Tetrahedron 64 (2008) 10821–10824

Contents lists available at ScienceDirect

Tetrahedron

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

Gomerones A–C, halogenated sesquiterpenoids with a novel carbon skeleton from *Laurencia majuscula*

Ana R. Díaz-Marrero, Inmaculada Brito, José M. de la Rosa, José Darias, Mercedes Cueto *

Instituto de Productos Naturales y Agrobiología del CSIC, Avenida Astrofísico F. Sánchez 3, 38206 La Laguna, Tenerife, Spain

ARTICLE INFO

Article history:
Received 8 August 2008
Received in revised form 4 September 2008
Accepted 5 September 2008
Available online 18 September 2008

Keywords:
Laurencia
Chamigrene
Gomerane

ABSTRACT

The structures and relative sterochemistry of three halogenated sesquiterpenoids gomerones A–C, **1**–**3**, isolated from *Laurencia majuscula* are described. They are representative of a novel carbon skeleton, gomerane, biogenetically derived from cyclization of a β-chamigrene precursor. A biogenetic relationship with related sesquiterpenoids from lower terrestrial plants Hepaticae of genera *Bazzania*, *Mannia*, *Mylia*, and *Rebouli* as well as higher plants of genera *Cupressus* and *Juniperus* has been established.

1. Introduction

Sesquiterpene cyclases are among nature's most versatile catalysts. These enzymes are responsible for the formation of more than 15 distinct sesquiterpene carbon skeletons in *Laurencia* species.[1] Among them, chamigrene appears to be the most common with over 110 naturally occurring metabolites. Interestingly, they are all derivatives of α- and β-chamigrene in a ratio close to unity.[2]

In this work we describe compounds **1**–**3** isolated from *Laurencia majuscula* (Ceramiales, Rhodomelaceae), a red alga commonly found at the southern coast of La Gomera. These compounds are representative of gomerane, a novel carbon skeleton formed by intramolecular cyclization of a β-chamigrene backbone. The name of this structure alludes to the location where the alga was collected.

Seaweeds of genus *Laurencia*, lower terrestrial plants Hepaticae (Liverworts) of genera *Bazzania*, *Mannia*, *Mylia*, and *Rebouli* as well as higher plants Cupressaceae of genera *Cupressus* and *Juniperus* have in common the exclusive ability to biosynthesize polycyclic sesquiterpene networks by internal cyclization of a chamigrene backbone. To date, marine sources have provided rhodolaurane[3] and aplydactane[4] in addition to the gomerane framework, Figure 1.

Terrestrial environments have yielded myltaylane,[5] cyclomyltaylane[6] (Liverworts),[7] as well as junicedrane,[8] and chamipinane[9] skeletons (Cupressaceae). It is interesting to highlight that all three marine skeletons, grouped as Class I, appear to evolve exclusively from cyclization of the exocyclic methylene carbon of a β-chamigrene framework and never from an α-chamigrene, despite both α- and β-chamigrene derivatives occur in *Laurencia*. On the other hand, Class II skeletons from a terrestrial environment, Figure 1, are likely to come from a chamigrenyl cation,[10] and therefore the central isoprenic methyl group remains intact.

2. Results and discussion

Vacuum flash chromatography of the acetone extract of *L. majuscula* gave two fractions from which compounds **1**–**3** were obtained by standard chromatographic procedures involving gel filtration, Si gel chromatography and HPLC.

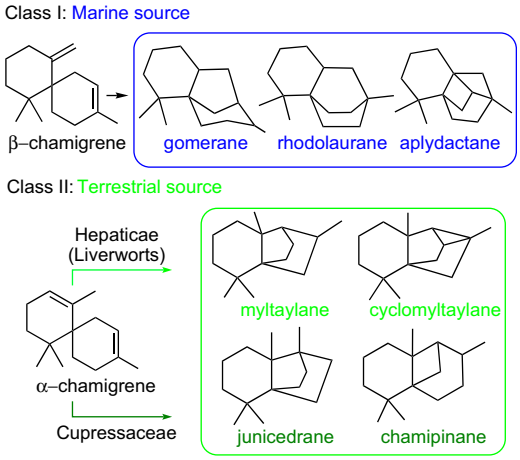


Figure 1. Class I and Class II of marine and terrestrial skeletons.

* Corresponding author. Tel.: +34 922252144; fax: +34 922260135.
E-mail address: mcueto@ipna.csic.es (M. Cueto).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.09.027

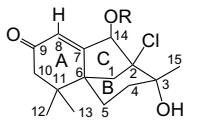
Table 1
NMR Data of compounds **1**–**3** [500 MHz, d ppm, (*J*) Hz, $CDCl_3$ or C_6D_6]

#	Compound **1**[b]			Compound **2**			Compound **3**	
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC	δ_H	δ_C
1	2.25 dd (*1.9*, *11.0*), 1.99 dd (*2.8*, *11.0*)	39.0	C-2, C-3, C-5, C-7, C-14	2.96 d (*12.4*), 2.18 dd (*3.1*, *12.4*)	43.9	C-2, C-3, C-5, C-6, C-7, C-14	2.95 d (*12.3*), 2.14 dd (*3.0*, *12.3*)	42.0
2	—	72.9		—	79.9[a]		—	79.4[a]
3	—	85.6		—	70.8[a]		—	73.6[a]
4	0.74 m, 1.45 ddd (*1.6*, *5.1*, *14.5*)	34.5	C-2, C-3	2.46 ddd (*1.8*, *4.9*, *15.9*), 1.72 ddd (*4.9*, *12.8*, *15.9*)	40.2	C-2, C-3, C-6	2.26 m 1.70 ddd (*5.4*, *12.8*, *12.8*)	39.0
5	1.56 ddd (*5.0*, *12.3*, *13.6*), 1.04 m	28.9	C-1, C-4, C-11	2.31 ddd (*4.9*, *13.3*, *13.3*), 1.98 dddd (*2.4*, *2.4*, *4.9*, *13.3*)	29.9		2.32 m 1.97 m	29.4
6	—	51.4		—	48.0		—	47.9
7	—	168.2		—	155.0		—	154.3
8	6.20 s	125.3	C-6, C-10, C-14	6.57 br s	125.0	C-6, C-14	6.57 s	125.1
9	—	197.4		—	204.6		—	199.8
10	1.93 d (*17.3*), 2.21 d (*17.3*)	48.7	C-6, C-8, C-9, C-11, C-12	2.21 d (*16.8*), 2.67 d (*16.8*)	48.9	C-9, C-11, C-13	2.21 d (*17.4*), 2.67 d (*17.4*)	49.0
11	—	37.5		—	38.4		—	38.4
12	0.55 s	24.8	C-6, C-10, C-11, C-13	1.14 s	24.3	C-6, C-10, C-11, C-13	1.13 s	24.3
13	0.74 s	22.9	C-6, C-10, C-11, C-12	0.99 s	24.0	C-6, C-10, C-11, C-12	0.99 s	23.9
14	3.69 s	73.8	C-8, C-6, C-1, C-2	—	196.3		—	198.2
15	0.96 s	25.6	C-2, C-3, C-4	1.90 s	29.7	C-2, C-3, C-4	1.70 s	27.8
OH_3	1.45 br s							
OH_{14}	1.83 d (*2.5*)							

[a] Interchangeable.
[b] NMR data recorded in C_6D_6.

Gomerone A (**1**) was a colorless oil. Its EIMS spectrum showed peaks at *m*/*z* 284/286, with relative intensities suggestive of a chlorine atom, which correspond to the empirical formula $C_{15}H_{21}O_3Cl$ $[M]^+$ (HREIMS) requiring five sites of unsaturation. Absorptions for an α,β-unsaturated carbonyl and hydroxyl groups at 1658 and 3433 cm^{-1}, respectively, were observed in the IR spectrum. Its NMR experiments acquired in C_6D_6 provided better resolution than in $CDCl_3$, avoiding overlapped signals. The ^{13}C NMR spectrum of **1**, Table 1, showed signals for 15 carbons whose multiplicities were determined from the DEPT spectral data: three methyl groups, four methylenes, two methines (one bearing a heteroatom and one olefinic), and six quaternary carbons (two bearing a heteroatom, one olefinic, and one ketone).

The ^1H NMR spectrum, Table 1, showed signals for only one olefinic proton at δ 6.20 indicative of the presence of a trisubstituted enone system. Therefore, the remaining three sites of unsaturation indicated that the molecule must be tricyclic. One proton geminal to a heteroatom at δ 3.69, eight methylene protons between δ 2.25 and 0.74, and upfield signals for three methyl groups at δ 0.55, 0.74, and 0.96 account for the remaining protons bonded to carbons. The presence of a quaternary carbon at δ 51.4, characteristic of a spiro-carbon,[2] suggested that this compound could be a chamigrene derivative and the absence of an additional methyl group expected for a sesquiterpene skeleton indicated that it has to be involved in the formation of the third cycle of the chamigrene-like skeleton. Formation of the monoacetylated derivative **1a** proved the presence of a secondary alcohol in **1**.



1: R = H
1a: R = COMe

A ^1H–^1H-COSY experiment established the presence of a single discrete spin system H_2-4–H_2-5. In the HMBC experiment, the mutual correlations of H_3-12/C-13, H_3-13/C-12 and their long-range correlations with an isolated C-10 methylene and with the quaternary carbons C-6 and C-11 secured the *gem*-dimethyl group. The correlations of H-14 with C-6 and C-8 and H-8 with C-10 established ring A whereas the H_3-15/C-2, C-3, C-4 correlations placed Me-15 at C-3 and the H_2-5/C-11 and H_2-1/C-2, C-3, C-5, C-7 correlations established ring B and the connection of both rings through the spiro-carbon C-6. The third ring of the molecule was confirmed by the long-range correlation of H-14 with C-1 and C-2 configuring the planar structure of **1**. Since the ^1H NMR singlet at δ 0.96 corresponds to a methyl group on a carbon bearing oxygen, the regiochemistry of the heteroatoms was established as depicted in **1**.

Gomerone B (**2**) and gomerone C (**3**) were colorless. Their EIMS spectra showed the same molecular ion at *m*/*z* 300/302/304 $[M]^+$ with relative intensities indicative of the presence of two chlorine atoms in the empirical formula $C_{15}H_{18}O_2Cl_2$ (HREIMS) and six sites of unsaturation for each molecule. Absorptions for α,β-unsaturated carbonyl groups were observed in their IR spectra. ^{13}C NMR data corresponding to **2** and **3**, Table 1, indicated the presence of 15 carbon atoms whose multiplicities were determined from the DEPT experiments: three methyl groups, four methylenes, only one methine (olefinic), and seven quaternary carbons (two bearing a heteroatom, one olefinic, and two ketones). Since the two oxygens given by the molecular formula of each product are ketones both molecules must be tricyclic.

Comparison of the NMR data of **2** and **3** with those of compound **1** (Table 1) showed the absence of any signal for a proton geminal to oxygen in the ^1H NMR spectra of **2** and **3** that suggested that the secondary hydroxyl group in **1** (C-14) was oxidized to a ketone in compounds **2** and **3**. ^{13}C chemical shifts of the carbonyl carbons and the presence of a single vinyl proton suggested that these ketones are involved in a dienone system. Hence, their planar structures are as depicted in **2** and **3**. These structures were corroborated by HMBC experiments. Structurally, compounds **2** and **3** contain six contiguous quaternary carbons of different nature: two sp^3 substituted by carbons, two sp^2 and two

sp^3 asymmetric stereocenters that render them challenging synthetic targets.

2.1. Relative stereochemistry

The relative stereochemistry of compounds **1** and **2** was determined on the basis of NOESY experiments. The configuration of C-3, C-6, and C-14 of compound **1** was deduced by the strong NOE effect observed between H-14 and H_3-15 that placed Me-15 in an equatorial disposition and in a *syn* relationship with respect to H-14 thus establishing, unambiguously, the relative configuration around the spiro-carbon. The downfield chemical shift of H_3-15 of compound **2** compared with that of **3** ($\Delta\delta$=0.20 ppm) was attributed to the deshielding effect caused by the carbonyl group of the cyclopentanone. This implies that in compound **2** Me-15 is located in a β-configuration, which was corroborated by the NOE effect observed between Me-15 and H-4α and H-4β. Although NOE experiments do not allow to establish the configuration of C-15 in compound **3**, MS data and comparison of the chemical shifts in the ^{1}H and ^{13}C NMR spectra of compounds **2** and **3** indicate that **3** represents the epimer at C-3 of compound **2**. The stereochemistry of compounds **1**–**3** is represented by the minimized structures in Figure 2.[11] We were unsuccessful either in preparing Mosher esters of **1** or in obtaining crystals for X-ray crystallography to establish the absolute configuration of compounds **1**–**3**.

2.2. Biogenetic pathway

The mechanism by which some sesquiterpene synthases convert the universal acyclic precursor farnesyl diphosphate is well known.[12] Initial ionization followed by rearrangement to a (3*R*)-nerolidyl diphosphate in the preferred *anti-endo* conformation allows cyclization to the (*R*)-bisabolyl cation **4**, the precursor of most of sesquiterpenes, Scheme 1.

Biogenesis of **1**–**3**, Scheme 1, may arise from **4** via path a. This pathway involves haloperoxidase[13] mediated chlorobromination of the endocyclic olefin; C-11–C-7 cyclization of **5** followed by a 1,4-hydride shift[12] to give a dihalocuprenyl carbocation that could lead to **6** after C-11 to C-6 bond migration and loss of hydrogen bromide. Deprotonation of **6** at C-14 gives 2-chloro-β-chamigrene **7**. This neutral intermediate, **7**, is not released from the enzyme active site but could be immediately activated at C-3 allowing ring closure from C-14 to C-2 to provide the gomerane backbone **8** (path b, Box 1) from which compounds **1**–**3** are derived. Although a vicinal dichlorination at the endocyclic double bond of **4** should not be excluded it seems unlikely since neither naturally occurring halogenated bisabolene nor chamigrene derivatives have been found to contain this pattern of halogenation. Thus, we suggest that the chlorine atom at C-2 of **7** is originated as shown in sequence **4**→**6**. Compound **7** could also be protonated at C-2 after ring closure from C-14 to C-3 (path c) to give the

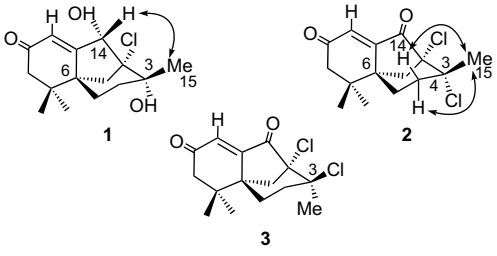
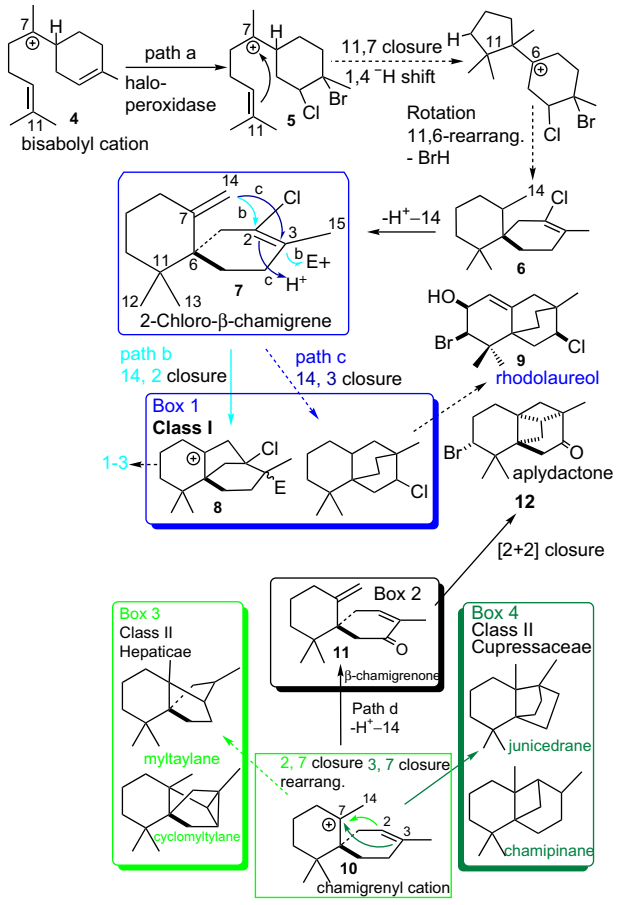


Figure 2. Gomerones A–C (**1**–**3**) and selected NOEs for **1** and **2**.



Scheme 1. Biogenesis of marine skeletons (Class I) and terrestrial skeletons (Class II).

rhodolaurane skeleton (Box 1) of rhodolaureol **9**.[3] The tetracyclic core of aplydactone **12**[4] appears to be generated by a different mechanism involving an intramolecular [2+2] cycloaddition (Box 2, Scheme 1, path d) of a conjugated enone **11** obtained from oxidation of a β-chamigrene originated by deprotonation of a chamigrenyl cation **10** (path d).

Although skeletal diversity from both marine and terrestrial environments derives from a common chamigrene backbone it seems that their origin follow similar chemical cyclization but acting in opposite directions, as shown by the arrows in **7** and **10**. The flow of electrons as represented in **7** favors the formation of a tertiary carbocation at C-7.

The ability of marine *Laurencia* and the terrestrial genera aforesaid to lead to specific skeletons from a common chamigrene backbone makes these genera interesting to explore and to compare at both genetic and molecular level the type of binding sites for specific protein–substrate interaction domain.[14]

The overall chemical features of secondary metabolites isolated from *Laurencia*, Hepaticae and Cupressaceae are very similar. It is difficult to discuss the evolution of lower terrestrial plants, especially because of the absence of fossil material of bryophytes.[7] Thus, our research comparing the chemical constituents of algae, bryophytes, and higher plants as well as their biochemical interrelationships may provide a platform to gain insight into the evolutionary correlation between these three plant groups. With the increasing availability of genomic sequences, it may be possible to resolve questions about their evolution by examining closely biosynthetic pathways and genes in related and even within phylo genetically distant species.

3. Experimental

3.1. General procedures

Optical rotations were measured on a Perkin–Elmer model 343 Plus polarimeter using a Na lamp at 25 °C. IR spectra were obtained with a Perkin–Elmer 1650/FTIR spectrometer. ^{1}H NMR and ^{13}C NMR, HSQC, HMBC, and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Two-dimensional NMR spectra were obtained with the standard Bruker software. EIMS and HRMS data were taken on a Micromass Autospec spectrometer. HPLC separations were performed with a Hewlett Packard 1050 (Jaigel-Sil semipreparative column, 10 μ, 20×250 mm) with hexane/EtOAc mixtures. The gel filtration column (Sephadex LH-20) used hexane/MeOH/CH_2Cl_2 (3:1:1) as solvent. The spray reagent for TLC was H_2SO_4/H_2O/AcOH (1:4:20).

3.2. Biological material

L. majuscula was collected by SCUBA diving off La Gomera (Canary Islands) at a depth of −1 to 3 m. A voucher specimen has been deposited at the Department of Marine Biology, Universidad de La Laguna, Tenerife, Canary Islands, Spain (deposit number: LAU006-111).

3.3. Extraction and isolation

Dry samples (2.0 kg) were extracted with acetone at room temperature, and were concentrated to give a dark residue (89.8 g). The extract was chromatographed by flash chromatography on silica gel. The fraction eluted with hexane/EtOAc (8:2) (8.7 mg) was further separated by gel filtration and HPLC to give compounds **2** (3.3 mg) and **3** (5.7 mg). The fraction eluted with hexane/EtOAc (1:1) (2.3 g) was further separated by gel filtration and HPLC to give compound **1** (2.4 mg).

3.3.1. Compound *1*

White powder; $[\alpha]_D^{20}$ −73 (*c* 0.30, $CHCl_3$); IR ν_{max} (film) 3433, 2965, 1658 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; EIMS *m*/*z* 284/286 $[M]^+$ (15, 5), 214/216 (48, 12), 71 (100); EIHRMS $[M]^+$ 286.1162 (calcd for $C_{15}H_{21}O_3^{37}Cl$, 286.1150); 284.1181 (calcd for $C_{15}H_{21}O_3^{35}Cl$, 284.1179).

3.3.2. Compound *2*

White powder; $[\alpha]_D^{20}$ +49 (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max} 245 nm (ε 7.800); IR ν_{max} (film) 2968, 1742, 1682 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; EIMS *m*/*z* 300/302/304 $[M]^+$ (14, 9, <1), 265/267 $[M-Cl]^+$ (100, 32); EIHRMS $[M]^+$ 302.0653 (calcd for $C_{15}H_{18}O_2^{35}Cl^{37}Cl$, 302.0653), 300.0692 (calcd for $C_{15}H_{18}O_2^{35}Cl_2$, 300.0684), 267.0962 $[M-Cl]^+$ (calcd for $C_{15}H_{18}O_2^{37}Cl$, 267.0966), 265.0991 $[M-Cl]^+$ (calcd for $C_{15}H_{18}O_2^{35}Cl$, 265.0995).

3.3.3. Compound *3*

White powder; $[\alpha]_D^{20}$ +121 (*c* 0.17, $CHCl_3$); IR ν_{max} (film) 2966, 1746, 1687 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; EIMS *m*/*z* 300/302/304 $[M]^+$ (10, 6, <1), 265/267 $[M-Cl]^+$ (100, 34); EIHRMS $[M]^+$ 302.0659 (calcd for $C_{15}H_{18}O_2^{35}Cl^{37}Cl$, 302.0654), 300.0697 (calcd for $C_{15}H_{18}O_2^{35}Cl_2$, 300.0684), 267.0963 $[M-Cl]^+$ (calcd for $C_{15}H_{18}O_2^{37}Cl$, 267.0966), 265.0992 $[M-Cl]^+$ (calcd for $C_{15}H_{18}O_2^{35}Cl$, 265.0995).

3.3.4. Acetylation of *1*

A solution of **1** (1.4 mg) in C_5H_5N (0.3 mL) was treated with Ac_2O (0.5 mL) and stirred at room temperature for 24 h. The reaction was quenched with H_2O and the mixture was extracted twice with EtOAc. The organic layer was washed with H_2O and 5% aqueous HCl, dried (Na_2SO_4), concentrated to give **1a** (1.1 mg).

Acknowledgements

This work was supported by the Ministerio de Educación y Ciencia (BIO2007-61745, SAF2006-03004) and DGUI Gobierno de Canarias (PIO42005, PUB2005/030). We are grateful to A. Corrales for her technical support and to Prof. J. Afonso-Carrillo (University of La Laguna) for the taxonomic classification of the alga.

Supplementary data

Supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.tet.2008.09.027.

References and notes

1. Martín, J. D.; Darias, J. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic: New York, NY, 1978; Vol. 1, pp 125–174.
2. Dorta, E.; Díaz-Marrero, A. R.; Cueto, M.; D'Croz, L.; Maté, J. L.; Darias, J. *Tetrahedron Lett.* **2004**, *45*, 7065–7068.
3. González, A. G.; Martín, J. D.; Martín, V. S.; Pérez, R.; Tagle, B.; Clardy, J. *J. Chem. Soc., Chem. Commun.* **1985**, 260–261.
4. Fedorov, S. N.; Radchenko, O. S.; Shubina, L. K.; Kalinovsky, A. I.; Gerasimenko, A. V.; Popov, D. Y.; Stonik, V. A. *J. Am. Chem. Soc.* **2001**, *123*, 504–505.
5. Takaoka, D.; Matsuo, A.; Kuramoto, J.; Nakayama, M.; Hayashi, S. J. *J. Chem. Soc., Chem. Commum.* **1985**, 482–483.
6. Takaoka, D.; Tani, H.; Matsuo, A. *J. Chem. Res., Synop.* **1988**, 130–131.
7. Asakawa, Y. Chemical constituents of the Bryophytes. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer: Vienna, New York, NY, 1995; Vol. 65, pp 509–520.
8. Barrero, A. F.; Álvarez-Manzaneda, E.; Lara, A. *Tetrahedron Lett.* **1995**, *36*, 6347–6350.
9. Cool, L. G. *Phytochemistry* **2005**, *66*, 249–260.
10. Srikrishna, A.; Yelamaggad, C. V.; Kumar, P. P. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2877–2881.
11. *PCModel (v. 7.0)*; Serena Software: Bloomington, IN, 1999.
12. (a) Cane, D. E. *Chem. Rev.* **1990**, *90*, 1089–1103; (b) Cane, D. E. *Acc. Chem. Res.* **1985**, *18*, 220–226.
13. Butler, A.; Carter-Franklin, J. N. *Nat. Prod. Rep.* **2004**, *21*, 180–188.
14. Wu, S.; Schoenbeck, M. A.; Greenhagen, B. T.; Takahashi, S.; Lee, S.; Coates, R. M.; Chappell, J. *Plant Physiol.* **2005**, *138*, 1322–1333.