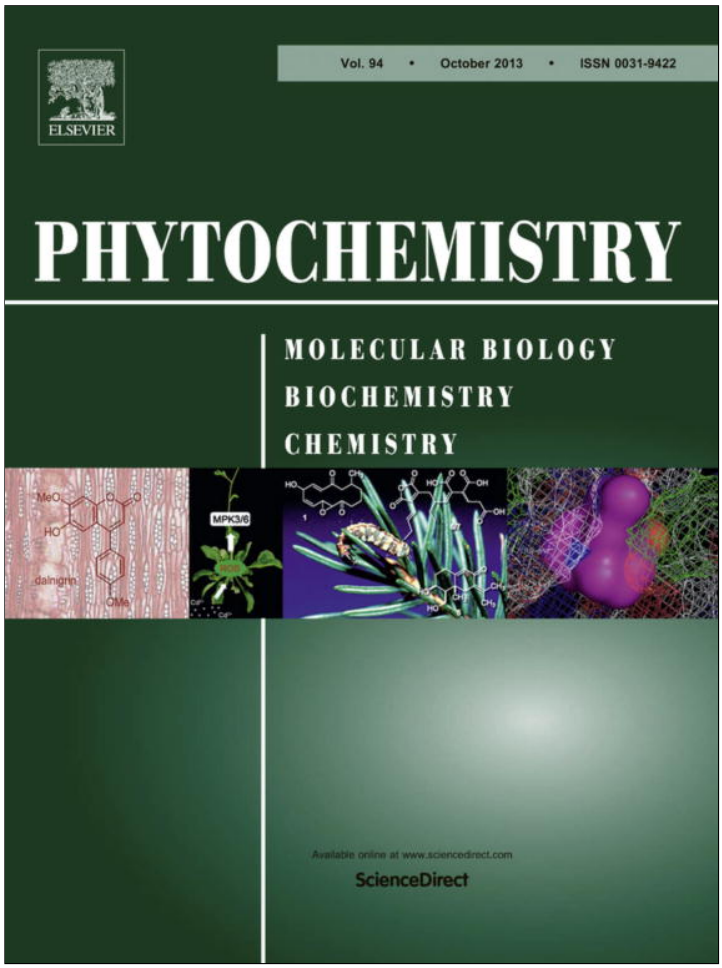


Phytochemistry 94 (2013) 260–267

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Agarofuran sesquiterpenes from *Schaefferia argentinensis*

Manuela E. García [a], Rubén D. Motrich [b], Beatriz L. Caputto [b], Marianela Sánchez [c], Jorge A. Palermo [c], Ana Estévez-Braun [d,*], Angel G. Ravelo [d,*], Viviana E. Nicotra [a,*]

[a] Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Universidad Nacional de Córdoba, 5000 Córdoba, Argentina
[b] Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC-CONICET), Universidad Nacional de Córdoba, 5000 Córdoba, Argentina
[c] Unidad de Microanálisis y Métodos Físicos Aplicados a la Química Orgánica (UMYMFOR-CONICET), Universidad de Buenos Aires, 1428 Buenos Aires, Argentina
[d] Departamento de Química Orgánica, Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG), Universidad de La Laguna, 38206 La Laguna, Spain

ARTICLE INFO

*Article history:*
Received 22 August 2012
Received in revised form 19 March 2013
Available online 1 July 2013

*Keywords:*
*Schaefferia argentinensis*
Celastraceae
Phytochemistry
Antiproliferative activity
Dihydro-β-agarofuran sesquiterpene

ABSTRACT

Sixteen dihydro-β-agarofuran sesquiterpenes were isolated from the aerial parts of *Schaefferia argentinensis* Speg. Their structures were determined by a combination of 1D and 2D NMR and MS techniques. The *in vitro* antiproliferative activity of the major sesquiterpenes was examined in T47D, MCF7, and MDA-MB231 human cancer cell lines, but was found to be marginal.



## 1. Introduction

Dihydro-β-agarofurans constitute a group of terpenoids isolated mainly from several genera from the Celastraceae (Gao et al., 2007). They are a large family of poly-esters of variously poly-oxygenated tricyclic scaffolds all based on a core C15 skeleton. This skeleton comprises A and B rings in the form of an axially dimethylated *trans*-decalin bicycle with a 1,3-diaxially fused, $Me_2C$-O bridge constituting the tetrahydrofuranyl C-ring. The basic polyhydroxy skeleton varies according to the position, number, and configuration of the ester residues in the dihydro-β-agarofuran sesquiterpene. Their chemistry and occurrence have already been reviewed (Gao et al., 2007; Muñoz et al., 1996; Spivey et al., 2002). The interest generated by polyesterified sesquiterpenes from the Celastraceae has increased in line with the complexity of the isolated substances and with the interesting biological activities that they exhibit, such as immunosuppressive (Wang et al., 2005), cytotoxic (Chen et al., 2006), and multidrug resistance (MDR) reversing activities (Perestelo et al., 2011).

*Schaefferia* Jacq. is a genus belonging to the Celastraceae family that comprises twenty-three species distributed in America and the West Indies (Simmons, 2004). As part of an intensive study of the bioactive metabolites from species of the Celastraceae family, the isolation of a set of eleven new and five already known dihydro-β-agarofurans with five different skeletons from *Schaefferia argentinensis* Speg. collected in Argentina is reported herein. In addition, data is provided of the antiproliferative activity shown by the major sesquiterpenes that were evaluated against human breast cancer cell lines.

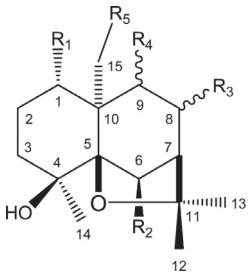
## 2. Results and discussion

The $CH_2Cl_2$ extract of the aerial parts of *S. argentinensis* yielded eleven new poly-ester dihydro-β-agarofuran-sesquiterpenes (**1**–**11**), along with five previously known sesquiterpenes (**12**–**16**) (Fig. 1). Their structures were elucidated on the basis of UV, IR, HRMS, and NMR spectroscopic data.

Compound **1** had the molecular formula $C_{26}H_{34}O_{10}$ as determined by HRESIQTOFMS. Its IR spectrum showed absorption bands for hydroxyl (3442 $cm^{-1}$) and ester (1735 $cm^{-1}$) groups, and the UV spectrum indicated the occurrence of aromatic ring absorptions at 275 and 258 nm. This was confirmed by analysis of the $^1H$ NMR and $^{13}C$ NMR spectroscopic data. The $^1H$ NMR spectrum displayed signals for methyls of two acetyl groups at δ 1.40 and 2.16 and signals of five aromatic protons at δ 7.90 (2H, *dd*, *J* = 8.0, 1.4 Hz), 7.39 (2H, *bt*, *J* = 8.0 Hz), and 7.52 (1H, *tt*, *J* = 8.0, 1.3 Hz) (Table 1). The resonances shown in the $^{13}C$ NMR spectrum at δ 169.9, 20.7, 170.3, and 21.3 were attributed to acetyl groups, whereas δ 167.3, 129.3, 129.6, 128.4, and 133.6 were assigned to a benzoyl group (Table 3). The core skeleton was derived from the $^1H$ NMR spectrum, which

* Corresponding authors. Tel.: + 54 351 4334170x147; fax: +54 351 4333030 (V.E. Nicotra).
*E-mail address:* vnicotra@fcq.unc.edu.ar (V.E. Nicotra).

0031-9422/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2013.06.003




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | OAc | OH | β OH | α OBz | OAc |
| **2** | OCin | OH | β OH | α OBz | OAc |
| **3** | OBz | OH | β OH | α OBz | OAc |
| **4** | OBz | OH | β OAc | α OBz | OAc |
| **5** | OAc | OAc | β OAc | β OBz | OAc |
| **6** | OAc | OAc | β OH | β OBz | OH |
| **7** | OCin | OAc | β OH | β OBz | OAc |
| **8** | OAc | OAc | α OAc | β OBz | OAc |
| **9** | OBz | OAc | α OH | α OBz | OAc |
| **10** | OAc | OAc | α OAc | α OBz | OAc |
| **11** | OAc | OAc | =O | α OBz | OAc |
| **12** | OH | OAc | α OAc | α OBz | OAc |
| **13** | OH | OAc | α OBz | α OBz | OAc |
| **14** | OAc | OAc | α OBz | α OBz | OAc |
| **15** | OAc | OAc | α OH | α OBz | OAc |
| **16** | OBz | OAc | α OAc | α OBz | OAc |

**Fig. 1.** Dihydro-β-agarofuran sesquiterpenes isolated from *Schaefferia argentinensis*. Ac: acetyl, Bz: benzoyl, Cin: cinnamyl.

showed three singlet signals corresponding to the geminal methyl groups $H_3$-12 (δ 1.68 *s*) and $H_3$-13 (δ 1.53 *s*), and a resonance at δ 1.54 *s* assigned to $H_3$-14; this assumption was confirmed by the cross-correlation peak between $H_3$-14 (δ 1.54) and the oxygenated quaternary carbon C-4 (δ 72.8) in the HMBC experiment. The $^{13}C$ NMR spectrum showed expected chemical shifts for the signals of carbons C-12, C-13, and C-14 at δ 26.3, δ 30.2, and δ 22.8, respectively. The proton resonance at δ 4.93 *d* (5.4 Hz) was assigned to H-6 indicating the presence of a hydroxyl group with a β orientation at C-6 in agreement with all substituted agarofurans at C-6 (Muñoz et al., 1996). The carbon signal at δ 76.8 assigned to C-6 is in accordance with this assumption. The presence of resonances at $\delta_H$ 4.88 *d* (12.8 Hz) and 4.36 *d* (12.8 Hz), and $\delta_C$ 61.4 in the NMR spectra suggested an oxygenated function at C-15. The complete assignments of the protonated carbons were made with the help of analysis of the HSQC spectrum, while a detailed examination of the $^1H$–$^1H$ COSY spectrum of compound **1** led to establishment of two spin systems: an $AY_2X_2$ system consisting of H-1 (δ 5.23 *dd*, *J* = 12.2, 4.4 Hz), $H_2$-2 (δ 1.79 *m* and δ 1.32 *m*), and $H_2$-3 (1.81 *m* and 1.69 *m*), and an AYX system consisting of H-7 (δ 2.50 *d*, *J* = 3.2 Hz), H-8 (4.15 *dt*, 9.4, 3.9 Hz), and H-9 (5.81 *d*, *J* = 9.4 Hz), respectively. Regiosubstitution of compound **1** was determined by an HMBC experiment, showing three-bond correlations between the resonances at $\delta_H$ 5.23 (H-1) and $\delta_H$ 4.88 (H-15a) and carboxyl signals of the acetate groups at $\delta_C$ 169.9 and 170.3, respectively, whereas the resonance at $\delta_H$ 5.81 (H-9) was correlated with the carboxyl signal of the benzoyl group at $\delta_C$ 167.3. Regarding the absolute configuration of compound **1**, all dihydro-agarofuran sesquiterpenes described to date have the same configuration of the stereocenters at C-1, C-4, C-5, C-6, C-7, and C-10: 1*S*,4*S*,5*S*,6*R*,7*R*,

**Table 1**
$^1H$ NMR spectroscopic data of compounds **1**–**8** in $CDCl_3$.[a]

| Position | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|
| 1 | 5.23 *dd* (12.2, 4.4) | 5.46 *dd* (12.5, 4.2) | 5.67 *dd* (12.1, 4.5) | 5.56 *m* | 5.33 *dd* (12.5, 4.0) | 5.39 *dd* (12.2, 4.1) | 5.49 *dd* (12.2, 4.6) |
| 2a | 1.79 *m* | 1.92 *m* | 1.95 *m* | 1.93 *m* | 1.90 *m* | 1.93 *m* | 1.86 *m* |
| 2b | 1.32 *m* | 1.48 *m* | nd | 1.75 *m* | 1.29 *m* | 1.44 *m* | 1.62 *m* |
| 3a | 1.81 *m* | 1.97 *m* | 1.96 *m* | 1.76 *m* | 1.87 *m* | 1.90 *m* | 1.95 *m* |
| 3b | 1.69 *m* | 1.79 *m* | 1.82 *m* | nd | 1.66 *m* | nd | 1.73 *m* |
| 6 | 4.93 *d* (5.4) | 5.02 *d* (5.4) | 5.04 *d* (5.0) | 5.26 *bs* | 5.96 *bs* | 6.48 *bs* | 6.79 *bs* |
| 7 | 2.50 *d* (3.2) | 2.59 *d* (3.2) | 2.58 *d* (3.2) | 2.39 *d* (4.2) | 2.34 *d* (3.3) | 2.21 *m* | 2.38 *d* (4.0) |
| 8 | 4.15 *dt* (9.4, 3.9) | 4.25 *m* | 4.24 *ddd* (9.6, 5.0, 3.2) | 5.51 *dd* (5.9, 4.2) | 5.70 *dd* (6.4, 3.4) | 4.19 *m* | 4.44 *m* |
| 9 | 5.81 *d* (9.4) | 5.94 *d* (9.3) | 6.00 *d* (9.6) | 5.70 *d* (5.9) | 5.54 *d* (6.5) | 5.29 *bs* | 5.65 *bd* (5.8) |
| 12 | 1.68 *s* | 1.78 *s* | 1.63 *s* | 1.61 *s* | 1.59 *s* | 1.43 *s* | 1.62 *s* |
| 13 | 1.53 *s* | 1.62 *s* | 1.79 *s* | 1.56 *s* | 1.49 *s* | 1.50 *s* | 1.59 *s* |
| 14 | 1.54 *s* | 1.65 *s* | 1.66 *s* | 1.64 *s* | 1.26 *s* | 1.28 *s* | 1.40 *s* |
| 15a | 4.88 *d* (12.8) | 5.09 *d* (13.0) | 5.10 *d* (12.8) | 5.00 *d* (13.2) | 4.47 *d* (12.8) | 4.66 *d* (12.8) | 5.05 *d* (13.0) |
| 15b | 4.36 *d* (12.8) | 4.48 *d* (13.0) | 4.59 *d* (12.8) | 4.79 *d* (13.2) | 4.45 *d* (12.8) | 4.50 *d* (12.8) | 4.71 *d* (13.0) |
| OH-C4 | 3.02 *d* (1.0) | 3.12 *s* | 3.13 *d* (1.0) | 2.95 *s* | 2.72 *s* | 2.63 *s* | 2.72 *s* |
| OH-C6 | 5.26 *d* (5.4) | 5.34 *d* (5.4) | 5.38 *d* (5.0) | 5.23 *s* | – | – | – |
| OH-C8 | 2.36 *d* (4.8) | 2.47 *d* (5) | 2.34 *d* (5.0) | – | – | 2.77 *d* (5.5) | 2.77 *d* (5.5) |
| AcO-C1 | 1.40 *s* | – | – | – | 1.55 *s* | 1.44 *s* | – |
| AcO-C6 | – | – | – | – | 2.05 *s* | 2.1 *s* | 2.14 *s* |
| AcO-C8 | – | – | – | 1.95 *s* | 1.83 *s* | – | – |
| AcO-C15 | 2.16 *s* | 2.25 *s* | 2.29 *s* | 2.18 *s* | 2.27 *s* | – | 2.36 *s* |
| BzO-C1 | – | – | 7.58 *dd* (8.3, 1.0) | 7.50 *dd* (8.2, 1.2) | – | – | – |
| | – | – | 7.19 *m* | 6.80 *t* (7.9) | – | – | – |
| | – | – | 7.18 *m* | 7.11 *m* | – | – | – |
| BzO-C9 | 7.90 *dd* (8.0, 1.4) | 7.86 *bd* (8.5) | 7.53 *dd* (8.3, 1.1) | 7.54 *dd* (8.3, 1.2) | 8.00 *bd* (7.8) | 7.96 *bd* (7.6) | 7.95 *bd* (7.8) |
| | 7.39 *bt* (8.0) | 7.27 *m* | 6.88 *bt* (7.8) | 7.12 *m* | 7.40 *bt* (7.8) | 7.40 *m* | 7.26 *m* |
| | 7.52 *tt* (8.0, 1.3) | 7.42 *bt* (7.3) | 7.41 *tt* (7.4, 1.1) | 7.33 *bt* (7.2) | 7.53 *bt* (7.5) | 7.53 *m* | 7.38 *m* |
| CinO-C1 | – | 5.64 *d* (16.0) | – | – | – | – | 5.70 *d* (16.0) |
| | – | 7.34 *d* (16.0) | – | – | – | – | 7.32 *m* |
| | – | 6.90 *bd* (8.0) | – | – | – | – | 6.91 *bd* (7.8) |
| | – | 7.19 *bt* (7.6) | – | – | – | – | 7.19 *bt* (7.6) |
| | – | 7.29 *m* | – | – | – | – | 7.29 *m* |

[a] Chemicals shifts (δ) downfield from TMS, *J* couplings (in parentheses) in Hz. 400.13 MHz.

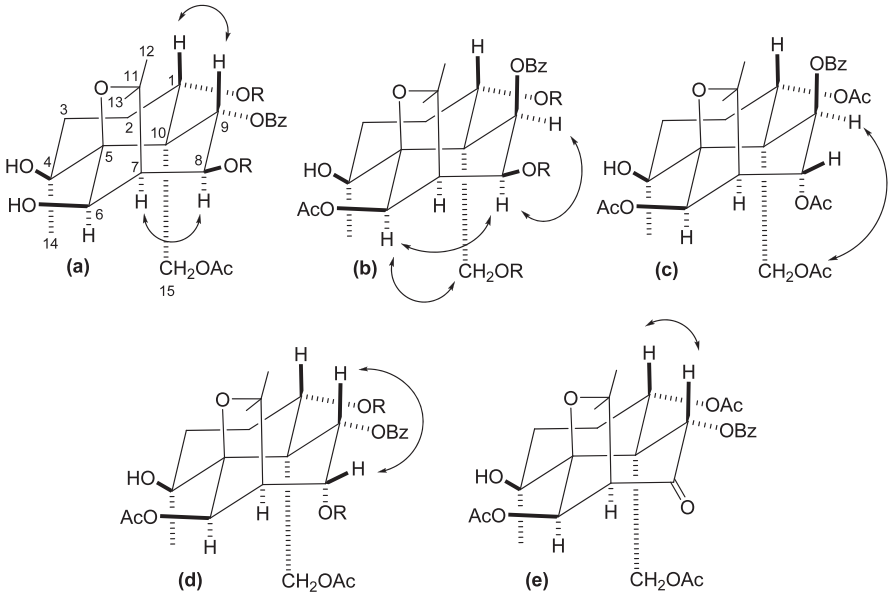



**Fig. 2.** Relevant NOE correlations of: (a) compounds **1**–**4**, (b) compounds **5**–**7**, (c) compound **8**, (d) compounds **9**–**10**, (e) compound **11**.

and 10*S*, respectively (Muñoz et al., 1996). The $\beta$ orientation of the hydroxyl group at C-8 was confirmed by a cross-correlation peak observed between H-8 ($\delta$ 4.15) and the resonance corresponding to H-7 ($\delta$ 2.50), while the $\alpha$ orientation of the benzoyl group at C-9 was established by a cross-correlation peak observed between H-9 ($\delta$ 5.81) and the signal corresponding to H-1 ($\delta$ 5.23) in the NOESY experiments (Fig. 2a). All NMR spectroscopic data provided clear evidence of the presence of a poly-esterified 2,3,13-trideoxy-isoeuoniminol skeleton (Spivey et al., 2002; Chang et al., 2006). Thus, the structure of compound **1** was established as (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*S*,10*S*)-1,15-diacetoxy-9-benzoyloxy-4,6,8-trihydroxy-dihydro-$\beta$-agarofuran.

The NMR spectroscopic data of sesquiterpenes **2**–**4**, (Tables 1 and 3) were closely related to those of **1**, showing a typical substitution pattern of the previously described compound. The main differences observed between compounds **1**–**4** were the type of esters and/or their relative position. The locations of the ester groups were established unequivocally through HMBC spectra. The $^1$H and $^{13}$C NMR spectroscopic data of compound **2** established that it was very similar to **1**, except that an acetyl group at C-1 in compound **1** was replaced by a cinnamoyl group in compound **2**. The presence of the cinnamoyl group was confirmed by analysis of the $^1$H NMR spectrum, which exhibited signals at $\delta$ 5.64 (1H, *d*, *J* = 16.0 Hz), $\delta$ 7.34 (1H, *d*, *J* = 16.0 Hz), $\delta$ 6.90 (2H, *bd*, *J* = 8.0 Hz), $\delta$ 7.19 (2H, *bt*, *J* = 7.6 Hz), and $\delta$ 7.29 (1H, *m*), while the $^{13}$C NMR spectrum showed resonances at $\delta$ 165.7, 117.1, 145.0, 133.7, 127.9, 128.2, and 130.1. The $^1$H and $^{13}$C NMR spectroscopic data of compound **3** (Tables 1 and 3) were consistent with a 2,3,13-trideoxy-isoeuoniminol sesquiterpene with a benzoyl group at C-1 unlike compounds **1** and **2**. Thus, resonances for two benzoyl groups were observed in its $^1$H NMR spectrum with signals at $\delta$ 7.58 (2H, *dd*, *J* = 8.3, 1.0 Hz), 7.19 (2H, *m*), and 7.18 (1H, *m*) for the benzoyl group at C-1, and 7.53 (2H, *dd*, *J* = 8.3, 1.1 Hz), 6.88 (2H, *bt*, *J* = 7.8 Hz), and 7.41 (1H, *tt*, *J* = 7.4, 1.1 Hz) for benzoyl group at C-9. The $^{13}$C NMR spectrum was in agreement with and supported the proposed structure. The $^1$H NMR and $^{13}$C NMR spectra of compound **4** were very similar to those of compound **3**. The most remarkable difference was the downfield shift of the H-8 resonance, which appears at $\delta$ 4.15–4.25 in compounds **1**–**3** and at $\delta$ 5.51 in **4**, with the signals corresponding to an acetyl group ($\delta_H$ 1.95, $\delta_C$ 169.6 and 20.9) at C-8.

Compound **5** had a molecular formula of $C_{30}H_{38}O_{12}$ by HRESIQ-TOFMS. The IR spectrum showed the presence of hydroxyl (3487 cm$^{-1}$) and ester carbonyl (1747 cm$^{-1}$) groups. The $^1$H NMR and $^{13}$C NMR (Tables 1 and 3) spectroscopic data indicated that compound **5** had four acetyls, one benzoyl, and one tertiary hydroxyl groups. The location of these groups was supported by the HMBC correlations between the signals of H-1 ($\delta$ 5.33 *dd*, *J* = 12.5, 4.0 Hz) and $CH_3CO_2$ at $\delta$ 169.3, H-6 ($\delta$ 5.96 *bs*) and $CH_3CO_2$ at $\delta$ 169.9, H-8 ($\delta$ 5.70 *dd*, *J* = 6.4, 3.4 Hz) and $CH_3CO_2$ at $\delta$ 169.2, $H_2$-15 ($\delta$ 4.47 *d* and $\delta$ 4.45 *d*, *J* = 12.8 Hz) and $CH_3CO_2$ at $\delta$ 170.3, H-9 ($\delta$ 5.54 *d*, *J* = 6.5 Hz) and $PhCO_2$ at $\delta$ 165.4, and OH-C4 ($\delta$ 2.72 *s*) and C-4 $\delta$ 70.0. The only difference between compound **5** and compounds **1**–**4** was the configuration at C-9. The relative configuration of **5** was established by the analysis of the coupling constants and confirmed by a NOESY experiment (Fig. 2b). The nOes observed between H-6 ($\delta$ 5.96) and H-8 ($\delta$ 5.70) and $H_2$-15 ($\delta$ 4.47 and $\delta$ 4.45), and between H-8 and H-9 ($\delta$ 5.54) indicated an $\alpha$ orientation of H-9. All the NMR data confirmed that compound **5** had a 2,3-dideoxy-isomagellanol core skeleton (Spivey et al., 2002). Thus, the structure of compound **5** was established as (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,6,8,15-tetra-acetoxy-9-benzoyloxy-4-hydroxy-dihydro-$\beta$-agarofuran.

Comparison of the $^1$H NMR and $^{13}$C NMR spectroscopic data of compounds **6** and **7** with those of compound **5** suggested the same core skeleton. The only differences observed between these compounds were the number of ester groups and/or their relative positions, which were confirmed by the cross-correlation peaks observed in the HMBC experiments. Compounds **6** and **7** could not be obtained in pure form by normal or reversed-phase TLC. Consequently, compound **6** was characterized from 2 mg of a mixture of compounds **6** and **12** [(1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,8,15-triacetoxy-9-benzoyloxy-1,4-dihydroxy-dihydro-$\beta$-agarofuran, in a 2:3 ratio] (González et al., 1993). The only difference between compounds **5** and **6** was the presence of two hydroxyl groups at C-8 and C-15 in compound **6** instead of the acetyl groups present in compound **5**. The $^1$H NMR spectroscopic spectrum of compound **6** (Table 1) displayed only two acetyl groups as singlets at $\delta$ 1.44 and 2.10 and signals of five aromatic protons at $\delta$ 7.96 (2H, *bd*, *J* = 7.6 Hz), 7.40 (2H, *m*), and 7.53 (1H, *m*). The resonances observed in the $^{13}$C NMR spectrum (Table 3) at $\delta$ 169.7, 20.6, 170.1, and 21.4 were attributed to acetyl groups, whereas $\delta$ 165.3, 130.0 $\times$ 2, 128.6,

and 133.8 were attributed to a benzoyl group. Compound **7** was characterized from 1.5 mg of a mixture of compounds **7** and an uncharacterized isomer in a 2:1 ratio. The $^1$H NMR data of compound **7** (Table 1) indicated the presence of four ester groups: two acetyl groups at C-6 and C-15 [$\delta$ 2.14 *s* (3H) and 2.36 *s* (3H)], a benzoyl group at C-9 [$\delta$ 7.95 (2H, *bd*, $J$ = 7.8 Hz), 7.26 (2H, *m*), and 7.38 (1H, *m*)], and a cinnamoyl group [$\delta$ 5.70 (1H, *d*, $J$ = 16.0 Hz), 7.32 (1H, *m*), 6.91 (2H, *bd*, $J$ = 7.8 Hz), 7.19 (2H, *bt*, $J$ = 7.6 Hz), and 7.29 (1H, *m*)]. The $^{13}$C NMR spectrum of compound **7** (Table 3) was in agreement with the proposed substitution pattern. The molecular formulae of the mixture components were unambiguously determined by HPLC-HRMS. Since compounds **6** and **7** could not be obtained in a pure form and were characterized from different mixtures, their structures were tentatively proposed as (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-1,6-diacetoxy-9-benzoyloxy-4,8-dihydroxy-dihydro-$\beta$-agarofuran and (1*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*R*,10*S*)-6,15-diacetoxy-9-benzoyloxy-1-cinnamoyloxy-4,8-dihydroxy-dihydro-$\beta$-agarofuran, respectively.

Compound **8** was established as (1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*R*,10*S*)-1,6,8,15-tetra-acetoxy-9-benzoyloxy-4-hydroxy-dihydro-$\beta$-agarofuran owing to the following observations: (i) compound 8 had a molecular formula of $C_{30}H_{38}O_{12}$ by HRESIQTOFMS; (ii) its $^1$H NMR spectrum (Table 2) displayed four acetate methyls as singlets at $\delta$ 1.62, 2.12, 1.89, and 2.30, and signals corresponding to a benzoyl group [$\delta$ 7.95 (2H, *bd*, $J$ = 8.0 Hz), 7.40 (2H, *m*), and 7.53 (1H, *m*)]; (iii) the resonances observed in the $^{13}$C NMR spectrum (Table 3) at $\delta$ 169.5, 20.8, 169.5, 21.4, 169.1, 20.8, 170.4, and 20.6 were attributed to acetyl groups, whereas the resonances at $\delta$ 164.6, 129.8 $\times$ 2, 128.8, and 133.8 were attributed to a benzoyl group; (iv) its regiosubstitution was determined through the following HMBC correlations: $\delta$ 5.39 (*dd*, $J$ = 11.6, 4.0 Hz, H-1)/$\delta$ 169.5 ($CH_3CO_2$); $\delta$ 6.42 (*bs*, H-6)/$\delta$ 169.5 ($CH_3CO_2$); $\delta$ 5.20 (*d*, $J$ = 2.9 Hz, H-8)/$\delta$ 169.1 ($CH_3CO_2$); $\delta$ 5.57 (*s*, H-9)/$\delta$ 164.6 ($PhCO_2$); and $\delta$ 4.61 and 4.52 (*d*, $J$ = 12.8 Hz, $H_2$-15)/$\delta$ 170.4 ($CH_3CO_2$); (v) the relative configurations at C-8 and C-9 were established by analysis of the coupling constants and confirmed by a NOESY experiment. The nOe effect observed (Fig. 2c) between H-9 ($\delta$ 5.57) and H-15b ($\delta$ 4.52) led us to propose a 4,15-dihydroxy-celapanol for compound **8** (Spivey et al., 2002; Chávez et al., 1999).

Compound **9** had a molecular formula of $C_{33}H_{38}O_{11}$ by HRESIQTOFMS. The $^1$H NMR spectrum of compound **9** (Table 2) showed the signals corresponding to two acetates [$\delta$ 2.14 (*s*, 3H) and 2.36 (*s*, 3H)], and two benzoates (ten aromatic protons in the $\delta$ 6.90–7.66 range). The positions of the acetyl groups were located at C-6 and C-15 from the following HMBC correlations: $\delta$ 6.79 (*bs*, H-6)/$\delta$ 169.8 ($CH_3CO_2$), and $\delta$ 5.08 and 4.80 (*d*, $J$ = 13.0 Hz, $H_2$-15)/$\delta$ 170.4 ($CH_3CO_2$). The benzoyl substituents were located at C-1 and C-9 by the HMBC correlations between the signals of H-1 ($\delta$ 5.68) and $PhCO_2$ at $\delta$ 165.3 and H-9 ($\delta$ 5.69) and $PhCO_2$ at $\delta$ 164.6. The NOESY correlation observed between H-8 and H-9 suggested that the orientations of OH-C8 and BzO-C9 were $\alpha$ (Fig. 2d). Accordingly, compound **9** was characterized as the isoalatol derivative (1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,15-diacetoxy-1,9-dibenzoyloxy-4,8-dihydroxy-dihydro-$\beta$-agarofuran (Spivey et al., 2002).

Comparison of the NMR spectroscopic data of compound **10** with those of compound **9** (Tables 2 and 3) suggested the same isoalatol core skeleton. The $^1$H NMR spectrum of **10** showed signals for four acetyl groups at $\delta$ 1.46, 2.12, 2.05, and 2.32, and for a benzoyl group [$\delta$ 8.04 (2H, *m*), 7.47 (2H, *m*), and 7.57 (1H, *m*)]. The positions of the ester groups were established through the cross-peak correlations observed in the HMBC experiment: $\delta$ 5.33 (*dd*, $J$ = 12.1, 4.8 Hz, H-1)/$\delta$ 169.66 ($CH_3CO_2$); $\delta$ 6.83 (*bs*, H-6)/$\delta$ 169.70 ($CH_3CO_2$); $\delta$ 5.58 (*m*, H-8)/$\delta$ 169.67 ($CH_3CO_2$); $\delta$ 5.05 and 4.53 (*d*, $J$ = 13.3 Hz, $H_2$-15)/$\delta$ 170.4 ($CH_3CO_2$), and $\delta$ 5.68 (*d*, $J$ = 5.9 Hz, H-9)/$\delta$ 164.4 ($PhCO_2$).

The HRESIQTOFMS of compound **11** showed a quasimolecular ion $[M+Na]^+$ at *m*/*z* 569 corresponding to a formula of $C_{28}H_{34}O_{11}Na$. The $^1$H NMR spectrum of compound **11** (Table 2) displayed signals for three acetyl groups at $\delta$ 1.46, 2.08, and 2.02, and a benzoyl group at $\delta$ 7.93 (2H, *dd*, $J$ = 8.0, 1.4 Hz), 7.39 (2H, *t*, $J$ = 7.9 Hz), and 7.52 (1H, *tt*, $J$ = 7.5, 1.2 Hz). The $^{13}$C NMR spectrum (Table 3) showed the expected chemical shifts corresponding to three acetyl groups and a benzoyl group. Compound **11** showed the typical $^1$H

**Table 2**
$^1$H NMR spectroscopic data of compounds **9**–**11** in $CDCl_3$.[a]

| Position | **8** | **9** | **10** | **11** |
|---|---|---|---|---|
| 1 | 5.39 *dd* (11.6, 4.0) | 5.68 *m* | 5.33 *dd* (12.1, 4.8) | 5.34 *dd* (12.5, 4.0) |
| 2a | 1.95 *m* | 1.91 *m* | 1.83 *m* | 1.76 *m* |
| 2b | 1.45 *m* | nd | 1.62 *m* | 1.38 *m* |
| 3a | 1.96 *m* | 1.77 *m* | 1.95 *m* | 1.88 *m* |
| 3b | 1.73 *m* | 2.00 *m* | 1.72 *m* | 1.73 *m* |
| 6 | 6.42 *bs* | 6.79 *bs* | 6.83 *bs* | 6.58 *bs* |
| 7 | 2.26 *d* (3.0) | 2.38 *d* (4.0) | 2.38 *d* (4.0) | 2.92 *bs* |
| 8 | 5.20 *d* (2.9) | 4.44 *q* (5.5) | 5.58 *m* | – |
| 9 | 5.57 *s* | 5.69 *d* (5.0) | 5.68 *d* (5.9) | 5.84 *s* |
| 12 | 1.56 *s* | 1.62 *s* | 1.66 *s* | 1.54 *s* |
| 13 | 1.63 *s* | 1.59 *s* | 1.56 *s* | 1.58 *s* |
| 14 | 1.26 *s* | 1.41 *s* | 1.37 *s* | 1.33 *s* |
| 15a | 4.61 *d* (12.8) | 5.08 *d* (13) | 5.05 *d* (13.3) | 5.01 *d* (12.0) |
| 15b | 4.52 *d* (12.8) | 4.80 *d* (13) | 4.53 *d* (13.3) | 4.27 *d* (12.0) |
| OH-C4 | 2.58 *s* | 2.74 *s* | 2.65 *s* | 2.63 *s* |
| OH-C6 | – | – | – | – |
| OH-C8 | – | 2.77 *d* (6.0) | – | – |
| AcO-C1 | 1.62 *s* | – | 1.46 *s* | 1.46 *s* |
| AcO-C6 | 2.12 *s* | 2.14 *s* | 2.12 *s* | 2.08 *s* |
| AcO-C8 | 1.89 *s* | – | 2.05 *s* | – |
| AcO-C15 | 2.30 *s* | 2.36 *s* | 2.32 *s* | 2.02 *s* |
| BzO-C1 | – | 7.59 *dd* (8.3, 1.1) | – | – |
| | – | 6.90 *bt* (7.8) | – | – |
| | – | 7.18 *m* | – | – |
| BzO-C9 | 7.95 *bd* (8.0) | 7.66 *dd* (8.4, 1.3) | 8.04 *m* | 7.93 *dd* (8.0, 1.4) |
| | 7.40 *m* | 7.20 *bt* (7.9) | 7.47 *m* | 7.39 *t* (7.9) |
| | 7.53 *m* | 7.41 *bt* (7.5) | 7.57 *m* | 7.52 *tt* (7.5, 1.2) |

[a] Chemicals shifts ($\delta$) downfield from TMS, $J$ couplings (in parentheses) in Hz. 400.13 MHz.

**Table 3**
$^{13}C$ NMR spectroscopic data of compounds **1–11** in $CDCl_3$.[a]

| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 76.8 | 76.4 | 76.6 | 78.0 | 72.0 | 72.5 | 77.5 | 72.5 | 77.4 | 78.2 | 76.0 |
| 2 | 24.2 | 24.6 | 24.6 | 25.1 | 23.9 | nd | 24.9 | 23.2 | 25.0 | 24.7 | 24.9 |
| 3 | 37.1 | 37.2 | 37.1 | 37.4 | 38.3 | 37.9 | 37.8 | 37.7 | 38.0 | 37.6 | 38.1 |
| 4 | 72.8 | 72.9 | 73.0 | 73.4 | 70.0 | 70.1 | 70.1 | 70.0 | 70.2 | 70.4 | 70.2 |
| 5 | 91.4 | 91.4 | 91.6 | 91.4 | 91.0 | 91.8 | 91.9 | 91.5 | 91.9 | 91.8 | 93.5 |
| 6 | 76.8 | 76.8 | 76.9 | 74.7 | 76.2 | 74.6 | 73.9 | 74.8 | 73.9 | 75.0 | 74.1 |
| 7 | 55.3 | 55.3 | 55.3 | 54.0 | 53.8 | 55.8 | 55.6 | 52.7 | 55.6 | 53.0 | 64.9 |
| 8 | 74.8 | 74.7 | 74.9 | 70.0 | 70.2 | 75.0 | 68.6 | 76.4 | 68.5 | 70.0 | 197.4 |
| 9 | 78.9 | 79.0 | 78.6 | 72.7 | 68.6 | 76.7 | 74.9 | 73.2 | 74.8 | 72.4 | 79.4 |
| 10 | 49.8 | 50.2 | 50.3 | 51.4 | 52.7 | nd | 52.7 | 53.7 | 52.9 | 51.9 | 51.7 |
| 11 | 84.3 | 84.3 | 84.3 | 83.2 | 84.4 | 82.9 | 82.6 | 82.5 | 82.4 | 82.3 | 84.7 |
| 12 | 26.3 | 26.5 | 30.1 | 24.8 | 26.4 | 25.5 | 24.1 | 29.6 | 23.9 | 24.3 | 24.3 |
| 13 | 30.2 | 30.1 | 26.2 | 30.0 | 30.1 | 29.6 | 29.2 | 25.1 | 29.3 | 29.4 | 29.2 |
| 14 | 22.8 | 22.9 | 22.8 | 22.0 | 23.8 | 23.2 | 23.0 | 22.7 | 23.1 | 22.4 | 23.6 |
| 15 | 61.4 | 61.0 | 61.3 | 60.9 | 64.0 | 64.9 | 60.4 | 64.7 | 60.3 | 60.3 | 60.4 |
| AcO-C1 | 169.9 | – | – | – | 169.3 | 169.7 | – | 169.5 | – | 169.7 | 169.6 |
| | 20.7 | – | – | – | 20.8 | 20.6 | – | 20.8 | – | 20.4 | 20.5 |
| AcO-C6 | – | – | – | – | 169.9 | 170.1 | 169.7 | 169.5 | 169.8 | 169.7 | 169.2 |
| | – | – | – | – | 21.4 | 21.4 | 21.4 | 21.4 | 21.5 | 21.3 | 21.2 |
| AcO-C8 | – | – | – | 169.6 | 169.2 | – | – | 169.1 | – | 169.7 | – |
| | – | – | – | 20.9 | 20.8 | – | – | 20.8 | – | 21.0 | – |
| AcO-C15 | 170.3 | 170.3 | 170.1 | 170.4 | 170.3 | – | 170.4 | 170.4 | 170.4 | 170.4 | 170.3 |
| | 21.3 | 21.5 | 21.3 | 21.4 | 21.2 | – | 21.0 | 20.6 | 21.2 | 21.1 | 20.6 |
| BzO-C1 | – | – | 165.0 | 165.3 | – | – | – | – | 165.3 | – | – |
| | – | – | 129.0 | 129.3 | – | – | – | – | 129.0 | – | – |
| | – | – | 128.9 | 129.2 | – | – | – | – | 129.1 | – | – |
| | – | – | 127.9 | 127.6 | – | – | – | – | 127.6 | – | – |
| | – | – | 132.4 | 132.5 | – | – | – | – | 132.4 | – | – |
| BzO-C9 | 167.3 | 167.3 | 166.7 | 164.5 | 165.4 | 165.3 | 164.8 | 164.6 | 164.6 | 164.6 | 165.1 |
| | 129.3 | 129.2 | 129.2 | 129.3 | 128.9 | 130.0 | 129.7 | 129.8 | 129.2 | 129.4 | 129.2 |
| | 129.6 | 129.7 | 129.1 | 129.2 | 130.3 | 130.0 | 129.7 | 129.8 | 129.3 | 129.8 | 129.6 |
| | 128.4 | 128.5 | 127.3 | 128.1 | 128.4 | 128.6 | 128.5 | 128.8 | 128.2 | 128.5 | 128.2 |
| | 133.6 | 133.2 | 132.7 | 133.0 | 133.6 | 133.8 | 133.0 | 133.8 | 132.8 | 133.4 | 133.5 |
| CinO-C1 | – | 165.7 | – | – | – | – | 165.9 | – | – | – | – |
| | – | 117.1 | – | – | – | – | 117.1 | – | – | – | – |
| | – | 145.0 | – | – | – | – | 145.0 | – | – | – | – |
| | – | 133.7 | – | – | – | – | 133.7 | – | – | – | – |
| | – | 127.9 | – | – | – | – | 127.9 | – | – | – | – |
| | – | 128.2 | – | – | – | – | 128.3 | – | – | – | – |
| | – | 130.1 | – | – | – | – | 130.0 | – | – | – | – |

[a] Chemical shifts ($\delta$) downfield from TMS; 100.03 MHz.

and $^{13}C$ NMR profiles of a 2,3,13-trideoxy-evoninol derivative (Spivey et al., 2002; Yoshihisa et al., 1987). This arrangement was characterized by the presence of three oxymethine protons in the $^{1}H$ NMR spectrum at $\delta$ 5.34 (*dd*, $J$ = 12.5, 4.0 Hz), 6.58 *bs*, 5.84 *s*, assigned to H-1, H-6, and H-9, respectively, and two oxymethylene protons at 5.01 (*d*, $J$ = 12.0 Hz) and 4.27 (*d*, $J$ = 12.0 Hz) corresponding to $H_2$-15. In addition, the carbonyl resonance at $\delta$ 197.4 in the $^{13}C$ NMR spectrum was assigned to a keto group at C-8. The position of the ester groups was established by the HMBC correlations between the signals of H-1 ($\delta$ 5.34) and $CH_3CO_2$ at $\delta$ 169.6, H-6 ($\delta$ 6.58) and $CH_3CO_2$ at $\delta$ 169.2, $H_2$-15 ($\delta$ 5.01 and 4.27) and $CH_3CO_2$ at $\delta$ 170.3, and H-9 ($\delta$ 5.84) and $PhCO_2$ at $\delta$ 165.1. The configuration of C-9 was determined on the basis of the nOe observed between H-9 and H-1 in the NOESY experiment (Fig. 2e).

**Table 4**
*In vitro* antiproliferative activity of dihydro-$\beta$-agarofurans against human solid breast tumor cell lines.[a]

| Compound | T47D | MCF7 | MDA |
|---|---|---|---|
| 1 | >100 | >100 | >100 |
| 2 | >100 | >100 | >100 |
| 3 | >100 | 97.3 (±15.1) | >100 |
| 5 | >100 | >100 | >100 |
| 9 | 68.1 (±13.6) | >100 | >100 |
| 11 | 73.9 (±5.7) | 91.4 (±21.6) | >100 |
| 13 | 72.9 (±9.8) | 74.9 (±23.6) | 96.0 (±23.6) |
| 15 | >100 | >100 | >100 |
| 16 | >100 | >100 | >100 |
| 5-Fluorouracyl | 43.0 (±14.0) | 8.3 (±1.2) | 68.4 (±12.5) |
| Camptothecin | 2.0 (±0.5) | 0.3 (±0.1) | 1.3 (±0.2) |

[a] Expressed as $GI_{50}$ values given in μM and determined as means of six experiments. Standard deviations are given in parentheses. $GI_{50}$ (concentration of the antiproliferative drug that inhibits the growth of cells by 50%).

In addition to the eleven new dihydro-$\beta$-agarofuran derivatives (compounds **1**–**11**), five previously known terpenoids (compounds **12**–**16**) were also isolated and identified by comparison with published spectroscopic and physical data as (1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,8,15-triacetoxy-9-benzoyloxy-1,4-dihydroxy-dihydro-$\beta$-agarofuran (compound **12**), (1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,15-diacetoxy-8,9-dibenzoyloxy-1,4-dihydroxy-dihydro-$\beta$-agarofuran (compound **13**), (1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-1,6,15-triacetoxy-8,9-dibenzoyloxy-4-hydroxy-dihydro-$\beta$-agarofuran (compound **14**), (1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-1,6,15-triacetoxy-9-benzoyloxy-4,8-dihydroxy-dihydro-$\beta$-agarofuran (compound **15**), (González et al., 1993), and (1*S*,4*S*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,8,15-triacetoxy-1,9-dibenzoyloxy-4,hydroxyl-dihydro-β-agarofuran (compound **16**) (González et al., 1989a), isolated from *Maytenus canariensis*.

Considering the cytotoxic activity shown by several dihydro-$\beta$-agarofuran sesquiterpenes (Chen et al., 2006; Zhu et al., 2008), the *in vitro* antiproliferative activity of sesquiterpenes **1**, **2**, **3**, **5**, **9**, **11**, **13**, **15**, and **16** was evaluated in three human breast cancer cell lines: T47D, MCF7, and MDA-MB231. Table 4 summarizes the results

expressed as 50% growth inhibition ($GI_{50}$). None of these compounds showed significant antiproliferative activity ($GI_{50}$ > 68 μM).

## 3. Conclusions

*Schaefferia* is one of the least studied genera from the Celastraceae family. To our knowledge, the only existing report corresponds to the phytochemical study of *S. cuneifolia* collected in Mexico (González et al., 1989b, 1991). From this species, five pentasubstituted agarofuran sesquiterpenes were isolated. In this paper the isolation and structural elucidation is reported of eleven new β-dihydroagarafuran sesquiterpenes functionalized at C-1, C-4, C-6, C-8, C-9, and C-15 (compounds **1**–**11**). Five already known compounds were also isolated. Compounds **12**, **13**, **15** and **16** were previously reported from *M. canariensis*. Compound **14** is herein reported for the first time as a natural product, since it has been obtained previously by benzoylation of compound **15** (González et al., 1993). The presence of dihydro-β-agarofuran sesquiterpenes in *S. argentinensis* confirms the value of these metabolites as chemotaxonomic markers of the Celastraceae family (Bruning and Wagner, 1978).

The information regarding the anti proliferative activity of these compounds suggests that the number, type and orientation of the ester groups are the key for cytotoxicity. Although the antiproliferative activity observed for the tested compounds in the present work was marginal ($IC_{50}$ > 68.1 μM), these results might contribute to deeper structure–activity relationship studies for this type of sesquiterpenoids.

## 4. Experimental

### 4.1. General

Optical rotations (as ORD measurements) were recorded on a JASCO P-810 Circular Dichroism Spectrometer. UV spectra were obtained with a Shimadzu-260 spectrophotometer, whereas IR spectra employed a Nicolet 5-SXC spectrophotometer. NMR experiments were performed on Bruker AVANCE II 400 MHz and AMX 500 MHz instruments at 298 K. Multiplicity determinations (DEPT) and 2D spectra (COSY, HSQC, HMBC, and NOESY) were obtained using standard Bruker software. Chemical shifts are given in ppm ($\delta$) downfield from TMS internal standard.

HRESIQTOFMS were measured on Micro TOFQ II Bruker Daltonics (MA, USA). UPLC-ESI-QTOF/MS was performed using a system: UPLC equipment Agilent 1200 L Series equipped with a binary pump. Tandem ESI source (Bruker Daltonics, MA, USA) operated in positive mode 2 L/min nebulizer gas ($N_2$) and drying gas 6 L/min at 200 °C (nitrogen), needle voltage (4500) and shield voltage (600 V). Mass spectrometer Micro TOFQ II Bruker Daltonics (MA, USA) was operated in positive scan mode (from *m*/*z* 100 to *m*/*z* 1000) and calibrated using NaCOOH (20 mM). Software for data analysis was Hystar 3.2 (Bruker Daltonics, MA, USA).

Chromatographic separations were performed by vacuum-liquid chromatography (VLC), column chromatography (CC) on silica gel 60 (0.063–0.200 mm), radial chromatography with a radial Chromatotron Model 7924 T on silica gel 60 $PF_{254}$ Merck (1 mm thick), and preparative TLC on silica gel 60 $F_{254}$ (0.2 mm thick) plates. Preparative TLC separations were performed under the following conditions: (i) the amount of sample applied was approximately 15 mg for 20 cm plate; (ii) the bands were visualized using ultraviolet light; (iii) compounds were eluted from the silica using $CH_2Cl_2$:MeOH (8:2). HPLC separations were performed using a Thermo Separations Refractomonitor IV RI detector and a Thermo Separations SpectraSeries UV 100 UV detector, HPLC grade solvents and YMC RP-18 (5 μm, 20 mm × 250 mm) columns.

### 4.2. Plant material

The aerial parts of *S. argentinensis* Speg. plants were collected in the Department Guaraní, Misiones, Argentina, in December 2008. A voucher specimen was deposited at Museo Botánico Córdoba (CORD), Universidad Nacional de Córdoba, under G. Barboza et al. 2096. The species were identified by G.E. Barboza (IMBIV-CONICET, UNC).

### 4.3. Extraction and isolation of compounds from S. argentinensis

Air-dried powdered aerial parts of *S. argentinensis* (263 g) were exhaustively extracted with EtOH (5 × 1 L), with the solvent evaporated under reduced pressure. The resulting residue was suspended in $H_2O$ and extracted with $CH_2Cl_2$ (5 × 300 mL). The $CH_2Cl_2$ extract was dried (anhydr. $Na_2SO_4$) filtered, and evaporated to dryness under reduced pressure. The resulting residue (12.4 g) was fractionated initially by VLC. Elution with *n*-hexane–EtOAc mixtures of increasing polarity (100:0–0:100) and EtOAc–MeOH (100:0–95:05) afforded five fractions containing dihydro-β-agarofuran-sesquiterpenes (Fractions 11–15). These fractions (760 mg) were subjected to silica gel 60 G CC using, for elution, $CH_2Cl_2$:MeOH mixtures of increasing polarity (100:0–80:20). The fraction eluted with $CH_2Cl_2$:MeOH 97:3 (275 mg) was subjected to addition silica gel 60 G CC. Elution with $CH_2Cl_2$:MeOH (100:0–80:20) afforded three fractions. Fraction I (20 mg) was processed by preparative TLC with $CH_2Cl_2$/MeOH (98:02), yielding five fractions. Fraction 2 was further purified by reversed-phase TLC with MeOH: $H_2O$ (80:20) to obtain compounds **11** (1.33 mg) and **5** (1.68 mg). Fraction II (112 mg) was fractionated by radial chromatography using $CH_2Cl_2$:MeOH mixtures of increasing polarity to obtain compound **16** (29.3 mg). Impure fractions obtained by chromatographic processes from fractions I and II, together with fraction III, were pooled (99 mg) and subjected to reversed-phase HPLC using 10 mm × 250 mm column, MeOH:$H_2O$ (70:30) as eluant and a flow rate of 5 mL/min to yield thirty-eight fractions. Purification of these fractions was carried out by preparative TLC ($CH_2Cl_2$/MeOH, 95:5), yielding compounds (in order of previous HPLC elution): **15** (3.3 mg), **1** (2.6 mg), **3** (1.3 mg), **4** (1.2 mg), **13** (1.1 mg), **9** (2.2 mg), **2** (3.3 mg), in addition to the following mixtures which could not be obtained pure by either normal phase TLC or reversed phase TLC: compounds **8** and **5** in a 3:7 ratio (2.3 mg), compounds **10**, **5**, and **8** in a ratio 5:3.5:1.5 (2.5 mg), compound **7** with an uncharacterized isomer in a ratio 2:1 (1.8 mg), compound **6** with **12** in a ratio 4:6 (2.0 mg), and compound **14** with **16** in a ratio 7:3 (1.4 mg).

Mass spectra of compounds obtained as mixtures were measured using UPLC-ESI-QTOF/MS. Detection was carried out using a PDA detector scanning between 200 and 800 nm, monitoring at 254 nm. The column used was Eclipse XDB-C18 (i.d. 3.0 mm × 100 mm; 1.8 μm) and operated at 40 °C. Mobile phase and elution conditions were as follows: (A) 0.5% HCOOH in ultrapure $H_2O$; (B) 0.5% HCOOH in $CH_3CN$ (HPLC grade, Merck). Flow 0.3 mL/min; program: from 90% A ($t$ = 0 min) to 90% B ($t$ = 20 min); 90% A ($t$ = 21 min) and stabilization during 10 min before next injection. Injection volume was 40 μl ($CH_3CN$).

#### 4.3.1. (1S,4S,5S,6R,7R,8S,9S,10S)1,15-diacetoxy-9-benzoyloxy-4,6,8-trihydroxy-dihydro-β-agarofuran (**1**)

White amorphous powder; $[\alpha]_{328}^{21}$ −3.2 (c 0.19, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 275 (2.79) nm, 258 (2.78) nm; IR (dry film) $\nu_{max}$ 3442, 2933, 2853, 1735, 1447, 1369, 1277, 1228, 1109, 1040, 758, 712 $cm^{-1}$; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIQTOFMS *m*/*z* $[M+Na]^+$ 529.2041 (calcd for $C_{26}H_{34}O_{10}Na$, 529.2044).

*4.3.2. (1S,4S,5S,6R,7R,8S,9S,10S)15-acetoxy-9-benzoyloxy-1-cinnamoyloxy-4,6,8-trihydroxy-dihydro-β-agarofuran (**2**)*

White amorphous powder; $[\alpha]_{328}^{21}$ −11.2 (c 0.08, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 282 (3.27) nm, 249 (3.18) nm; IR (dry film) $\nu_{max}$ 3445, 2936, 2869, 1741, 1457, 1281, 1241, 1107, 1045, 843, 755, 709 $cm^{-1}$; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIQTOFMS *m/z* $[M+Na]^+$ 617.2363 (calcd for $C_{33}H_{38}O_{10}Na$, 617.2357).

*4.3.3. (1S,4S,5S,6R,7R,8S,9S,10S) 16-acetoxy-1,9-dibenzoyloxy-4,6,8-trihydroxy-dihydro-β-agarofuran (**3**)*

White amorphous powder; $[\alpha]_{328}^{21}$ −4.3 (c 0.10, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 278 (3.02) nm, 251 (3.07) nm; IR (dry film) $\nu_{max}$ 3451, 2927, 2854, 1735, 1451, 1276, 1239, 1112, 1045, 710 $cm^{-1}$; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIQTOFMS *m/z* $[M+Na]^+$ 591.2213 (calcd for $C_{31}H_{36}O_{10}Na$, 591.2201).

*4.3.4. (1S,4S,5S,6R,7R,8S,9S,10S)8,15-diacetoxy-1,9-dibenzoyloxy-4,6-dihydroxy-dihydro-β-agarofuran (**4**)*

White amorphous powder; $[\alpha]_{274}^{21}$ +9.1 (c 0.06, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 275 (3.22) nm, 242 (3.17) nm; IR (dry film) $\nu_{max}$ 3457, 2954, 2924, 2857, 1666, 1460, 1375, 1260, 844 $cm^{-1}$; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIQTOFMS *m/z* $[M+Na]^+$ 633.2302 (calcd for $C_{33}H_{38}O_{11}Na$, 633.2306).

*4.3.5. (1S,4S,5S,6R,7R,8S,9R,10S)1,6,8,15-tetra-acetoxy-9-benzoyloxy-4-hydroxy-dihydro-β-agarofuran (**5**)*

White amorphous powder; $[\alpha]_{328}^{21}$ −1.6 (c 0.14, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 275 (2.83) nm, 250 (2.92) nm; IR (dry film) $\nu_{max}$ 3487, 2930, 2847, 1747, 1457, 1367, 1280, 1233, 1094, 1046, 715 $cm^{-1}$; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIQTOFMS *m/z* $[M+Na]^+$ 613.2259 (calcd for $C_{30}H_{38}O_{12}Na$, 613.2255).

*4.3.6. (1S,4S,5S,6R,7R,8S,9R,10S)1,6-diacetoxy-9-benzoyloxy-4,8,15-trihydroxy-dihydro-β-agarofuran (**6**)*

White amorphous powder; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIQTOFMS *m/z* $[M+Na]^+$ 529.2075 (calcd for $C_{26}H_{34}O_{10}Na$, 529.2044).

*4.3.7. (1S,4S,5S,6R,7R,8S,9R,10S)6,15-diacetoxy-9-benzoyloxy-1-cinnamoyloxy-4,8-dihydroxy-dihydro-β-agarofuran (**7**)*

White amorphous powder; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIQTOFMS *m/z* $[M+H]^+$ 637.2702 (calcd for $C_{35}H_{41}O_{11}$, 637.2643).

*4.3.8. (1S,4S,5S,6R,7R,8R,9R,10S)1,6,8,15-tetra-acetoxy-9-benzoyloxy-4-hydroxy-dihydro-β-agarofuran (**8**)*

White amorphous powder; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 2 and 3; HRESIQTOFMS *m/z* $[M+H]^+$ 591.2500 (calcd for $C_{30}H_{39}O_{12}$, 591.2436).

*4.3.9. (1S,4S,5S,6R,7R,8R,9S,10S)6,16-dicetoxy-1,9-dibenzoyloxy-4,8-dihydroxy-dihydro-β-agarofuran (**9**)*

White amorphous powder; $[\alpha]_{328}^{21}$ −4.6 (c 0.13, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 274 (2.99) nm, 254 (3.01) nm; IR (dry film) $\nu_{max}$ 3451, 2939, 2869, 1723, 1447, 1368, 1274, 1106, 1065, 755, 709 $cm^{-1}$; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 2 and 3; HRESIQTOFMS *m/z* $[M+Na]^+$ 633.2289 (calcd for $C_{33}H_{38}O_{11}Na$, 633.2290).

*4.3.10. (1S,4S,5S,6R,7R,8R,9S,10S)1,6,8,15-tetra-acetoxy-9-benzoyloxy-4-hydroxy-dihydro-β-agarofuran (**10**)*

White amorphous powder; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 2 and 3; HRESIQTOFMS *m/z* $[M+H]^+$ 591.2482 (calcd for $C_{30}H_{39}O_{12}$, 591.2436).

*4.3.11. (1S,4S,5S,6R,7R,9S,10S)1,6,15-triacetoxy-9-benzoyloxy-4-hydroxy-8-oxo-dihydro-β-agarofuran (**11**)*

White amorphous powder; $[\alpha]_{328}^{21}$ +1.9 (c 0.09, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 275 (2.92) nm, 268 (2.92) nm, 247 (3.03) nm; IR (dry film) $\nu_{max}$ 3460, 2920, 2842, 1738, 1460, 1226, 762 $cm^{-1}$; for $^1H$ NMR and $^{13}C$ NMR spectroscopic data, see Tables 2 and 2; HRESIQTOFMS *m/z* $[M+Na]^+$ 569.2002 (calcd for $C_{28}H_{34}O_{11}Na$, 569.1993).

### 4.4. Biological assays

#### 4.4.1. Materials

All starting materials were commercially available research-grade chemicals and used without further purification. RPMI 1640 medium without Phenol Red and Fetal Bovine Serum (FBS) were purchased from Gibco, BRL, Invitrogen (Carlsbad, CA, USA), trichloroacetic acid (TCA) and glutamine from Merck (Darmstadt, Germany), and penicillin G, streptomycin, dimethyl sulfoxide (DMSO) and sulforhodamine B (SRB) from Sigma (St. Louis, MO, USA).

#### 4.4.2. Cell culture

Human breast cancer cell lines T-47D, MCF7 and MDA-MB231 (ATCC-Bethesda, MD, USA) were used in this study. Cells were maintained in 25 $cm^2$ culture flasks in RPMI 1640 without Phenol Red supplemented with 5% FBS, 2 mM L-glutamine and 1 mM sodium pyruvate at 37 °C and 5% $CO_2$ in a 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (100 U/ml penicillin G and 0.1 mg/ml Streptomycin). Single cell suspensions displaying > 97% viability by trypan blue dye exclusion were subsequently counted. After counting, cells were plated at appropriate cell densities onto 96-well microtiter plates. Cells were seeded in a volume of 100 μL per well at densities of 10,000 (MCF7), 15,000 (T-47D), and 20,000 (MDA-MB231) cells per well, based on their doubling times.

#### 4.4.3. Chemosensitivity testing

Chemosensitivity tests were performed using the SRB assay of the NCI with slight modifications (Skehan et al., 1990; Papazisis et al., 1997). Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, diluent control) in a final culture medium volume of 200 μL. Each agent was tested in sixtuplicate at different dilutions in the range 1–100 μM. The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which cells were precipitated with 50 μL ice-cold $Cl_3CO_2H$–$H_2O$ (1:1, v/v) carefully added to the culture medium surface and fixed for 60 min at 4 °C. Plates were washed five times with milli-Q water and air dried overnight at room temperature. The SRB assay was then performed. Briefly, 70 μL/well of a SRB solution [0.4%, w/v in a $Cl_3CO_2H$–$H_2O$ (1:99, v/v) solution] was added and incubated for 20 min at room temperature. Plates were washed 5 times with 200 μL/well of a $Cl_3CO_2H$–$H_2O$ (1:99, v/v) solution and air dried overnight at room temperature. SRB was then solubilized with 200 μL/well of Tris 10 mM and incubated on a shaker for 10 min at room temperature. The optical density (OD) of each well was measured at 492 nm using a microplate reader (Bio-Rad). Values were corrected for background OD from wells only containing medium. Drug activity was expressed as the relative inhibition activity calculated

with respect to untreated control cells (C) at each drug concentration level. Relative inhibition activity was calculated as follows: % growth inhibition = 100 − (corrected mean OD sample × 100/corrected mean OD diluent control). For $IC_{50}$ determinations, a dose–response curve between the compound concentrations and percent growth inhibition was plotted. $IC_{50}$ values were calculated using curve-fitting method with statistical analysis software. As positive controls, 5-fluorouracyl and camptothecin (Sigma–Aldrich, St. Louis, MO, USA) were included in the assays.

## Acknowledgements

This work has been funded by CONICET (Argentina), Ministerio de Ciencia y Tecnología de Córdoba (Argentina), SeCyT-UNC, Spanish MICINN (SAF 2009-13296-CO2-01) and ICIC (Instituto Canario de Investigacion del Cancer). M.E.G. thanks CONICET (Argentina) for the fellowship granted. NMR assistance by G. Bonetto is gratefully acknowledged.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2013.06.003.

## References

Bruning, R., Wagner, H., 1978. Übersicht Über Die Celastraceen-Inhaltsstoffe: Chemie, Chemotaxonomie, Biosynthese, Pharmakologie. Phytochemistry 17, 1821–1858.

Chang, F., Chen, I., Liao, S., Issa, H., Hayashi, K., Nozaki, H., Wu, Y., Lee, K., 2006. Three new agarofuran sesquiterpenes reissantins F-H from *Reissantia buchananii*. Planta Med. 72, 92–96.

Chávez, H., Callo, N., Estévez-Braun, A., Ravelo, A., González, A., 1999. Sesquiterpene polyol esters from the leaves of *Maytenus macrocarpa*. J. Nat. Prod. 62, 1576–1577.

Chen, J., Chou, T., Duh, C., Chen, I., 2006. Cytotoxic dihydroagarofuranoid sesquiterpenes from the stem of *Microtropis fokienensis*. J. Nat. Prod. 69, 685–688.

Gao, J., Wu, W., Zhang, J., Konishi, Y., 2007. The dihydro-β-agarofuran sesquiterpenoids. Nat. Prod. Rep. 24, 1153–1189.

González, A.G., Jiménez, I.A., Ravelo, A.G., Bazzocchi, I.L., 1989a. β-Agarofuran sesquiterpenes from *Maytenus canariensis*. Phytochemistry 28, 173–175.

González, A.G., Mendoza, J.J., Ravelo, A.G., Luis, J.G., Jiménez, I.A., Vázquez, J.T., Domínguez, X.A., Chang, M., 1989b. Nuevos sesquiterpenos de la *Schaefferia cuneifolia*. Rev. Latinoamer. Quím. 20, 91–94.

González, A.G., Mendoza, J.J., Ravelo, A.G., Luis, J.G., Domínguez, X.A., 1991. Nuevo triterpeno pentacíclico de *Schaefferia cuneifolia* (Celastraceae). Rev. Latinoamer. Quím. 22, 3–5.

González, A.G., Jiménez, I.A., Ravelo, A.G., Sazatornil, J.G., Bazzocchi, I.L., 1993. New sesquiterpenes with antifeedant activity from *Maytenus canariensis* (Celastraceae). Tetrahedron 49, 697–702.

Muñoz, O., Peñaloza, A., González, A.G., Ravelo, A.G., Bazzochi, I.L., Alvarenga, N.L., 1996. The Celastraceae from Latin America. Chemistry and biological activity. Stud. Nat. Prod. Chem. 18, 739–783.

Papazisis, K.T., Geromichalos, G.D., Dimitriadis, K.A., Kortsaris, A.H., 1997. Optimization of the sulforhodamine B colorimetric assay. J. Immunol. Methods 208, 151–158.

Perestelo, N.R., Sánchez-Cañete, M.P., Gamarro, F., Jiménez, I.A., Castanys, S., Bazzocchi, I.L., 2011. Overcoming human P-glycoprotein-dependent multidrug resistance with novel dihydro-β-agarofuran sesquiterpenes. Eur. J. Med. Chem. 46, 4915–4923.

Simmons, M.P., 2004. Celastraceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants, vol. 6. Springer, Berlin, pp. 29–64.

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.

Spivey, A.C., Weston, M., Woodhead, S., 2002. Celastraceae sesquiterpenoids: biological activity and synthesis. Chem. Soc. Rev. 31, 43–59.

Wang, X., Gao, W., Yao, Z., Zhang, S., Zhang, Y., Takaishi, Y., Duan, H., 2005. Immunosuppressive sesquiterpenes from *Tripterygium wilfordii*. Chem. Pharm. Bull. 63, 607–610.

Yoshihisa, T., Kunie, U., Kiyoshi, K., Masayuki, S., Toshiaki, T., 1987. Polyhydroxyagarofuran derivatives from *Tripterygium wilfordii* H.. Phytochemistry 26, 2581–2584.

Zhu, Y., Miao, Z., Ding, J., Zhao, W., 2008. Cytotoxic dihydroagarofuranoid sesquiterpenes from the seeds of *Celastrus orbiculatus*. J. Nat. Prod. 71, 1005–1010.