New Sesquiterpene Derivatives from the Red Alga *Laurencia scoparia*. Isolation, Structure Determination, and Anthelmintic Activity[1]

Danilo Davyt,[†] Rafael Fernandez,[†] Leopoldo Suescun,[‡,▽] Alvaro W. Mombrú,[‡] Jenny Saldaña,[§] Laura Domínguez,[§,#] Javier Coll,[⊥] Mutue T. Fujii,[∥,⊗] and Eduardo Manta*[,†]

Facultad de Química, Universidad de la República, Avenida Gral. Flores 2124, Montevideo, Uruguay, and Instituto de Botânica, Avenida Miguel Estéfano 3687, 04301-902 São Paulo, Brazil

Received May 7, 2001

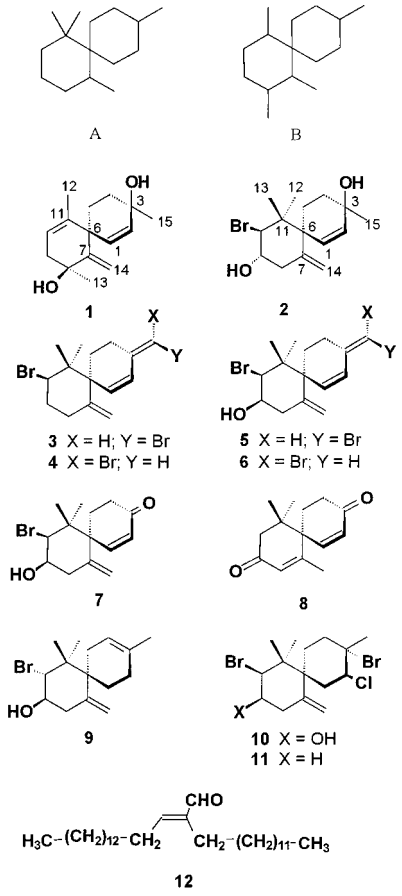
Eleven sesquiterpenes (**1**−**11**) and one long chain aldehyde (**12**) have been isolated from the dichloromethane extract of the red alga *Laurencia scoparia*. Four of them are new natural products. Scopariol (**1**) is a new natural product with an unusual rearranged chamigrane-type structure. The other three are *β*-chamigrenes: isorigidol (**2**), (+)-3-(*Z*)-bromomethylidene-10*β*-bromo-*β*-chamigrene (**3**), and (−)-3-(*E*)-bromomethylidene-10*β*-bromo-*β*-chamigrene (**4**). The in vitro activity of compounds **1**−**12** against the parasitant stage of *Nippostrongylus brasiliensis* (L4) has been studied.

In our search for new anthelmintic compounds from South American marine species[2,3] we included some red seaweeds of the genus *Laurencia* Lamouroux from the Brazilian coasts. *Laurencia* is the most prolific source of secondary metabolites from algae.[4] These metabolites have biological activity reported,[5,6] but there have been no previous reports on anthelmintic activity. At the same time, these chemical studies of metabolites from *Laurencia* are intended to contribute to the taxonomic investigation of Brazilian species of this complex genus.[7]

Most of the metabolites obtained from CH_2Cl_2 extracts of *Laurencia scoparia* were chamigrane-type sesquiterpenoids with common skeleton system A. However compound **1** is a new natural product with a nonhalogenated rearranged chamigrane-type sesquiterpenoid with the skeleton system of B. This unusual type of compound was only isolated from sea hare of the genus *Aplysia*.[8,9] Also a halogenated compound with skeleton system B was isolated from *Laurencia* sp.[10]

Three other new natural products (**2**−**4**) were also isolated. All of them were *β*-chamigrane-type sesquiterpenes. Compound **2** was an isomer of rigidol[11] with the unusual vicinal bromine-hydroxyl diequatorial substitution of ring A. To the best of our knowledge this is the second report of the isolation of a *β*-chamigrane compound with this substitution. Its structure was proposed by spectroscopic methods. This structure was confirmed, and the absolute configuration was also determined by X-ray crystallography.

The anthelmintic activities of compounds **1**−**12** using *Nippostrongylus brasiliensis* in vitro model have been evaluated.



Four new and seven previously reported sesquiterpenes and one long-chain aldehyde were isolated and characterized from *L. scoparia* CH_2Cl_2 extracts.

Compound **1** was obtained as a white powder. The molecular formula $C_{15}H_{22}O_2$ was established by HREIMS and NMR spectroscopy (see Tables 1 and 2). NMR spectroscopy showed that the structure of **1** contained six sp^2-hybridized carbon atoms corresponding with one *exo*-cyclic double bond [δ_C 154.6 (s), 110.2 (t), δ_H 5.46 (d), 4.86 (d)]

* To whom correspondence should be addressed. Tel: (598 2) 924 18 05. Fax: (598 2) 924 19 06. E-mail: emanta@bilbo.edu.uy.
▽ To whom correspondence regarding X-ray structure should be addressed.
To whom correspondence regarding biological assays should be addressed.
⊗ To whom correspondence regarding plant material should be addressed.
† Cátedra de Química Farmacéutica, Universidad de la República.
‡ Cátedra de Cristalografía, Universidad de la República.
§ Cátedra de Farmacología, Universidad de la República.
⊥ Cátedra de Botánica, Universidad de la República.
∥ Instituto de Botânica, Seção de Ficologia.

10.1021/np0102307 CCC: $20.00
Published on Web 11/10/2001

Table 1. ^{13}C NMR (100 MHz, $CDCl_3$) Spectral Data for Compounds **1**−**4**[a]

C no.	**1**	HMBC	**2**	HMBC	**3**	HMBC	**4**
1	136.5	5′	132.3		137.3		133.7
2	133.7	15	135.8	15	127.1	15	129.3
3	67.0	1, 15	66.8	1, 15	137.8	1, 15	140.1
4	33.2	2, 15	33.6	2, 15	26.5		24.7
5	25.7	1	22.7	1	27.8		25.8
6	48.3	2, 5′, 14, 14′	50.9	2, 8, 12, 13, 14, 14′	52.4	2, 12, 13, 14, 14′	52.3
7	154.6	5, 9, 13	144.8	8, 8′	147.0		146.8
8	72.5	9, 13, 14, 14′	38.8	14, 14′	32.8	14, 14′	32.9
9	42.6	13	71.4	8, 8′, 10	35.2		34.8
10	121.9	9, 12	74.9	8, 12, 13	64.1	12, 13	64.2
11	137.9	5, 9, 12	42.3	12, 13	42.7	12, 13	42.6
12	20.1	9?	19.1	13	18.5		18.5
13	29.5		26.0	12	26.3		26.2
14	110.2		115.7	8	115.7	8	115.3
15	30.3	1	29.6		101.6		105.6

[a] ^{13}C NMR assignments supported by HMQC and DEPT experiments.

Table 2. ^1H NMR (400 MHz, $CDCl_3$) Spectral Data for Compounds **1**−**4**[a]

H no.	**1**	**2**	**3**	**4**
1	5.25 (dd, 10.0,1.4)	5.69 (dd, 10.3, 1.9)	6.00 (d, 10.4)	5.81 (d, 10.3)
2	5.83 (dd, 10.0, 1.0)	5.84 (dd, 10.3, 1.6)	6.65 (d, 10.4)	6.22 (d, 10.3)
4	1.6−1.7 (m)	1.54 (ddd, 13.6, 13.5, 3.0)	1.98 (dm, 13)	2.65 (bd, 15.6)
		1.66 (m)	1.75 (td, 13, 3.6)	1.96 (bd, 15.6)
5	2.08 (m)	1.98 (ddd, 13.5, 13.4, 3.0)	2.15−2.3 (m)	2.05 (bd, 14)
	1.67 (m)	1.78 (br d, 13.4)		1.73 (ddd, 14, 13, 3)
8		2.41 (ddt, 14.0, 11.0, 2.0)	2.45 (tm, 14.2)	2.44 (btd, 14, 6)
		2.69 (dd, 14.0, 6.2)	2.15−2.3 (m)	2.2−2.3 (m)
9	2.28 (ddd, 16.3, 6.0, 1.2)	3.86 (ddd, 11.0, 10.5, 6.2)	2.15−2.3 (m)	2.2−2.3 (m)
	2.18 (bd, 16.3)			
10	5.51 (dq, 6.0, 1.2)	4.47 (d, 10.5)	4.57 (dd, 12.6, 4.6)	4.61 (dd, 12.7, 4.5)
12	1.73 (3H, dd, 1.2, 1.2)	1.09 (3H, s)	1.16 (3H, s)	1.15(3H, s)
13	1.45 (3H, s)	1.12 (3H, s)	1.08 (3H, s)	1.08 (3H, s)
14	5.46 (d, 1.3)	5.06 (t, 2.0)	5.01 (s)	4.98 (s)
	4.86 (d, 1.3)	4.74 (t, 2.0)	4.7 (s)	4.68 (s)
15	1.34 (3H, s)	1.24 (3H, s)	5.9 (s)	6.11 (bs)

[a] Multiplicity and coupling constants in Hz are in parentheses.

and two *endo*-cyclic double bonds [δ_C 136.5 (d), 133.7 (d), 121.9 (d), 137.9 (s)]; two tertiary hydroxyl functions [δ_C (72.5 (s), 67.0(s)] that were confirmed by IR band at 3350 cm^{-1}; and, finally, one olefinic [δ_H 1.73 (dd)] and two hydroxy vicinal methyl groups [δ_H 1.45 (s), 1.34 (s)]. All remaining carbon atoms were sp^3 hybridized, indicating that the structure of **1** had to be bicyclic. The presence of a signal at δ_C 48.3 (s) consistent with a spiro-atom confirms that **1** is closely related to a chamigrane sesquiterpene.

2D NMR COSY spectra of the sesquiterpenic diol **1** showed cross-peaks between the signal at δ_H 5.51 (dq) and methylene proton signals at δ_H 2.28 (ddd), 2.18 (bd), and between this last proton signal and methyl proton signals at δ_H 1.45, corresponding to a molecular fragment of ring A, $-COH(CH_3)-CH_2-CH{=}C(CH_3)-$. The two other olefinic proton signals at δ_H 5.23 (dd) and 5.83 (dd) were strongly coupled to each other, showing also a long-range coupling with the methylene proton signal at δ_H 1.67 (m). This methylene proton signal was coupled with three other proton signals [2.08 (m), 1,6−1,7 (m)] forming a $-CH_2CH_2-$ system. These data were in agreement with a substitution of hydroxyl and methyl in position 3 of ring B.

The correlations showed by the HMBC spectrum indicated the rearranged chamigrane-type structure of **1** (see Figure 1 and Table 1). Correlations between δ_C 154.6 and δ_H 1.45; δ_C 72.5 and δ_H 5.46, 4.86; and δ_C 42.6 and δ_H 1.45 were important to confirm the position of the hydroxyl group in ring A, and correlations between δ_C 133.7, 67.0, 33.2 and δ_H 1.45 allowed us to confirm the hydroxyl position in the ring B.

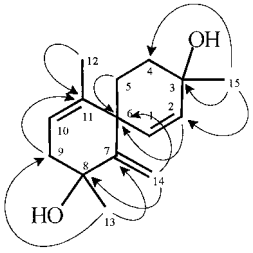


Figure 1. Main HMBC correlations for compound **1**.

2D NMR ROESY spectra of diol **1** showed cross-peaks between the *exo*-cyclic olefin proton signal at δ_H 4.86 and CH_3-15[12] (δ_H 1.34), and between the CH_3-13 (1.45) proton signal and CH_2-5 (2.08 and 1.67) proton signals, in agreement with the relative configuration 3*R**,6*R**,8*S**. The trivial name of scopariol was suggested for compound **1**.

Compound **2** was a colorless crystalline solid. ^1H and ^{13}C NMR spectra examination indicated the presence of 15 carbon atoms in the molecule and similar characteristics with respect to other chamigrane-type compounds isolated previously such as **1**, **5**, and **7**. NMR spectroscopy (see Tables 1 and 2), IR, and EIMS showed that compound **2** contained two carbon−carbon double bonds [one *endo* 132.3 (d), 135.8 (d), and one *exo*-cyclic 144.8 (s), 115.7 (t)], two hydroxyl groups [3380 cm^{-1}, *m*/*z* 296/298 ($M^+ - H_2O$), one secondary, 71.0 (d), and another tertiary, 66.8, (s)], and finally one secondary bromo-function [74.9 (d), *m*/*z* 314/316 (2/2) (M^+), 217 ($M^+ - H_2O - Br$)]. Also the presence of a spiro carbon [50.9 (s)] and a gem dimethyl fragment [42.3

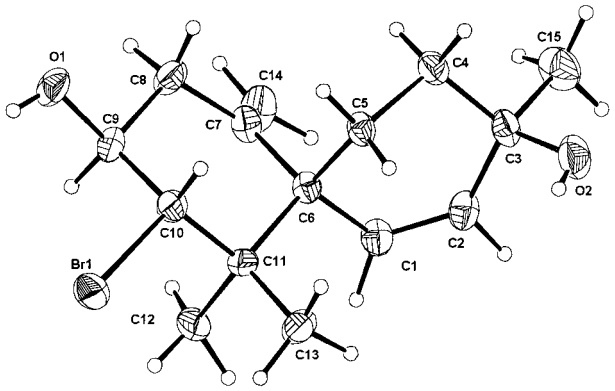


Figure 2. Molecular structure of one molecule of isorigidol (**2**), with displacement ellipsoids at the 30% probability level. Hydrogen atoms are represented as spheres of arbitrary radii.

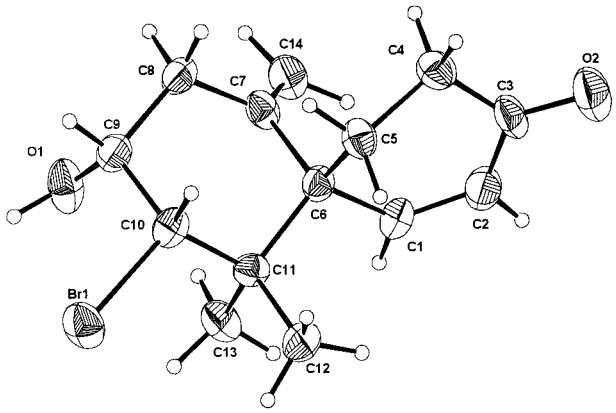


Figure 3. Molecular structure of one molecule of ma'ilione (**7**), with displacement ellipsoids at the 30% probability level. Hydrogen atoms are represented as spheres of arbitrary radii.

(s), 19.1 (q), 26.0 (q)] were in agreement with a β-chamigrane structure for compound **2**.

The COSY spectrum of **2** showed the correlations of the protons (see Table 2) at δ 4.47 (d), 3.86 (ddd), 2.69 (dd), and 2.41 (ddt) coupled to each other in a A−M−X_2 system, corresponding with the molecular fragment −CHBr−CHOH−CH_2− of ring A. It also showed a correlation of *endo* double bond proton signals [5.69 (dd), 5.84 (dd)] and between the methylenes of a −CH_2−CH_2− fragment [1.54 (ddd), 1.66 (m), 1.98 (ddd), 1.78 (bd)]. Consequently a quaternary carbon bearing a hydroxyl group separates these fragments in the ring B as in scopariol.

This structure is in agreement with the HMBC spectrum data (see Table 1). Furthemore, the coupling constant of 10.5 Hz measured for H9−H10 (in axial−axial position) is the same as the one determined for the corresponding protons in compound **9**. Thus the β-chamigrane **2** is an isomer of rigidol[11] with the unusual diequatorial substitution of the bromine and hydroxyl of ring A. X-ray diffraction analysis of a good crystal was performed (see Figure 2), rendering the structure confirmation and the absolute configuration of 3*R*,6*S*,9*S*,10*S*.[13,14] The trivial name of isorigidol is suggested for compound **2**.

Compounds **3** and **4** showed identical EIMS and a very similar NMR spectra. In addition these data resembled the NMR spectral data for two isomeric compounds **5**[15] and **6**,[16] both of which were major metabolites from this extract. Compound **3** had the molecular formula $C_{15}H_{20}Br_2$ determined by HREIMS, and its spectroscopic data agreed with a non-hydroxylated derivative of **5**. Spectroscopic data of compound **4** corresponded with an *E*-isomer of compound **3**.

Also seven known chamigranes, **5**, **6**, **7**,[17] **8**,[18] **9**,[19] **10**,[20] **11**,[21] and one long chain aldehyde, **12**,[22] were isolated and their structures determined by comparing their spectroscopic data with those previously reported. The configuration [6*S*,9*R*,10*S*] of ma'ilione (**7**) was established by X-ray crystallography (Figure 3).[13,14]

Compounds **2**, **5**, **6**, **7**, **8**, **9**, **11**, and **12** demonstrated moderate in vitro anthelmintic activity (lower than 100 μM), and none of the compounds assayed were more active than the reference drugs (e.g., albendazole, EC_{50}: 0.34 μM). It is interesting to observe the activity shown by isomeric pairs of compounds **3**, **4** and **5**, **6**. In both pairs of compounds the corresponding *Z*-isomers (**3** and **5**) demonstrated a higher activity (30- and 2-fold more) than the *E*-isomers, respectively (**4** and **6**).

Experimental Section

General Experimental Procedures. Melting points were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. IR spectra were recorded on a FT IR Shimadzu DR-8031. NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chemical shifts are related to TMS as internal standard. Multiplicities of ^{13}C NMR were assigned using a standard DEPT experiment. LREIMS were recorded on a GCMS Shimadzu QP 1100-EX spectrometer and HREIMS were recorded on a Micromass Autospec spectrometer. MPLC chromatography was carried out with silica gel 60 for flash chromatography (J. T. Baker, 40 μm average particle diameter). Chromatographic separations were monitored by TLC analyses, performed on 0.25 mm silica gel plastic sheets (Macherey−Nagel, Polygram SIL G/UV 254). Spots were visualized using UV light (254 nm), iodine vapor, or 50% phosphomolybdic acid in EtOH.

Plant Material. *L. scoparia* was collected in September 1997 and September 2000 at Praia Brava, coast of Ubatuba, State of Sao Paulo, Brazil. The specimens were identified taxonomically and voucher specimens deposited at the Herbarium of the Botanical Institute, Brazil (SP 336317 and 336318).

Extraction and Isolation. The air-dried algae of each collection (50 and 172 g, respectively) were extracted separately three times with dichloromethane for 1 day each time. Both extracts showed identical spots by TLC. Solvents were removed by evaporation at reduced pressure, yielding 2.2 and 9.2 g of dark green oils, respectively. The residue of 9.2 g was fractionated on a silica gel 60 flash chromatography column, with mixtures of increasing polarity of *n*-hexane−EtOAc−MeOH as eluent. Selected fractions were further purified by a Sephadex LH-20 column with *n*-hexane−$CHCl_3$−MeOH (1:1:1). Crude compounds were purified by medium-pressure liquid chromatography on silica gel 100 with *n*-hexane−EtOAc mixtures to obtain pure compounds before performing the spectroscopy and biological experiments.

Scopariol (1): [(−)-(4*S**,6*R**,9*R**)-1,4,9-trimethyl-5-methylidenespiro[5.5]undec-1,7-diene-4,9-diol], white amorphous solid (4.3 mg, 0.002%); mp 188−190 °C; $[\alpha]^{25}_D$ −8.8° (*c* 0.16, $CHCl_3$); IR (film) λ_{max} 3350, 2922, 2851, 2360, 1116 cm^{-1}; ^{13}C NMR, see Table 1, ^1H NMR, see Table 2; LREIMS 70 eV *m*/*z* 234 $[M]^+$ (0.4), 216 (8), 201 (9), 183(9), 173 (30), 133 (17), 119 (16), 105 (20), 91 (18), 43 (100); HREIMS *m*/*z* 234.1584 (calcd for $C_{15}H_{22}O_2$, 234.1620, M).

Isorigidol (2): [(−)-(2*S*,3*S*,6*S*,9*R*)-2-bromo-1,1,9-trimethyl-5-methylidenespiro[5.5]undec-7-ene-3,9-diol], colorless crystals (5 mg, 0.003%); mp 138−140 °C; $[\alpha]^{25}_D$ −11.5° (*c* 0.32, CH_2Cl_2); IR (film) λ_{max} 3430, 1645 cm^{-1}; ^1H NMR, see Table 1, ^{13}C NMR, see Table 1; LREIMS 20 eV *m*/*z* 316/314 $[M]^+$ (2/2), 301 (9), 299 (11), 298 (13), 296 (13), 217 (100), 199 (43), 173 (30), 161 (30), 133 (32), 119 (32), 105 (51).

(2*R,6*S**,9*Z*)-2-Bromo-9-bromomethylidene-1,1-dimethyl-5-methylenespiro[5.5]undec-7-ene (3):** colorless oil (30 mg, 0.017%); $[\alpha]_D^{25}$ +9.0° (*c* 1.4, $CHCl_3$); IR (film) λ_{max} 2970, 1636, 1451, 1431, 1387, 1369, 1304, 746 cm^{-1}; ^{13}C NMR, see Table 1, ^1H NMR, see Table 2; LREIMS 70 eV *m*/*z* 362/360/358 $[M]^+$ (22/45/22), 281 (95), 279 (100), 237 (21), 171 (38), 115 (38), 91 (56), 173 (30), 69 (61), 41 (65); HREIMS *m*/*z* 357.9964 (calcd for $C_{15}H_{20}{}^{79}Br_2$, 357.9932, M).

(2*R,6*S**,9*E*)-2-Bromo-9-bromomethylidene-1,1-dimethyl-5-methylenespiro[5.5]undec-7-ene (4):** colorless oil (11 mg, 0.006%); $[\alpha]_D^{25}$ −17.4° (*c* 0.23, $CHCl_3$); IR (film) λ_{max} 2972, 1636, 1447, 1385, 1370, 1300, 872 cm^{-1}; ^{13}C NMR see Table 1; ^1H NMR, see Table 2; LREIMS 70 eV *m*/*z* 362/360/358 $[M]^+$ (13/27/14), 293 (4), 281 (98), 279 (100), 238 (18), 171 (41), 115 (32), 91 (54), 69 (34), 41 (69).

Anthelmintic in Vitro Assay. The effect of compound on parasitant stage (L4) of *N. brasiliensis* was evaluated as reported previously.[23]

Tests were carried out in tissue-culture 24-well plates. Appropriate dilutions in DMSO were prepared for each compound in order to obtain the desired concentration after the addition of 10 μL into each well. At least 6 wells were set up for each concentration. The percentage of dead worms was determined on day 5 and corrected by controls. Statistical analysis was performed using the Student's test. A probability greater than 0.05 was not considered significant. Albendazole, levamisole, and febendazole were used as reference drugs (EC_{50}: 0.34, 0.21, and 0.12 μM, respectively).

Acknowledgment. This work was supported by grants from SAREC (Swedish Agency for Research Co-operation with Developing Countries), PEDECIBA (Programa de Desarrollo de las Ciencias Básicas, Project URU/84/002), and CONICYT/BID (Project No. 19), CSIC Universidad de la República (Project No. 151/95). We thank Dr. Javier Fernández, Universidad de La Laguna (Tenerife, Spain), for his assistance in obtaining HRMS of the samples.

References and Notes

(1) Dedicated to Professor Raúl Mariezcurrena on occasion of his designation as Emeritus Professor of Facultad de Química, Universidad de la República.

(2) Davyt, D.; Entz, W.; Manta, E.; Navarro, G.; Norte, M. *Nat. Prod. Lett.* **1997**, *9*, 305−312.

(3) Davyt, D.; Entz, W.; Fernandez, R.; Mariezcurrena, R.; Mombrú, A.; Saldaña, J.; Dominguez, L.; Coll, J.; Manta, E. *J. Nat. Prod.* **1998**, *61*, 1560−1563.

(4) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49, and previous reports in this series.

(5) Rashid, M. A.; Gustafson, K. R.; Cardellina, J. H.; Boyd, M. R. *Nat. Prod. Lett.* **1995**, 255−259.

(6) Munro M. H. G.. Luibrand R. T.. Blunt J. W.. Scheuer P. J., Eds. *Biorganic Marine Chemistry*; Springer-Verlag: Berlin, 1987; pp 93−176.

(7) Masuda, M.; Abe, T.; Sato, S.; Susuki, T.; Susuki, M. *J. Phycol.* **1997**, *33*, 196−208.

(8) Fedorov, S. N.; Shubina, L. K.; Kalinovsky, A. I.; Lyhakova E. G.; Stonk, V. A. *Tetrahedron Lett.* **2000**, *41*, 1979−1982.

(9) Sakai, R.; Higa T.; Jefford, C. W.; Bernardinelli, G. *Helv. Chim. Acta* **1986**, *69*, 91−105.

(10) Bittner, M. L.; Silva, M.; Paul, V. J.; Fenical, W. *Phytochemistry* **1985**, *24*, 987−989.

(11) König, G. M.; Wright, A. D. *J. Nat. Prod.* **1997**, *60*, 967−970.

(12) We use the chamigrene common numbering for the structural formulas and spectroscopic data; for IUPAC nomenclature and numbering for retrieval purposes see Experimental Section.

(13) Suescun, L.; Mombrú, A. W.; Mariezcurrena, R.; Davyt, D.; Fernández, R.; Manta E. *Acta Crystallogr.* **2001**, C*57*, 286−288.

(14) Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. 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)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

(15) Susuki, M.; Kurosawa, E. *Tetrahedron Lett.* **1978**, *48*, 4805−4806.

(16) Schmitz, F.; Michaud, D.; Schmidt, P. *J. Am. Chem. Soc.* **1982**, *104*, 6415−6423.

(17) Juagdan, E. G.; Kalindi, R.; Scheuer, P. J. *Tetrahedron* **1997**, *53*, 521−528.

(18) Susuki, M.; Kurosawa, E.; Kurata, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3795−3796.

(19) Wright, A. D.; Coll, J. C.; Price, I. R. *J. Nat. Prod.* **1990**, *53*, 845−861.

(20) Gonzalez, A. G.; Martín, J. D.; Martín, V. S.; Martinez-Ripoll, M.; Fayos, J. *Tetrahedron Lett.* **1979**, 2717−2718.

(21) Gonzalez, A. G.; Martín, J. D.; Martín, V. S.; Norte, M. *Tetrahedron Lett.* **1979**, 2719−2722.

(22) De Rosa, S.; De Giulio, A.; Iodice, C.; Alcaraz, M. J.; Paya, M. *Phytochemistry* **1995**, *40*, 995−996.

(23) Gordon, S.; Costa, L.; Incerti, M.; Manta, E.; Saldaña, J.; Domínguez, L.; Mariezcurrena, R.; Suescun, L. *Il Farmaco* **1997**, *52*, 603−608.

NP0102307