel Metabolites from *Thapsia transtagana*

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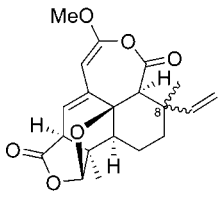
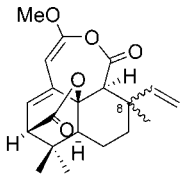
Received December 16, 2004

ABSTRACT



1 C-8 α-methyl, β-vinyl
2 C-8 β-methyl, α-vinyl



3 C-8 β-methyl, α-vinyl
4 C-8 α-methyl, β-vinyl

Four novel and unusual C-19 compounds from *Thapsia transtagana*, named transtaganolides A–D, have been isolated. Their structures were established by physical methods, including X-ray analysis of transtaganolides A and B. This is the first time that a 7-methoxy-4,5-dihydro-3*H*-oxepin-2-one ring has been found in a natural product.

Thapsia transtagana Brot. is a perennial herb that grows in the southwest of the Iberian Peninsula and northwestern Morocco.[1] Although traditionally *T. transtagana* has been misidentified as *Thapsia garganica* and *Thapsia decussata* Lag.,[2] there are nevertheless significant morphological and anatomical differences among these three species. *T. garganica* roots are the source of thapsigargin,[3] a guaianolide that inhibits the ubiquitous SERCA-ATPase enzymatic system involved in calcium homeostasis.[4]

Metabolites isolated from *T. transtagana* are closely related to those found from *T. garganica*.[5] In our research project aimed at the search for new thapsigargin analogues, we decided to reinvestigate the chemical composition of *T. transtagana*, collected in Bouznika (northwestern Morocco).

Roots of *T. transtagana* were ground and extracted with dichloromethane in a Soxhlet apparatus, yielding an oily residue that was purified by column chromatography using increasing polarities of EtOAc/hexanes mixtures. The fraction eluted with 1:4 EtOAc/hexanes was subjected to further purification by column chromatography, yielding haplono-

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10.1021/ol047421w CCC: $30.25
Published on Web 02/04/2005

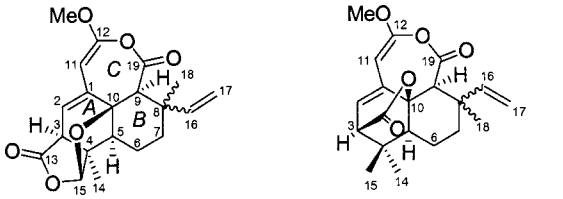

Figure 1. Novel metabolites isolated from *Thapsia transtagana*.

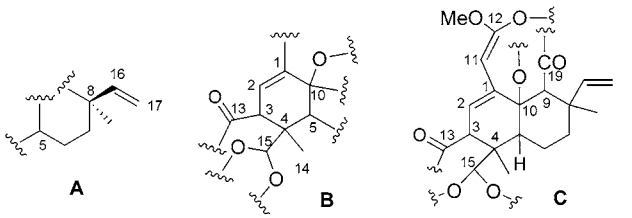

Figure 2. Partial structures (**A**−**C**) of **1**.

lide.[6] The fraction eluted with 1:3 EtOAc/hexanes provided, after purification by column chromatography, thapsitranstagin[7] and four novel C-19 compounds, which we have named transtaganolides A−D, **1**−**4**.

Transtaganolide A (**1**) showed in its HRCIMS spectrum a molecular ion at *m*/*z* 358.1415 in accordance with the molecular formula $C_{20}H_{22}O_6$, indicating 10 degrees of unsaturation. Its ^{13}C NMR spectrum showed the presence of two carbonyl groups and six vinyl carbons, which accounted for five degrees of unsaturation, thus indicating the pentacyclic nature of the compound.

The ^1H NMR spectrum displayed the presence of a vinyl group, as could be deduced from the presence of a double doublet at δ_H 6.89 (17.9, 11.2 Hz) attributable to H-16, coupled with two doublets at δ_H 5.11 (H-17, dd, 11.1, 1.1 Hz) and 5.03 (H-17′, dd, 17.8, 1.1 Hz). The signal corresponding to H-16 showed in the HMBC spectrum a correlation with a quaternary carbon at δ_C 38.1 (C-8), which was in turn coupled in the HMBC spectrum with the signal of a methyl group centered at δ_H 1.22 (3H-18, s). In addition, C-8 was the dead end of a CH_2-CH_2-CH- sequence, confirmed by HMBC and $^1H-^1H$ COSY correlations. This information led us to locate the methyl and vinyl groups on C-8 and propose the partial structure **A** (Figure 2).

H-5, identified at δ_H 1.96 (dd, 12.1, 6.2 Hz), showed in the HMBC spectrum a coupling with C-4 (δ_C 48.6), which in turn displayed correlations with the singlet signals at δ_H 1.30 (3H-14) and 5.61 (H-15, s). Additionally, C-15 was identified by its correlation in the gHSQC spectrum at δ_C 109.6. The remarkable downfield chemical shift of the latter carbon suggested the presence of two oxygen atoms on C-15, indicating the presence of an acetal group.

H-15 could also be reached through another correlation sequence starting at C-4, which showed in the HMBC spectrum a correlation with H-3 (δ_H 3.11, d, 6.0 Hz), which in turn was coupled with the carbonyl C-13 (δ_C 175.5) and finally coupled to H-15 (δ_H 5.61). In addition, C-14 and C-15 showed in the HMBC spectrum a correlation to H-3. This proton showed in the HMBC spectrum couplings with the carbon signals corresponding to C-2 and C-1 at δ_C 119.4 and 143.7, respectively, indicating the presence of a double bond between C-1 and C-2. Finally, H-3 showed in the DQF-COSY spectrum a cross-coupling signal with H-2 at δ_H 5.60 (dd, 5.9, 1.1 Hz), which in turn displayed in the HMBC spectrum a correlation with C-10 at δ_C 87.5 (s). All these data accounted for a partial structure **B** (Figure 2).

The following cross-coupling correlations found in the HMBC spectrum were diagnostic to complete the Decalin ring: C-10 showed a correlation with H-9 (δ_H 2.99, s), which in turn showed a correlation with C-7 (δ_C 39.1), C-8 (δ_C 38.1, s), and C-16 (δ_C 143.7). The value of the chemical shift of C-10 suggested the presence of an oxygenated function on this carbon. The HMBC spectrum allowed the establishment of new relations: C-2 with H-11; H-11 with C-12; and C-12 with a singlet signal at δ_H 3.70 attributable to a methoxyl group. The chemical shift of C-11 (δ_H 84.1) suggested the presence of an electron-rich double bond.

The gHSQC spectrum showed a correlation of H-9 with a signal at δ_C 52.5 corresponding to C-9. Finally, H-9 and a carbonyl group, assigned to C-19 (δ_C 164.0), were also connected, indicating the presence of an ester moiety on C-9. Bringing all the pieces together, we were able to establish the planar structure **C** (Figure 2).

The remaining task was to disclose the connectivity of the ester groups. In the upper part of the molecules, several possibilities were considered, but most of them were rejected

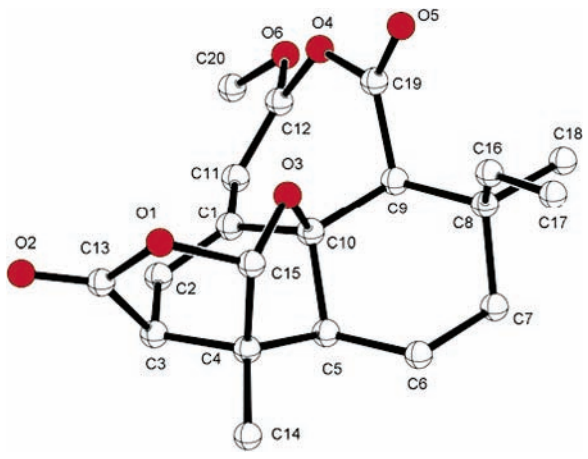

Figure 3. X-ray model of **1**.

(6) Haplonolide has recently been reported as a component of *Haplophyllum vulcanicum* (Rutaceae), an annual herb of central Anatolia (Turkey): Gunes, H. S. *J. Fac. Pharm. Gazi* **2001**, *18*, 35−41.

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due to the fact that the planar arrangement of the conjugated double bonds C-1/C-2 and C-11/C-12 would make it impossible to close the ring with a carbon different than C-19. It also seemed logical that one of the oxygen atoms on C-15 would be linked to the carbonyl group C-13, leaving the other one attached at C-10. All the insight thus resulted in the complete planar structure of transtaganolide A (**1**).

A detailed study of the one- and two-dimensional NOESY experiments for transtaganolide A (**1**) not only displayed the spatial relationships among the different protons but also confirmed some of the structural features described above. H-5 displayed correlations with H-9 and H-3, confirming the relative stereochemistry of C-3, C-5, and C-9 in agreement with a 1,3-diaxial interaction, allowing the protons of the ring fusion to be placed on the same face of the molecule. H-9 also displayed a correlation with H-18, establishing the relative stereochemistry of C-8. The correlation of H-15/H-14 and H-14/H-5 allowed the oxygen attached to C-15 and C-10 to be placed on the β-side of the molecule. In this sense, it is also worth noting the significant NOE between H-16 and H-15.

A single-crystal X-ray diffraction study of **1** was carried out, from which its relative stereochemistry was determined.[8] The drawing depicted in Figure 3 supports the deduction concerning the structure made by both NMR and mass spectrometry. Transtaganolide B (**2**) showed ^{13}C NMR signals almost identical to those of **1**. Thus, the molecular ion was present in the HRCIMS at *m*/*z* 358.1412, in agreement with a molecular formula $C_{20}H_{22}O_6$. As a result of the different stereochemistry of C-8, NOE effects were observable between H-9 and H-16 and between H-15 and the methyl H-18.

The differences in chemical shift in the ^{13}C NMR spectrum were below a range of 3 ppm, except in the case of C-18, which displayed a signal at δ_C 19.9 (δ_C 28.3 in **1**, $\Delta\delta_C$ 8.4). Additionally, H-16 also experienced an upfield shift of 1.08 ppm (δ_H 6.89 in **1**, δ_H 5.81 in **2**) in their ^{1}H NMR spectrum. These facts suggested a structure closely related to **1**. The difference in the chemical shift of methyl C-18 suggested a different configuration at C-8. The rest of the spectroscopic data were in agreement with a C-8-epimer of **1**. A single crystal of **2** was submitted to X-ray analysis, which confirmed the relative stereochemistry proposed for this compound (see Experimental Section).[8]

HRMS and ^{13}C NMR analysis of transtaganolide C (**3**) indicated it to have the molecular formula $C_{20}H_{24}O_5$. From ^{1}H and ^{13}C NMR, MS, and IR data, it was deduced that the molecule contained two carbonyl groups and six vinyl carbons. As the molecular formula of **3** required nine degrees of unsaturation, **3** was deduced to be tetracyclic.

From a preliminary inspection of the ^{1}H and ^{13}C NMR spectra, some instantly recognizable features were observed. The occurrence of a methoxyl group was observed by the presence of a singlet signal at δ_H 3.72 (3H), whose corresponding carbon was observed in the gHSQC spectrum at δ_C 56.3. One of the carbonyl groups showed a resonance signal in the ^{13}C NMR at δ_C 172.0 and was assigned to a δ-lactone. The other carbonyl group was located at an unusually upfield value at δ_C 162.5, corresponding to C-19 (vide infra).

The analysis for the elucidation of the bicyclic ring C1/C10 in **3** was similar to **1**. Thus, the occurrence of a vinyl group and a methyl group, located on C-8, was deduced from the presence in the ^{1}H NMR spectrum of the signals at δ_H 5.81 (H-16, dd, 17.4, 10.8 Hz), 5.05 (H-17, d, 10.7 Hz), 5.08 (H-17′, d, 17.4 Hz), and 1.61 (3H-18, s).

The DQF-COSY and HMBC spectra also confirmed the occurrence of a CH_2-CH_2-CH- sequence from C-8 to C-5, similar to that depicted in Figure 2 (partial structure **A**). Once H-5 was identified at δ_H 1.30 (dd, 11.5, 6.5 Hz), the HMBC spectrum showed a long-range coupling with C-4 (δ_C 33.2), which displayed further correlations with two singlet signals at δ_H 1.09 (3H-15) and 0.98 (3H-14), placing two geminal methyl groups on C-4. The gHSQC spectrum allowed the identification of the signal corresponding to C-15 and C-14 at δ_C 24.8 and 29.6, respectively. C-4, C-14, and C-15 could be linked to H-3 through a coupling signal observed in the HMBC spectrum. H-3 also displayed a coupling signal with C-1 and C-2 at δ_C 137.9 and 123.5, respectively. The carbonyl group C-13 (δ_C 172.0) was also located by the presence of a long-range coupling with H-3. The DQF-COSY spectrum confirmed the vicinal relationship between H-3 and H-2 (δ_H 6.98, dd, 6.5, 1.1 Hz). Finally, also observable was the presence of a correlation between H-2 and an oxygenated carbon at δ_C 87.4, which was assigned to C-10. The linkage between rings **A** and **B** to provide the Decalin ring was completed through long-range CH couplings involving C-10 and H-9 (δ_H 3.24, s) and H-9 with C-8 (δ_C 38.4, s).

The elucidation of the C-ring was carried out according to the following facts. On one hand, C-2 showed in the ^{13}C

(8) Crystal structure analysis: Intensity data were collected, at 293 °K, on a Enraf-Nonius Kappa CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71070 Å). Data reduction and cell parameter refinement were carried out with the programs COLLECT[12a] and DENZO.[12b] The two structures were solved by direct methods using SIR97.[12c] Refinement was performed with SHELXL-93[12d] using full-matrix least squares with anisotropic thermal parameters for all non-H atoms. The hydrogen atoms were placed at idealized positions. Molecular graphics were computed with PLATON[12e]

(9) Crystal data for **1**: $C_{20}H_{22}O_6$, MW = 358.4, orthorhombic, space group $P2_12_12_1$, a = 10.052(4), b = 10.322(4), c = 17.378(9) Å, V = 1803.1(2) Å^3, Z = 4, ρ_c=1.34 g cm^{-3}, $F(000)$ = 760, μ(Mo Kα) = 0.097 mm^{-1}; R = 0.0553, R_w = 0.1338, and S = 1.14 for 2302 observed reflections (θ_{max} = 28.6°, $I > 4\sigma(I)$ criterion) and 236 parameters. Maximum and minimum residual in the final difference map: 0.28 and 0.26 e Å^3.

(10) Crystal data for **2**: $C_{20}H_{22}O_6$, MW = 358.4, tetragonal, space group $I4$, a = 19.327(9), c = 9.814(6) Å, V = 3666(3) Å^3, Z = 8, ρ_c = 1.30 g cm^{-3}, $F(000)$ = 1520, μ(Mo Kα) = 0.096 mm^{-1}; R = 0.0575, R_w = 0.1348 and S = 1.04 for 1910 observed reflections (θ_{max} = 28.5°, $I > 4\sigma(I)$ criterion) and 236 parameters. Maximum and minimum residual in the final difference map: 0.20 and 0.19 e Å^3.

(11) Crystallographic data (excluding the structure factor tables) have been deposited with the Cambridge Crystallographic Data Center, deposition nos. 257586 (transtaganolide A) and 257587 (transtaganolide B). 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-(0)1223-306033 or e-mail: deposit@ccdc.cam.ac.uk].

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Table 1. NMR Data for Compounds **1**−**4** in $CDCl_3$

entry	**1** δ_C	**1** δ_H (*J* in Hz)	**2** δ_C	**2** δ_H (*J* in Hz)	**3** δ_C	**3** δ_H (*J* in Hz)	**4** δ_C	**4** δ_H (*J* in Hz)
1	143.7		144.0		131.9		137.5	
2	119.4	5.60 dd (5.9, 1.1)	119.2	5.58 dd (6.0, 1.1)	123.5	6.08 dd (6.5, 1.1)	123.8	6.08 dd (6.5, 1.2)
3	51.3	3.11 d (6.0)	51.4	3.12 dt (6.0, 0.9)	53.8	3.07 d (6.5)	53.9	3.04 d (6.6)
4	48.6		48.6		33.2		33.2	
5	49.4	1.96 dd (12.1, 6.2)	49.3	1.95 dd (12.1, 6.3)	47.9	1.30 dd (11.5, 6.5)	48.2	1.34 dd (12.6, 5.3)
6	19.9	α 1.60 dddd (13.3, 6.2, 2.7, 2.2) *β* 1.47 dddd (13.3, 13.3, 12.3, 2.3)	19.2	α 1.73 dddd (12.9, 6.2, 3.7, 2.5) *β* 1.51 dddd (13.0, 13.0, 13.0, 2.4)	19.8	2H, 1.70−1.60 m	20.3	2H 1.65−1.53 m
7	39.1	α 1.39 ddd (13.3, 13.3, 2.7) *β* 1.82 ddd (12.7, 3.7, 2.2)	36.8	α 1.43 ddd (13.3, 11.9, 2.3) *β* 1.63 ddd (13.3, 3.7, 2.3)	38.3	α 1.44 ddd (13.0, 13.0, 3.3) *β* 1.65 m	40.3	α 1.39 ddd (13.0, 13.0, 3.3) *β* 1.89 ddd (13.0, 3.3, 3.3)
8	38.1		37.9		38.4		38.3	
9	52.5	2.99 s	49.6	3.09 s	50.4	3.24 s	53.1	3.14 s
10	87.5		87.5		87.4		87.4	
11	84.1	4.99 br s	84.2	4.97 t (0.8)	79.1	5.01 d (1.1)	79.2	5.02 d (1.1)
12	154.4		154.3		156.7		156.6	
13	175.5		175.5		172.0		171.9	
14	15.6	1.30 s	15.7	1.33 s	29.6	0.98 s	29.8	0.96 s
15	109.6	5.61 s	109.6	5.66 t (0.7)	24.8	1.09 s	24.7	1.02 s
16	143.7	6.89 dd (17.9, 11.2)	146.5	5.81 dd (17.4, 10.8)	146.5	5.81 dd (17.4, 10.8)	142.9	6.98 dd (17.8, 11.2)
17	111.5	5.11 ddt (11.1, 1.1, 0.6) 5.03 dd (17.8, 1.1)	112.5	5.09 dd (10.8, 0.7) 5.06 dd (17.4, 0.7)	112.8	5.05 br d (10.7) 5.08 br d (17.4)	111.9	5.11 dd (11.1, 1.1) 5.03 dd (17.8, 1.1)
18	28.3	1.22 s	19.9	1.55 s	19.1	1.61 s	28.4	1.21 s
19	164.0		163.7		162.5		162.7	
OMe	56.3	3.70 s	56.3	3.68 s	56.3	3.72 s	56.3	3.72 s

NMR spectrum a signal at δ_C 123.5, which displayed in the HMBC spectrum a long-range C−H coupling with a vinyl proton at δ_H 5.01 (H-11, d, 1.1 Hz). The gHSQC spectrum allowed the identification of C-11 at δ_C 79.1. On the other hand, the vinyl carbon located at δ_C 137.9 (C-1) presented a correlation in the HMBC spectrum with H-2. H-11 showed a correlation with a carbon located at δ_C 156.7 (C-2), indicating the presence of two conjugated double bonds between C-1/C-2 and C-11/C-12. C-12 presented a coupling signal with the singlet at δ_H 3.72 attributed to the methoxyl group. Finally, an ester group was placed on C-9 through a HMBC correlation between H-9 (δ_H 3.24, s) and C-19 (δ_C 162.5), suggesting a similar arrangement in the C-ring as in **1**. The presence of the C ring required the formation of a *δ*-lactone ring between the oxygen atom on C-10 and the carbonyl C-13 leading to the planar structure proposed for **3**.

The relative stereochemistry of the different centers was confirmed through one- and two-dimensional NOESY experiments. Irradiation of H-9 resulted in enhancements of the signals of H-5 and H-3, in good agreement with the α-axial orientation of these three protons. The relative α orientation of the vinyl group was determined by irradiation of H-16, resulting in an increase of the signals belonging to H-9 and H-5.

Transtaganolide D (**4**) was the C-8 epimer of **3**. As in the case of **1** and **2**, the differences in the chemical shift of equivalent carbons were small, except for C-18 ($\Delta\delta_{C-18}$ −9.3 ppm). One- and two-dimensional NOESY experiments confirmed the epimeric relationship between **3** and **4**. Thus, irradiation of H-9 resulted in enhancements of the signals corresponding to H-5 and H-18, confirming the opposite relative configuration of C-8.

Although the biogenetic origin of transtaganolides A−D cannot easily be inferred directly from their structures, a mixed route leading to these compounds can be presumed. It is worth noting the high oxidation degree of these metabolites, a common pattern found in those metabolites isolated from the genus *Thapsia*.

Finally, the presence of a 7-methoxy-4,5-dihydro-3*H*-oxepin-2-one ring in transtaganolides A−D is a remarkable feature that provides a unique architecture in these molecules, making them attractive targets not only for the evaluation of their biological properties but also as a challenge to the synthetic chemist.

Acknowledgment. This investigation was supported by the Spanish Ministerio de Educación y Cultura (Grant BQU2001-3076) and Consejería de la Presidencia de la Junta de Andalucía (Grant A36-02). A.S. is thankful to the Agencia Española de Cooperación Internacional for financial support. We are also grateful to Prof. María J. Ortega for her helpful comments.

Supporting Information Available: Experimental details on the isolation of **1**−**4**; physical and spectral data and NMR spectra of **1**−**4**; and X-ray data of **1** and **3** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

OL047421W