Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 62 (2006) 3259–3265

Structural elucidation and bioactivity of novel secondary metabolites from *Carex distachya*

Antonio Fiorentino,* Brigida D'Abrosca, Angelina Izzo, Severina Pacifico and Pietro Monaco

Dipartimento di Scienze della Vita, Seconda Università di Napoli, via Vivaldi 43, I-81100 Caserta, Italy

Received 25 October 2005; revised 2 January 2006; accepted 19 January 2006

Abstract—Four new carexanes and a new seco-derivative metabolite have been isolated and characterized from the herbaceous plant *Carex distachya* Desf. All of the structures have been elucidated on the basis of spectroscopic data. These compounds derive from the cyclization of prenylate stylbenoid precursors. The seco-carexane is formed by a further oxidative cleavage of the C-7–C-8 bond. The absolute configurations have been determined by Mosher's method using appropriate chemical correlations. All of the carexanes A–H have been tested for their phytotoxicity against *Lactuca sativa*. The bioassays showed an inhibitory effect on seed germination for all compounds described in this report.

1. Introduction

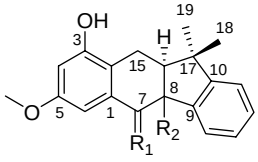
The organization of ecosystems is based on complex interactions among life forms living in close union. The search for food, the struggle for the reproduction, and defense from predators have induced organisms to develop suitable strategies to assure their survival, and the plant organisms interact no less aggressively than animals. In fact, on account of their stillness, plants have developed physical and chemical mechanisms to make contact with other plants and/or with animals living in the same ecosystem. Amongst such chemical strategies, the development of natural products is the most sophisticated mode to insure their existence. A variety of secondary plant metabolites are released into the soil, either as exudates from living plant tissues or by decomposition of plant residues.[1,2] Some of these chemicals play an important role in chemical interactions in natural plant communities and are known as allelochemicals.

Although allelopathic science is a relatively new field of study, there is convincing evidence that allelopathic interactions between plants play a crucial role in both natural and manipulated ecosystems.[1,3] These interactions are an important factor in determining species distribution and abundance within some ecosystems and for the success of many invasive plants.

In the search for allelochemicals from plants found near the Mediterranean area,[4,5] we recently reported the isolation and characterization of three new secondary metabolites, named carexanes A–C (**1**–**3**), from the leaves of *Carex distachya*,[6] a herbaceous plant growing in a Mediterranean bushland. These compounds showed a new tetracyclic molecular skeleton and should derive from the prenylation and successive cyclization of stilbene precursors, and are believed to be structurally interesting.[7]

Literature data suggested that other *Carex* species produce oligostilbenes,[8,9] constituted by two to four monomers of resveratrol (3,5,4′-trihydroxystilbene), most of them showing antimicrobial activity.

In further investigations on the same source we isolated five new compounds. In this paper, we report the elucidation of five new metabolites, named carexane D–H, and the phytotoxicity evaluation of these metabolites against *Lactuca sativa*, the test organism currently used for phytotoxic assays.



carexane A (**1**)	R_1 = βOH,αH	R_2 = βH
carexane B (**2**)	R_2 = O	R_2 = αOH
carexane C (**3**)	R_1 = O	R_2 = βOH
carexane D (**4**)	R_1 = O	R_2 = βH
9	R_1 = αOH,βH	R_2 = βH

Keywords: *Carex distachya* Desf; Carexanes; Prenyl stilbenes; NMR analysis; Phytotoxicity; *Lactuca sativa*.
* Corresponding author. Tel.: +39 0823 274576; fax: +39 0823 274571; e-mail: antonio.fiorentino@unina2.it

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.01.066

2. Results and discussion

Compound **4** has been isolated as an amorphous powder and named carexane D. The elemental analysis and the presence of 20 carbons in the ^{13}C NMR spectrum justified the molecular formula $C_{20}H_{20}O_3$. The EIMS spectrum showed the molecular ion at *m/z* 308 confirming the presence of 11 unsuturations. The ^{1}H NMR spectrum (Table 1) showed six aromatic protons, two of them appear as doublets at δ 7.02 and 6.60, a doublet at δ 4.13, a methoxyl at δ 3.76, two methylene protons as double doublets at δ 3.07 and 2.33, a doublet of double of doublets at δ 2.72 and two singlet methyls at δ 1.16 and 1.27.

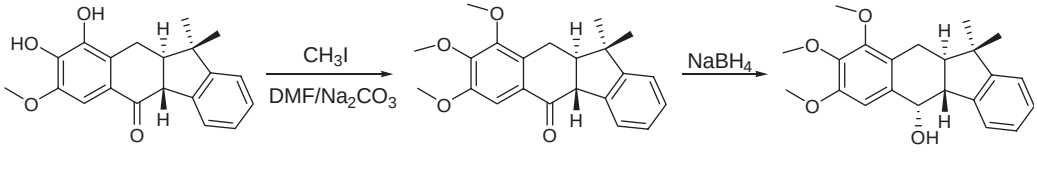
A DQ-COSY experiment showed cross-peaks of the two methylene protons with each other and with the methine at δ 2.72, which was, in turn, correlated with the doublet at δ 4.13. The ^{13}C NMR spectrum, on the basis of a DEPT experiment, identified three methyls, a methylene, eight methines, seven tetrasubstituted carbons and a carbonyl carbon. The NMR values of the signals confirmed the presence of two aromatic rings in the molecule.[6] The doublet at δ 4.13, bonded to the carbon at δ 53.7, was heterocorrelated, in an HMBC experiment, to the carbons at δ 20.9, 47.2, 50.8, 138.7, 152.5, and 199.5. The carbonyl showed correlations with the aromatic proton at δ 7.02 and with the doublet of double of doublet at δ 2.72. The signal at δ 7.02 showed interactions with the carbons at δ 108.6, 160.1 and with the tetrasubstituted carbons at δ 135.0 and 125.7, which were both correlated with the methylene protons. These data confirm the presence of a carexane skeleton possessing a C-7 carbonyl group, a methoxyl on the C-5 carbon, and a hydroxyl bonded to the C-3 carbon. The constant coupling (Table 1) of the H-8 and H-16 protons indicated their trans-orientation on the basis of the generalized Karplus equation.[10] To establish the absolute configurations to the chiral carbons, the carexane D was reduced in MeOH with $NaBH_4$. The products of the reaction were purified by HPLC and identified, by NMR spectroscopic analysis, as the known carexane A[6] and its epimer **9**, confirming an *R* configuration for both C-8 and C-16 carbons and also for the compound **4**.

Compound **5**, named carexane E, showed 20 signals in the ^{13}C NMR spectrum and a molecular peak, in the EIMS spectrum, in accordance with the molecular formula $C_{20}H_{20}O_4$. The ^{1}H NMR spectrum (Table 1) showed five aromatic protons as a doublet at δ 7.36 and four signals ranging from δ 7.12 to 7.19 ppm. In the aliphatic region of the spectrum a doublet at δ 4.06, a doublet of double of doublets at δ 2.68, a methylene as doublet of doublets at δ 3.08 and 2.35 were evident, besides a methoxyl at δ 3.85 and two methyls at δ 1.18 and 1.23. The ^{13}C NMR spectrum showed five methines and seven tetrasubstituted carbons, in the aromatic region. In the aliphatic region, a methylene, two aliphatic methines, three methyls, a quaternary carbon, besides a carbonyl carbon at δ 198.5, were present. The latter carbon showed correlations, in the HMBC experiment, with

Table 1. ^{1}H and ^{13}C NMR data of carexane D–G in CD_3OD

Position	Carexane D (**4**)		Carexane E (**5**)		Carexane F (**6**)		Carexane G (**7**)	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1	—	135.0	—	127.5	—	127.6	—	127.6
2	—	125.7	—	124.7	—	123.9	—	123.9
3	—	157.0	—	143.0	—	142.9	—	143.9
4	6.60d (2.4)	108.6	—	141.6	—	142.3	—	140.9
5	—	160.1	—	148.0	—	148.4	—	148.2
6	7.02d (2.4)	101.6	7.15s	101.9	7.08s	102.5	7.24s	103.8
7	—	199.5	—	198.5	—	197.4	—	196.5
8	4.13d (6.9)	53.7	4.06d (6.6)	53.4	—	82.0	—	80.3
9	—	138.7	—	138.8	—	143.5	—	142.4
10	—	152.5	—	152.4	—	153.2	—	155.5
11	7.17m	123.3	7.15m	123.3	7.17m	123.0	7.32m	123.5
12	7.20m	128.8	7.19m	128.7	7.28m	129.8	7.35m	130.0
13	7.18m	127.8	7.16m	127.8	7.25m	127.8	7.25m	127.5
14	7.38m	126.0	7.36d (6.9)	125.8	7.67m	127.0	7.94d (6.9)	127.5
15	3.07dd (6.0, 16.8) 2.33dd (8.4, 16.8)	20.9	3.08dd (6.0, 16.5)	21.3 2.35dd (9.0, 16.5)	3.08d (4.8)	19.7	3.09dd (10.9, 16.5) 2.95dd (5.4, 16.5)	20.9
16	2.72ddd (6.0, 6.9, 8.4)	50.8	2.68ddd (6.0, 6.6, 9.0)	51.0	2.71t (4.8)	57.3	2.22dd (10.9, 5.4)	58.7
17	—	47.2	—	47.2	—	46.1	—	44.8
18	1.16s	24.0	1.18s	23.9	0.75	26.6	1.39	27.8
19	1.27s	28.3	1.23s	28.2	1.43	29.4	1.41	26.9
OMe	3.76	55.7	3.85	56.4	3.83	56.4	3.89	56.5

s=singlet, d=doublet, dd=double doublet, ddd=double double doublet, m=multiplet, t=triplet; *J* values (Hz) are reported in brackets.



5 **10** **11**

Scheme 1. Chemical modifications used to determinate the absolute configurations of carexane E.

Table 2. NMR data of carexane H (**8**) in CD_3OD

Position	^1H (δ)	J (Hz)	DQ-COSY	^{13}C (δ)	DEPT	HMBC
1	—	—	—	135.2	C	—
2	—	—	—	121.3	C	—
3	—	—	—	158.3	C	—
4	6.51d	2.7	H-6	105.5	CH	C-2, C-3, C-5, C-6
5	—	—	—	159.7	C	—
6	6.83d	2.7	H-4	107.6	CH	C-2, C-4, C-5, C-7
7	—	—	—	172.4	C	—
8	—	—	—	210.1	C	—
9	—	—	—	135.6	C	—
10	—	—	—	164.4	C	—
11	7.56d	7.8	H-12	124.1	CH	C-9, C-10, C-12, C-13, C-17
12	7.63t	7.8	H-11, H-13	136.1	CH	C-10, C-11, C-13, C-14
13	7.37t	7.8	H-12, H-14	128.5	CH	C-9, C-12, C-14
14	7.62d	7.8	H-13	124.4	CH	C-8, C-9, C-10, C-12
15	3.38dd	7.5, 13.8	H-16	23.6	CH_2	C-1, C-2, C-3, C-8, C-16, C-17
	3.24dd	6.6, 13.8				C-1, C-2, C-3, C-8, C-16, C-17
16	2.97dd	6.6, 7.5	H-15	61.2	CH	C-2, C-8, C-10, C-15, C-17
17	—	—	—	43.6	C	—
18	1.28s	—	—	27.0	CH_3	C-10, C-16, C-17, C-19
19	1.31s	—	—	28.6	CH_3	C-10, C-16, C-17, C-18
OMe	3.77s	—	—	55.7	CH_3	C-5

s=singlet, d=doublet, dd=double doublet, ddd=double double doublet, m=multiplet, t=triplet; J values (Hz) are reported in brackets.

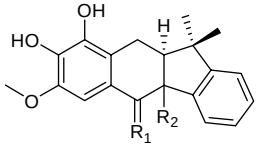
Table 3. Bioactivity of carexanes A–F and H on the germination, root elongation and shoot elongation of *L. sativa*

Carexanes	Germination						Root elongation						Shoot elongation					
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M
A	−2.0	−7.0	−5.0	−7.0	−3.0	−5.0	+11.5	+12.6	+9.1	+9.0	+5.1	−1.7	+2.5	+2.0	−4.3	+1.9	+2.8	+22.1
B	−25.0	−24.0	−36.0[a]	−39.0[a]	−35.0[a]	−28.0[b]	+1.9	+18.1[b]	+13.3[b]	+18.7[a]	+8.2	+7.4	+1.7	+14.5[b]	+11.8	+12.6	+10.4	−3.8
C	−4.0	−9.0	−10.0	−17.0[b]	−12.0[b]	−21.0[a]	+17.5[b]	+12.0	+12.8	+8.3	+2.1	+4.8	−4.4	+11.4	+11.0	+9.8	+8.4	+9.5
D	−46.0[a]	−34.0[a]	−15.0	−7.0	−6.0	−3.0	−43.5[a]	+1.2	+39.5[a]	+28.8[b]	+23.8	+32.7[b]	−36.5[b]	−14.5[b]	+16.3	+19.8[b]	+14.5	+19.9
E	−10.0	−6.0	−6.0	−3.0	−8.0	−7.0	+15.5[b]	+0.8	−2.0	+0.9	+5.0	−3.3	−24.5[a]	−7.9	+7.2	−7.1	−2.0	−10.3
F	−8.0	−7.0	−8.0	−11.0	−7.0	−8.0	+0.2	+14.3	+20.1[b]	+49.4[a]	+34.1[b]	+37.5[a]	−0.1	+5.1	+10.1	+29.4[a]	+34.8[a]	+37.9[b]
H	−24.0[a]	−20.0[a]	−11.0	−7.0	−6.0	+2.0	−22.3[b]	+0.2	+31.6[b]	+18.2[b]	+28.6	+52.1[a]	−29.4	−28.4	+1.2	+6.7	+12.0	+32.9[a]

Value are presented as percentage differences from control and are significantly different with $P>0.05$ for Student's t-test.
[a] $P<0.01$.
[b] $0.01<P<0.05$.

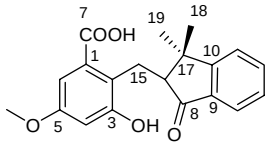
an aromatic proton at δ 7.15 and with both the methines at δ 4.06 and 2.68, suggesting a 7-oxocarexane skeleton. The NMR spectroscopic data indicated the presence of a further hydroxyl group in the A ring. In fact, heterocorrelation between the methylene protons and the carbon at δ 143.0 was evident; between the H-6 aromatic proton with the carbons at δ 141.6 and 148.0 and between this latter with the methoxyl protons at δ 3.85. These data were in good agreement with the proposed structure for carexane E. The absolute configurations to the carbons C-8 and C-16 were determined in this way: the coupling constant of the H-8 and H-16 protons indicated their trans-orientation. The reduction of compound **5** with $NaBH_4$ failed, probably due to the presence of a hydrochinon moiety in the molecule. Therefore, the compound was first methylated with CH_3I, and then reduced with $NaBH_4$ to produce the carexane **11**. The coupling constant (4.0 Hz) of the H-7 proton, geminal to the hydroxyl group, was in accordance with a cis-orientation in respect to the H-8 proton, which is trans in respect to the H-16 proton. The absolute configuration of the C-7 carbon was established by using a modified Mosher method.[11] The negative and positive $\Delta\delta_{R-S}$ values the H-8, and the H-6 protons were found, respectively, on the right and left sides of the MTPA plane, indicated an *S* configuration for C-7 and, consequently, an *R* configuration for the C-8 and C-16 carbons (Scheme 1).

Carexanes F and G (compounds **6** and **7**) were identified as two isomers on the basis of their ^{13}C NMR and EIMS spectra. The molecular formula found $C_{20}H_{20}O_5$, and the spectroscopic data, indicated that these compounds were 4-hydroxy derivatives of carexanes B and C, respectively. In fact the differences in chemical shifts (Table 1) were attributed to the presence of a further hydroxyl group at the C-4 carbon. The hypothesised structures were confirmed by two-dimensional NMR (HSQC, HMBC, NOESY) and EIMS data.



carexane E (**5**)	R_1 = O	R_2 = βH
carexane F (**6**)	R_1 = O	R_2 = αOH
carexane G (**7**)	R_1 = O	R_2 = βOH

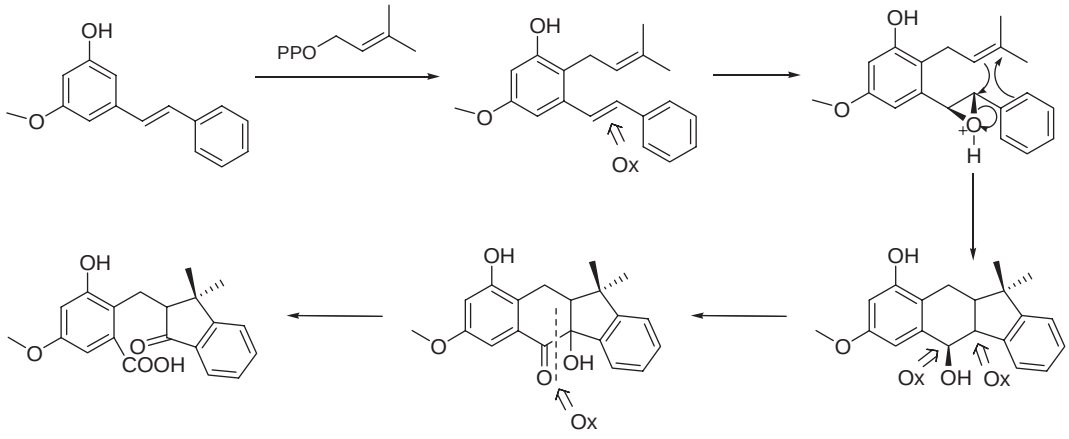
Compound **8** has been isolated as a colourless oil and named carexane H. The elemental analysis and the ^{13}C NMR spectroscopic data were in accordance with a molecular formula $C_{20}H_{20}O_5$. The EIMS spectrum showed a molecular peak at *m/z* 340, confirming the presence of 11 unsaturations in the molecule.



carexane H (**8**)

In the aromatic region of the ^1H NMR spectrum (Table 2), six protons were present: three protons were overlapped in the range from 7.70 to 7.50 ppm besides a triplet at δ 7.31 and two *meta* coupled doublets at δ 6.83 and 6.51. In the aliphatic region of the spectrum, a methoxyl at δ 3.77, two doublet of doublets at δ 3.38 and 3.24, a methine at δ 2.97 and two methyls at δ 1.28 and 1.31 were observed. The DQ-COSY experiment showed correlations between the methylene protons and the methine at δ 2.97, between the aromatic doublets at δ 6.51 and 6.83 and between the remaining four aromatic protons. The ^{13}C NMR spectrum exhibited 20 signals, which were identified on the basis of a DEPT experiment as three methyls, a methylene, seven methines, and nine quaternary carbons. In particular, a carbonyl carbon at δ 210.1 and a carboxyl at δ 172.4 were present.

The HMBC experiment (Table 2) showed heterocorrelations with the aromatic protons and the carbons at δ 159.7, 158.3, 121.3 and 107.6. The latter signal showed a correlation, in the HSQC spectrum, with the proton at δ 6.83. This latter showed correlations with the carbons at δ 159.2, 135.2, 121.3 and the carboxyl group. These data confirmed the presence of the A ring with an hydroxyl and methoxyl groups bonded at the C-3 and C-5 carbons. The C-2 carbon at δ 121.3 were correlated to the methylene protons and to the methine at δ 2.97 bonded to the carbon at δ 61.2. The latter proton resulted correlated to both the methyls, to the carbons at δ 43.6 and 164.2 and to the carbonyl at δ 210.1, which was in turn, correlated with the methylene protons. These data suggested, for the compound **8**,



Scheme 2. Biosynthetic pathway proposed for the carexanes.

a 7,8-*seco*-carexane structure derived by a oxidative cleavage of the C-7–C-8 bond. In fact the C-7 and C-8 carbon were oxidized at carboxyl and carbonyl groups, respectively.

All of the compounds, with exception of the less abundant carexane G, have been tested on the dicotyledonous *L. sativa* L.[12,13] and the results are reported in Table 3. The inhibitory effect on germination, on the contrary, a stimulating effect was shown against plant growth. The most active compounds on the germination were carexanes B and D. This latter and the carexane H showed a similar behaviour: on the seed germination showed a good dose-response relationship, while on the plant growth they were active at the highest concentration used and became stimulating at the lower doses.

No many articles report the isolation of prenylated stilbenes from natural sources.[14] These compounds are identified as cytotoxic against ovarian cancer cell lines.[15] The tetracyclic prenylated structures of the carexanes A–G and the derivative carexane H have been reported for the first time. They could originate by the prenylation of a stilbene precursors, cyclization and successive modifications, as hypothesised in the Scheme 2.

3. Experimental

3.1. General procedures

NMR spectra were recorded at 300 MHz (for ^{1}H) and 75 MHz (for ^{13}C) on a Varian 300 spectrometer Fourier transform NMR spectrometer in CD_3OD at 25 °C. Proton-detected heteronuclear correlations were measured using a gradient heteronuclear single-quantum coherence (HSQC), optimised for $^{1}J_{HC}=140$ Hz, and a gradient heteronuclear multiple bond coherence (HMBC), optimised for $^{n}J_{HC}=8$ Hz. UV spectra were performed in MeOH solution on UV-1601 Shimadzu spectrophotometer. Optical rotations were measured in MeOH solution on a Perkin-Elmer 141. Electron ionization mass spectra (EIMS) were obtained with a HP 6890 instrument equipped with a MS 5973 N detector.

The preparative HPLC apparatus consisted of a pump (Shimadzu LC-10AD), a refractive index detector (Shimadzu RID-10A) and a Shimadzu Chromatopac C-R6A recorder. Preparative HPLC was performed using RP-8 (Luna 10 μm, 250×10 mm i.d., Phenomenex) column. Analytical TLC was performed on Merck Kieselgel 60 F_{254} or RP-8 F_{254} plates with 0.2 mm layer thickness. Spots were visualized by UV light or by spraying with H_2SO_4–AcOH–H_2O (1/20/4). The plates were then heated for 5 min at 110 °C. Preparative TLC was performed on Merck Kieselgel 60 F_{254} plates, with 0.5 or 1 mm film thickness. Flash column chromatography (FCC) was performed on Merck Kieselgel 60 (230–400 mesh) at medium pressure. Column chromatography (CC) was performed on Merck Kieselgel 60 (70–240 mesh).

3.2. Plant material, extraction and isolation of the metabolites

Plants of *C. distachya* Desf (Cyperaceae) were collected in June 2004, in the vegetative state, in Castelvolturno, near Caserta (Italy), and identified by Dr. Assunta Esposito of the Second University of Naples. A voucher specimen (CE278) has been deposited at the Herbarium of the Dipartimento di Scienze della Vita of Second University of Naples.

Fresh leaves of *C. distachya* (6 kg) were extracted with hexane for 5 days at 4 °C in the dark. The organic solution was distilled under reduced pressure by a Rotavapor® to obtain 30.0 g of crude extract. The hexane extract was chromatographed on SiO_2, with hexane and EtOAc solutions, to give three fractions I–III.

Fraction I, eluted with hexane–EtOAc (5/1), was rechromatographed by Sephadex LH-20® eluting with hexane–$CHCl_3$–MeOH (3/1/1) to obtain a fraction, which was purified by TLC eluting with hexane–EtOAc (4/1) to give pure carexane D (12.4 mg).

Fraction II, eluted with hexane–EtOAc (4/1), was rechromatographed by Sephadex LH-20® eluting with hexane–$CHCl_3$–MeOH (3/1/1) to obtain the carexane E (40.2 mg) and two further fractions. The first one was purified by preparative RP-8 HPLC eluting with MeOH–MeCN–H_2O (2/2/1) to give pure carexanes A (3.2 mg), B (5.0 mg) and C (4.3 mg). The second fraction was chromatographed by TLC with $CHCl_3$–Me_2CO (9/1) to have pure carexane H (2.1 mg).

Fraction III, eluted with hexane–EtOAc (3/2), was rechromatographed by Sephadex LH-20 eluting with hexane–$CHCl_3$–MeOH (3/1/1) to obtain a fraction, which was purified by preparative RP-8 HPLC eluting with MeOH–MeCN–H_2O (2/2/1) to give pure carexanes F (2.0 mg) and G (1.0 mg).

3.2.1. Characterization of the carexanes D–H. *Carexane D* (**4**). Amorphous white powder; UV (MeOH) λ_{max} nm (log ε): 334.4 (2.86), 273.4 (3.39), 213.0 (3.84); ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1; EIMS *m/z* 308 $[M]^+$, 293 $[M-CH_3]^+$, 291 $[M-OH]^+$; $[\alpha]_D^{25}$ +136.5 (*c* 0.14, MeOH). Anal. Calcd for $C_{20}H_{20}O_3$: C, 77.90; H, 6.54. Found: C, 77.54; H, 6.91.

Carexane E (**5**). Amorphous white powder; UV (MeOH) λ_{max} nm (log ε): 308.0 (3.84), 213.2 (4.19); ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1; EIMS *m/z* 324 $[M]^+$, 309 $[M-CH_3]^+$, 307 $[M-OH]^+$; $[\alpha]_D^{25}$ +80.2 (*c* 0.41, MeOH). Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21. Found: C, 74.32; H, 6.51.

Carexane F (**6**). Amorphous white powder; UV (MeOH) λ_{max} nm (log ε): 314.4 (3.48), 203.2 (3.92); ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1; EIMS *m/z* 416 $[M]^+$, 401 $[M-CH_3]^+$; $[\alpha]_D^{25}$ −64.3 (*c* 0.06, MeOH). Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92. Found: C, 70.78; H, 5.84.

Carexane G (**7**). Amorphous white powder; UV (MeOH) λ_{max} nm (log ε): 301.8 (3.69), 204.4 (4.12); ^{1}H NMR

(300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1; EIMS *m/z* 340 $[M]^+$, 325 $[M-CH_3]^+$, 323 $[M-OH]^+$; $[\alpha]_D^{25}$ +203.8 (*c* 0.13, MeOH). Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92. Found: C, 70.67; H, 6.01.

Carexane H (**8**). Colourless oil; UV (MeOH) λ_{max} nm (log ε): 287.6 (3.11), 240.4 (3.53), 205.4 (4.16); ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1; EIMS *m/z* 340 $[M]^+$, 325 $[M-CH_3]^+$, 295 $[M-CO_2H]^+$; $[\alpha]_D^{25}$ 0 (*c* 0.10, MeOH). Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92. Found: C, 70.76; H, 5.89.

3.2.2. Reduction of carexanes D. To a solution of compound **4** (6 mg, 0.019 mmol) in MeOH (0.5 ml) 2 mg of $NaBH_4$ (0.053 mmol) were added. The solution was kept under magnetic stirring for 1 h and then treated with AcOH (1 drop) and dried under nitrogen flow. The mixture were purified by TLC [EtOAc–$CHCl_3$ (1/4)] to give carexane A (1 mg) and product **9** (4 mg). Compound **9**: ^1H NMR (300 MHz, CD_3OD) δ: 7.38 (1H, m, H-14), 7.06 (3H, m, H-11–H-13), 6.48 (1H, d, J=2.1 Hz, H-6), 6.22 (1H, d, J=2.1 Hz, H-4), 4.99 (1H, d, J=4.2 Hz, H-7), 3.70 (3H, s, OMe), 3.65 (1H, dd, J=4.2, 9.3 Hz, H-8), 2.81 (1H, dd, J=8.1, 15.0 Hz, H-15), 2.74 (1H, dd, J=7.8, 15.0 Hz, H-15), 2.55 (1H, ddd, J=7.8, 8.1, 9.3 Hz, H-16), 1.29 (3H, s, H-18), 1.27 (3H, s, H-19); ^{13}C NMR (300 MHz, CD_3OD) δ: 159.6 (C-5), 155.4 (C-10), 155.1 (C-3), 144.0 (C-1), 142.2 (C-9), 127.8 (C-12), 127.2 (C-13), 126.3 (C-14), 117.7 (C-2), 104.3 (C-4), 101.1 (C-6), 72.8 (C-9), 55.6 (OMe), 51.8 (C-8), 48.0 (C-16), 46.7 (C-17), 33.5 (C-19), 25.8 (C-18), 21.0 (C-15); EIMS *m/z* 310 $[M]^+$, 295 $[M-CH_3]^+$. Anal. Calcd for $C_{20}H_{22}O_3$: C, 77.39; H, 7.14. Found: C, 77.56; H, 7.64.

3.2.3. Methylation and reduction of carexane E. To 1 ml of dry DMF, saturated with anhydrous Na_2CO_3, 15 mg of carexane E (0.046 mmol) were added. After 5 min 10 µl of CH_3I were added and the mixture was kept under magnetic stirring for 1 h (Scheme 1). The mixture was then treated with H_2O (15 ml) and extracted with EtOAc (2×15 ml). The organic extract was dried with anhydrous Na_2SO_4 and evaporated in vacuo to afford the methyl derivative **10** (10 mg): ^1H NMR (300 MHz, CD_3OD) δ: 7.38 (1H, m, H-14), 7.23–7.18 (3H, m, H-11–H-13), 7.15 (1H, s, H-6), 4.14 (1H, d, J=6.9 Hz, H-8), 3.86 (9H, s, OMe), 3.11 (1H, d, J=6.3, 16.8 Hz, H-15), 2.74 (1H, ddd, J=6.3, 6.9, 7.8 Hz, H-16), 2.38 (1H, dd, J=8.7, 16.8 Hz, H-15), 1.28 (3H, s, H-18), 1.20 (3H, s, H-19); EIMS *m/z* 352 $[M]^+$, 337 $[M-CH_3]^+$. Anal. Calcd for $C_{22}H_{26}O_4$: C, 74.98; H, 6.86. Found: C, 75.11; H, 6.95. To a solution of compound **10** (10 mg, 0.028 mmol) in MeOH (0.5 ml) 3 mg of $NaBH_4$ (0.080 mmol) were added. The solution was kept under magnetic stirring for 1 h and then treated with AcOH (1 drop) and dried under nitrogen flow. The mixture was purified by TLC EtOAc–$CHCl_3$–hexane (2/8/1) to give **11** (6 mg) as the main product. ^1H NMR (300 MHz, CD_3OD) δ: 7.38 (1H, m, H-14), 7.22–7.04 (3H, m, H-11–H-13), 6.58 (1H, s, H-6), 5.00 (1H, d, J=4.0 Hz, H-7), 3.82 (9H, s, OMe), 3.63 (1H, dd, J=4.0, 9.9 Hz, H-8), 2.85 (1H, dd, J=5.7, 16.0 Hz, H-15), 2.76 (1H, dd, J=8.1, 16.0 Hz, H-15), 2.55 (1H, ddd, J=5.7, 8.1, 9.9 Hz, H-16), 1.29 (6H, s, H-18 and H-19); EIMS *m/z* 354 $[M]^+$, 339 $[M-CH_3]^+$. Anal. Calcd for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.61; H, 7.64.

3.2.4. Preparation of (*S*) and (*R*)-MTPA esters of compound 11. (*R*)-(−)-MTPA chloride (5 µl, 26 µmol) was added to a solution of pure compound **11** (1.5 mg, 4.2 µmol) in dry pyridine (50 µl). After 6 h under magnetic stirring at room temperature, EtOAc (5 ml) and H_2O (5 ml) were added to the reaction mixture. The organic layer, separated by centrifugation at 4000 rpm for 10 min, gave a crude extract, which was purified by preparative TLC eluting with hexane–EtOAc (7/3). The (*S*)-MTPA ester had the ^1H NMR spectral data (300 MHz, CD_3OD) δ: 6.77 (1H, s, H-6), 6.34 (1H, d, J=3.9 Hz, H-7), 4.21 (1H, m, H-8), 3.81 (3H, s, OMe), 3.79 (3H, s, OMe), 3.73 (3H, s, OMe). The (*R*)-MTPA ester had the ^1H NMR spectral data (300 MHz, CD_3OD) δ: 6.73 (1H, s, H-6), 6.31 (1H, d, J=3.8 Hz, H-7), 3.93 (1H, m, H-8), 3.76 (3H, s, OMe), 3.72 (3H, s, OMe), 3.69 (3H, s, OMe).

3.3. Phytotoxicity test

Seeds of *L. sativa* L. (cv Napoli V.F.), collected during 2003, were obtained from Ingegnoli S.p.a. All undersized or damaged seeds were discarded and the assay seeds were selected for uniformity. Bioassays used Petri dishes (50 mm diameter) with one sheet of Whatman No. 1 filter paper as support. In four replicate experiments, germination and growth were conducted in aqueous solutions at controlled pH. Test solutions (10^{-4} M) were prepared using (2-[*N*-morpholino]ethanesulfonic acid (MES; 10 mm, pH 6) and the rest (10^{-5}–10^{-9} M) were obtained by dilution. Parallel controls were performed. After the addition of 25 seeds and 2.5 ml test solns, Petri dishes were sealed with Parafilm® to ensure closed-system models. Seeds were placed in a growth chamber KBW Binder 240 at 25 °C in the dark. Germination percentage was determined daily for 5 days (no more germination occurred after this time). After growth, plants were frozen at −20 °C to avoid subsequent growth until the measurement process. Data are reported as percentage differences from control in the graphics. Thus, zero represents the control, positive values represent stimulation of the parameter studied and negative values represent inhibition.

Statistical treatment. The statistical significance of differences between groups was determined by a Student's *t*-test, calculating mean values for every parameter (germination average, shoot and root elongation) and their population variance within a Petri dish. The level of significance was set at $P<0.05$.

References and notes

1. Rice, E. L. *Allelopathy*; Academic: Orlando, 1984; pp 266–291.
2. Putnam, A.; Tang, C. S. *The Science of Allelopathy*; Wiley: New York, 1986; pp 171–188.
3. Mallik, M. A. B.; Puchala, R.; Grosz, F. A. *J. Chem. Ecol.* **1994**, *20*, 957–967.

4. Della Greca, M.; Fiorentino, A.; Isidori, M.; Previtera, L.; Temussi, F.; Zarrelli, A. *Tetrahedron* **2003**, *59*, 4821–4825.
5. D'Abrosca, B.; Della Greca, M.; De Maria, P.; Fiorentino, A.; Golino, A.; Izzo, A.; Monaco, P. *Tetrahedron* **2006**, *62*, 640–646.
6. D'Abrosca, B.; Fiorentino, A.; Golino, A.; Monaco, P.; Oriano, P.; Pacifico, S. *Tetrahedron Lett.* **2005**, *46*, 5269–5272.
7. Hill, R. A.; Sutherland, A. *Nat. Prod. Rep.* **2005**, *22*, 559–562.
8. Kawabata, J.; Mishima, M.; Kurihara, H.; Mizutani, J. *Phytochemistry* **1995**, *40*, 1507–1510.
9. Meng, Y.; Bourne, P. C.; Whiting, P.; Šik, V.; Dinan, L. *Phytochemistry* **2001**, *57*, 393–400.
10. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.
11. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
12. Macías, F. A.; Castellano, D.; Molinillo, J. M. G. *J. Agric. Food Chem.* **2000**, *48*, 2512–2521.
13. D'Abrosca, B.; Della Greca, M.; Fiorentino, A.; Monaco, P.; Zarrelli, A. *J. Agric. Food Chem.* **2004**, *52*, 4101–4108.
14. Ioset, J. R.; Marston, A.; Gupta, M. P.; Hostettmann, K. *J. Nat. Prod.* **2001**, *64*, 710–715.
15. van der Kaaden, J. E.; Hemscheidt, T. K.; Mooberry, S. L. *J. Nat. Prod.* **2001**, *64*, 103–105.