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Phytochemistry 67 (2006) 971–977
PHYTOCHEMISTRY
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Structures of bioactive carexanes from the roots of *Carex distachya* Desf

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Received 22 February 2006; received in revised form 4 April 2006

Abstract

Four metabolites, named carexanes I–L, have been isolated from the roots of *Carex distachya* Desf, an herbaceous plant living in the Mediterranean maquis, together with three known compounds, already isolated from the aerial part of the plant. All the compounds have been characterized on the basis of their spectroscopic properties. Carexane I derived from the lose of a proton from the C-18 carbon of an intermediate isopropyl cation. Its stereostructure has been elucidated by Mosher's method, NOESY/ROESY experiments and computational calculations.

The bioactivity on seed germination and root/shoot growth of *Lactuca sativa* L. of all the isolated compounds is also reported.

Keywords: *Carex distachya* desf; Cyperaceae; *Lactuca sativa*; Carexanes; Prenyl-stilbene derivatives; Phytotoxicity; Spectroscopic analysis; Computational calculations

1. Introduction

Plants produce a number of compounds that are not directly used for growth. These compounds, named secondary metabolites, can help plant survival by attracting pollinators, deterring herbivores, or offering protection against stress imposed by the environment (Harborne, 1997). Some of these substances may have a role in the allelopathy, playing an important part in chemical interactions in natural plant communities (Callaway and Aschehoug, 2000; Dayan et al., 2000; Fitter, 2003). In fact, a number of plants have been reported to possess inhibitory effects on the growth and population of neighbouring or successional plants by releasing allelopathic substances into the soil through leaching, root exudation, volatilization, and decomposition of plant residues (Rice, 1984; Turk and Tawaha, 2003).

In the study of the role of Mediterranean plants in the allelopathy, we isolated and characterized new phytotoxic compounds from weeds and spontaneous species growing in the South of Italy (D'Abrosca et al., 2001, 2005a, 2006). In a chemical characterization of the Mediterranean carix (*Carex distachya*), we have recently reported the isolation of new secondary metabolites, named carexanes, from the leaves of plants (D'Abrosca et al., 2005b; Fiorentino et al., 2006). These unusual tetracyclic compounds are derived from the prenylation and successive cyclization of stilbene precursors.

In this work, we report the isolation, characterization and phytotoxicity of new substances, with a carexane skeleton, from the root of *C. distachya*. These compounds have been characterized on the basis of their spectroscopic features.

2. Results and discussion

Continuing the phytochemical study of Mediterranean plants, we recently investigated *C. distachya*, a herbaceous plant growing in the Mediterranean bush. From the leaf extracts we isolated and characterized eight new compounds, named carexanes A–H, whose structures were derived from the modification of a stilbene precursor (D'Abrosca et al., 2005b; Fiorentino et al., 2006).

In pursuing the study of *C. distachya*, we investigated the root of the plant, and from the EtOAc extract we

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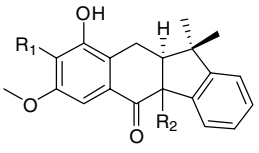
doi:10.1016/j.phytochem.2006.04.003

isolated, besides the carexanes E–G (**1**–**3**), the four new compounds **4**–**7**.

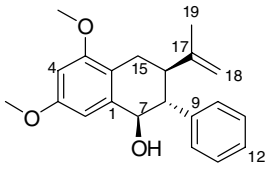
The compound **4**, named carexane I, has been characterized as a tetrahydronaphthalene derivative (Fig. 1). Its EI MS spectrum showed a molecular ion at *m*/*z* 324. This data together with the presence of 21 signals in the ^{13}C NMR spectrum suggested a molecular formula $C_{21}H_{24}O_3$, indicating the presence of 10 unsaturations in the molecule.

The ^{1}H NMR spectrum showed seven aromatics as two protons *meta*-coupled doublets at δ 6.48 and 6.62, two multiplets at δ 6.82 and 7.09 integrated for two and three protons, respectively. The correlations between these later signals in the DQ-COSY experiment, and their chemical shifts were in accordance with a phenyl group in the molecule. This hypothesis was confirmed by EI MS spectrum that showed the characteristic fragment at *m*/*z* 77.

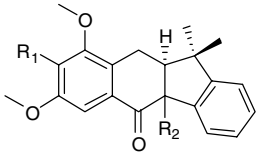
In downfield region of the ^{1}H NMR spectrum, a doublet proton at δ 4.76, two singlets at δ 4.75 and 4.41 and two methoxyls were also evident. Finally, two methines at δ 3.53, and 2.94, two diasterotopic methylene protons at δ 2.69 and 2.21 and a singlet methyl at δ 1.79 resonated in the upfield region of the spectrum. The DQ-COSY experiment showed correlations between the doublet at δ 4.76 with the signal at δ 3.53, which correlated with the signal at δ 2.94, which was, in turn, coupled with the diasterotopic methylenes.



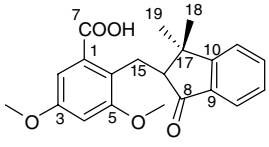
carexane D (**1**) $R_1 = H, R_2 = \beta H$
carexane E (**2**) $R_1 = OH, R_2 = \beta H$
carexane F (**3**) $R_1 = OH, R_2 = \alpha OH$



carexane I (**4**)



carexane J (**5**) $R_1 = H, R_2 = \alpha OH$
carexane K (**6**) $R_1 = OH, R_2 = \beta H$



carexane L (**7**)

Fig. 1. Structures of carexanes isolated from the roots of *C. distachya*.

The ^{13}C NMR showed 21 signals, identified, on the basis of a DEPT experiment, as three methyls, two methylenes, 10 methines and six tetrasubstituted carbons. The two singlets at δ 4.75 and 4.41 were correlated, in the HSQC experiment, with a methylene carbon at δ 111.7. Furthermore the chemical shift of the methyl proton at δ 1.79, correlated to the carbon at δ 22.8, suggested the presence of an isopropenyl group in the molecule. In fact, both the singlets at δ 4.75 and 4.41 correlated, in the HMBC experiment, with the methyl carbon and, vice versa, the methyl protons correlated with the carbon at δ 111.7. All these protons correlated with an olefinic carbon at δ 147.1, and with the methine at δ 40.5, bonded to the proton at δ 2.94. This latter proton was heterocorrelated with the methylene carbon at δ 24.1, with the methine carbons at δ 51.4 and 73.2, bonded to the protons at δ 3.53 and 4.76, respectively, and with the aromatic tetrasubstituted carbon at δ 140.6.

The two *meta*-coupled protons correlated with the carbons at δ 140.0, 120.2, 160.5, which showed interactions with the methoxyl group at δ 3.81. Furthermore the proton at δ 6.48, showed correlations with the carbons at δ 159.1 and 55.7. The carbon at δ 159.1 also resulted correlated to the methylene protons at δ 2.69 and 2.21, while the proton at δ 6.62 correlated with the carbinol carbon at δ 73.2. These data, together with the other correlations reported in Table 1, indicated the presence of a tetrahydronaphthalene skeleton bonding two *meta* methoxyl groups on the aromatic moiety, and an hydroxyl, a phenyl and 2-propenyl groups on the saturated ring as reported in the Fig. 1.

This structure could be derived from a prenylated open-chained derivative that cycled to a isopropyl cation. This intermediate could be attacked by the aromatic ring giving rise to the tetracyclic carexanes, or lose a proton from the C-18 carbon giving rise to the three cyclic carexane I (Scheme 1).

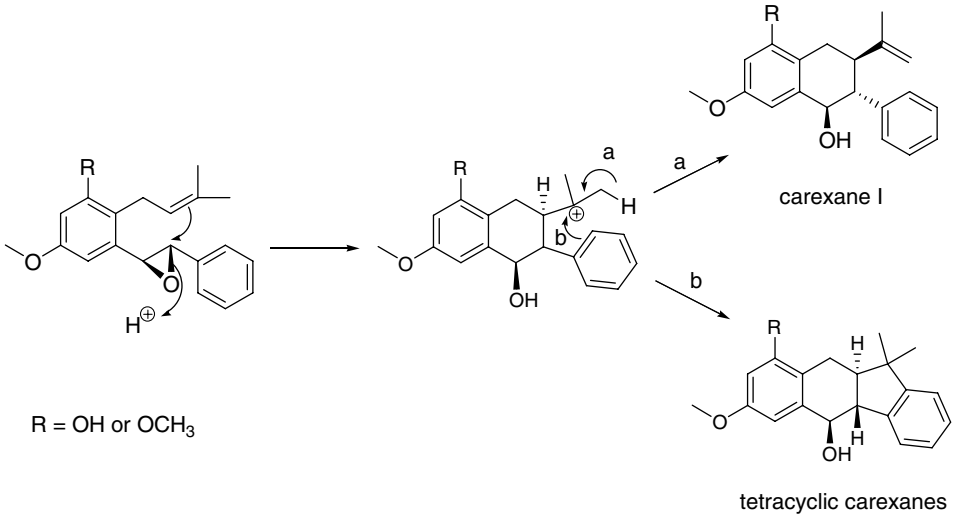
The stereochemistry of the molecule was determined by the modified Mosher's method (Ohtani et al., 1991), NOESY experiment, and computational calculations.

The negative and positive $\Delta\delta_{R\text{-}S}$ values of the H-8, and the H-6 protons were found, respectively, on the right and left sides of the MTPA plane indicating an *R* configuration for the C-7 carbon. To determine the absolute configuration of the C-8 and C-16 chiral carbons, we computed the four possible stereoisomers (Fig. 2) using molecular mechanic method (MM+) as implemented in HyperChem 7.5. Computational results can be produced with high accuracy for entire molecular systems, starting from optimized three-dimensional structure of a compound[9]. The observed coupling constant values of the H-7 and H-8 protons (Table 1) were in agreement with dihedral angles of about 60° or 110°. The minimized stereostructure 7*R*,8*S*,16*S* (Fig. 2B) was rejected because it showed a *trans* diaxial orientation for both H-8 and H-16, in accordance with a large value of the coupling constant. The NOESY and ROESY experiments showed NOE between the H-10 and H-14 aromatics

Table 1
NMR data of compound **4**[a]

Position	δ_H	δ_C	DEPT	HMBC (H → C)
1	–	140.0	C	–
2	–	120.2	C	–
3	–	159.1	C	–
4	6.48 *d* (2.4 Hz)	98.0	CH	C2, C3, C5, C6
5	–	160.5	C	–
6	6.62 *d* (2.4 Hz)	106.5	CH	C2, C4, C5, C7
7	4.76 *d* (2.7 Hz)	73.2	CH	C1, C2, C6, C8, C9
8	3.53 *dd* (2.7, 3.3 Hz)	51.4	CH	C1, C7, C9, C10, C14, C15, C16
9	–	140.6	C	–
10	6.82 *m*	129.9	CH	C8, C9, C11, C12
11	7.09 *m*	128.6	CH	C9, C10, C12
12	7.09 *m*	127.4	CH	C10/14, C11/13
13	7.09 *m*	128.6	CH	C9, C12, C14
14	6.82 *m*	129.9	CH	C8, C9, C12, C13
15	2.69 *dd* (17.4, 4.5 Hz) 2.21 *dd* (17.4, 12.3 Hz)	24.1	CH_2	C1, C2, C3, C8, C16, C17
16	2.94 *dt* (12.3, 3.3 Hz)	40.5	CH	C7, C8, C9, C16, C17, C18, C19
17	–	147.9	C	–
18	4.75 *s* 4.41 *s*	111.7	CH_2	C16, C17, C19
19	1.79 *s*	22.8	CH_3	C16, C17, C18
OMe	3.80 *s*	55.7	CH_3	C4, C5
OMe	3.81 *s*	55.8	CH_3	C5, C6

[a] Registered in CD_3OD, at 300 MHz (for ^1H) and 75 MHz (for ^{13}C). *J* values (Hz) are reported in brackets.



Scheme 1. Plausible biosynthetic pathway of carexane I.

with the H-7, H-8 and H-16 aliphatic protons, indicating a *trans* orientation of the aryl and the isopropenyl groups. The NOE observed between the H-8 proton and the methyl at δ 1.79 confirmed this hypothesis (Fig. 3). These observations were in agreement with the stereostructure 7*R*,8*R*,16*R* (Fig. 2A). In fact, the calculated distances, measured in this minimized structure, between the H-10/14 and H-7 (2.47 Å), the H-10/14 and H-16 (2.67 Å), the H-8 and H-19 proton (2.58 Å), confirmed the hypothesized structure for carexane I.

Compound **5**, named carexane J, showed a molecular formula $C_{21}H_{22}O_4$ in accordance with the molecular ion at *m*/*z* 340 in the EI MS spectrum, and with the 21 signal in the ^{13}C NMR spectrum.

The ^1H NMR spectrum showed, in the aromatic region, two doublets due to two *meta* protons at δ 7.01 and 6.78, four protons as multiplet at δ 7.65, 7.28, 7.22 and 7.17. Two methoxyls (δ 3.90 and 3.79) two methyls (δ 1.43 and 0.72) a methylene as doublet (δ 3.05) and a methine as a triplet (δ 3.72), in the remaining part of the spectrum, were also evident. These data, together with those of the ^{13}C NMR (Table 2) suggest a structure similar to that of carexane C (D'Abrosca et al., 2005b). The differences were due to the presence of another methoxyl on the A ring instead of the hydroxyl at the C-3 carbon. The HMBC experiment confirmed the hypothesis showing heterocorrelations between the carbon at δ 159.3 and the methylene H-15 at δ 3.05, and the methoxyl at δ 3.90 and the H-4 proton at

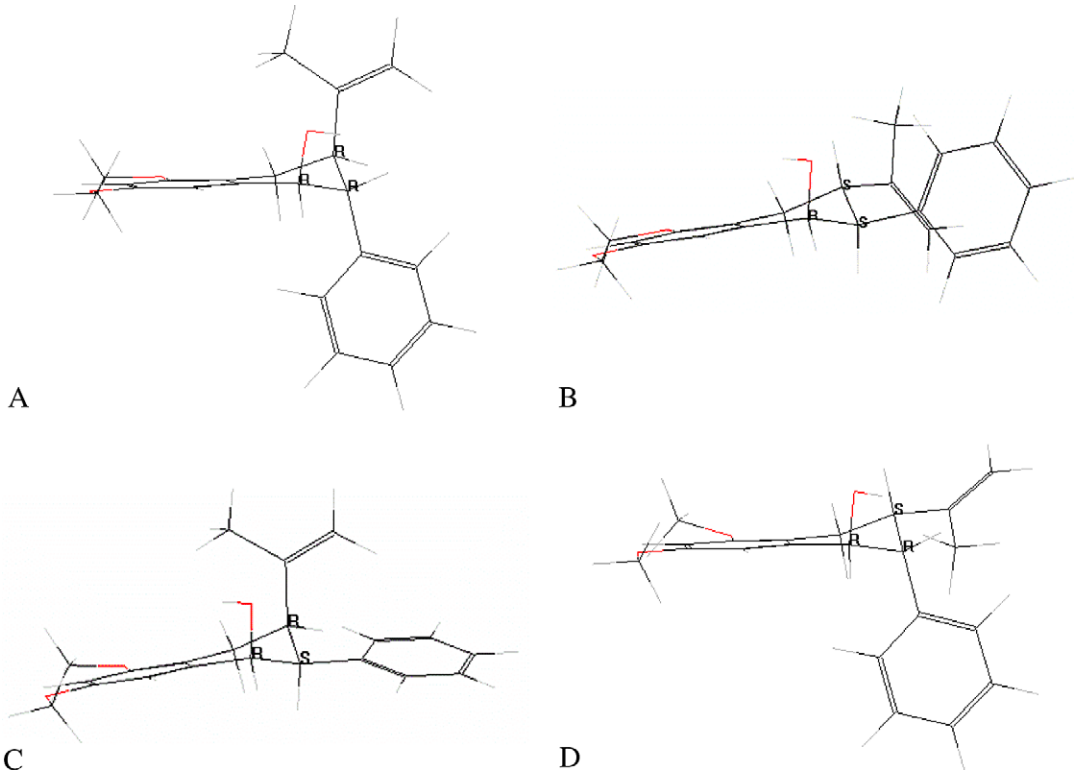


Fig. 2. Geometry-optimized structure of MM+ low-energy conformations of the four possible stereostructures (A–D) of carexane I. (A) Stereostructure 7*R*,8*R*,16*R*. (B) Stereostructure 7*R*,8*S*,16*S*. (C) Stereostructure 7*R*,8*S*,16*R*. (D) Stereostructure 7*R*,8*R*,16*S*.

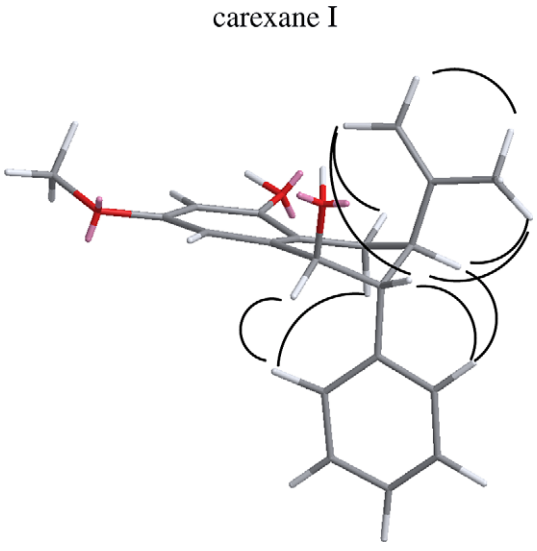


Fig. 3. Selected NOE observed in the NOESY/ROESY experiments of carexane I.

δ 6.78. The further correlations present in the HMBC experiment confirmed the structure hypothesized.

Compound **6**, named carexane K, was an isomer of the previous compound. In fact it showed the same molecular formula $C_{21}H_{22}O_4$.

The ^{1}H NMR spectrum showed five aromatic protons, two of them appear as a sharp singlet at δ 7.36 and a multiplet at δ 7.38, a doublet at δ 4.14, two methoxyls at δ 3.88 and 3.78, two methylene protons as double doublets at δ 3.12 and 2.41, a doublet of double of doublet at δ 2.76 and two singlet methyls at δ 1.22 and 1.27. The DQ-COSY experiment showed correlations between the doublet at δ 4.14 and the signal at δ 2.76, which was, in turn, correlated with both the diasterotopic methylenes. The ^{13}C NMR spectrum (Table 2) suggested a structure similar to that of carexane

Table 2
^{13}C NMR data of carexanes J–L in CD_3OD.

Position	**5** Carexane J	**6** Carexane K	**7** Carexane L
1	134.1	128.8	133.9
2	125.9	124.4	123.3
3	159.3	145.5	158.4
4	105.7	148.9	106.5
5	160.8	148.9	159.8
6	102.6	105.9	107.6
7	198.4	198.0	170.6
8	82.3	53.4	209.7
9	143.0	138.7	135.5
10	153.1	152.4	164.3
11	123.1	123.4	124.1
12	130.0	128.8	135.7
13	127.9	127.9	128.8
14	127.1	125.9	124.5
15	19.1	21.6	23.5
16	57.1	51.2	60.8
17	46.1	47.2	43.7
18	26.7	23.9	26.9
19	29.3	28.2	28.9
OMe	56.6	60.7	57.7
OMe	55.9	56.6	55.6

E (Fiorentino et al., 2006) with two methoxyls in the A ring. The position of the second methoxyl group was determined with an HMBC experiment. In fact correlations were evident between the C-5 carbon at δ 148.9 with the methoxyl at δ 3.78 and with the aromatic singlet. This was in turn correlated with the carbonyl at δ 198.0 and with the H-4 carbon at δ 147.6. The other methoxyl group correlated, in the same experiment, with the carbon at δ 145.5, which was correlated with the methylene protons at δ 3.12 and 2.41. These data allowed the second methoxyl to the C-3 carbon to be positioned.

The last isolated compound (compound **7**) was identified as the 3-methoxy derivative of carexane H (Fiorentino et al., 2006). In fact the EI MS spectrum showed a molecular peak at m/z 354 in accordance with a molecular formula $C_{21}H_{22}O_5$. Furthermore, the differences in the ^1H and ^{13}C NMR (Table 2) spectra were in good accordance with the hypothesized structure.

All of the metabolites were tested for their phytotoxicity on seeds of *Lactuca sativa*. The results of the bioassay are reported in Fig. 4. The metabolites induced a weak decrease of germination (20%) of test organism. Furthermore the

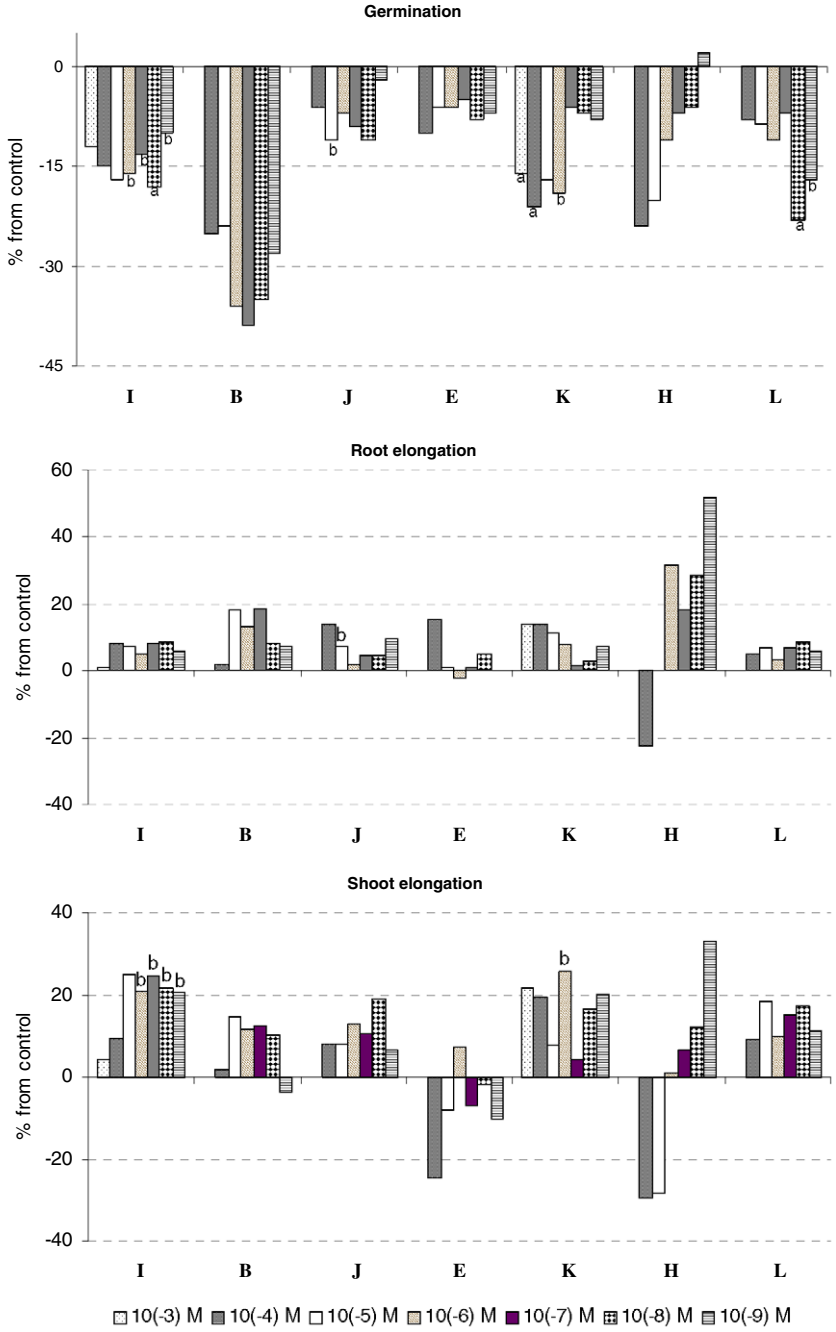


Fig. 4. Effect of carexanes I–L from *C. distachya* on germination (A), root length (B) and shoot length (C) of *Lactuca sativa* L (Data of carexanes B, E and H, are reported by Fiorentino et al. (2006)). Values are presented as percentage differences from control and are not significantly different with $P > 0.05$ for Student's t-test. (a) $P < 0.01$; (b) $0.01 < P < 0.05$.

substances showed a stimulating effect on seedling growth. This effect was more evident on shoot elongation. Compound **1** stimulated shoot elongation to the lower tested concentrations; compound **3** determined an increase of shoot elongation for all the concentrations.

The results of carexanes J, K, and L were compared with those of the corresponding demethylated carexanes B, E, and H, previously isolated from leaves of *C. distachya* (Fiorentino et al., 2006). It was observed, for example, that carexane B induced a higher inhibition of the germination in respect to the corresponding methylated carexane J. Instead opposite dose–response effect was observed for carexanes H and L for all the tested concentrations. These data suggested that methylation represents a mechanism adopted from the vegetal organism to reduce its phytotoxicity.

3. Experimental

3.1. General experiment procedures

NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian Mercury 300 spectrometer Fourier transform NMR, in CD_3OD and $CDCl_3$ solns at 25 °C. Proton-detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{HC} = 145$ Hz) and HMBC (optimized for $^nJ_{HC} = 8$ Hz). UV spectra were performed on UV-1601 Shimadzu spectrophotometer in MeOH solution. Optical rotations were measured on a Perkin–Elmer 343 polarimeter. Electronic ionization mass spectra (EI-MS) were obtained with a HP 6890 instrument equipped with a MS 5973 N detector.

The 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 Merk Kieselgel 60 F_{254} or RP-18 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 120 °C. Prep. 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), Baker Bond Phase C18 (0.040–0.063 mm), Fluka Reversed phase silica gel 100 C8 (0.040–0.063 mm) or on Sephadex LH-20® (Pharmacia).

3.2. Plant material

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

3.3. Extraction and isolation

Fresh roots of *C. distachya* (5.4 kg) were extracted with EtOAc in a refrigerated chamber at 4 °C. After three days the solution was filtered on Whatman paper, dried with Na_2SO_4 and concentrated under vacuum, yielding 100.0 g of crude residue, which was stored at −80 °C until purification.

3.3.1. Organic extract fractionation

The EtOAc extract was chromatographed on silica gel eluting with CH_2Cl_2–EtOAc solutions to obtain two fractions (I and II).

Fraction I, eluted with CH_2Cl_2–EtOAc (19:1), was purified by Sephadex LH-20® eluting with Ex–$CHCl_3$–MeOH (3:1:1) to give three fractions A–C. Fraction A was purified by TLC with hexane–EtOAc (4:1) to have pure carexane I (**4**, 35.0 mg), carexane K (**6**, 6.0 mg) and a mixture which was purified by RP-8 HPLC (MeCN–MeOH–H_2O, 1:2:2) constituted of carexane J (**5**, 4.0 mg) and carexane L (**7**, 2.0 mg). Fraction B was purified on SiO_2 column eluting with hexane–EtOAc (3:2) to have pure carexane E (**2**, 70.0 mg). Fraction C gave pure carexane D (**1**, 50.0 mg).

Fraction II, eluted with CH_2Cl_2–EtOAc (4:1), was purified by Sephadex LH-20® eluting with Ex–$CHCl_3$–MeOH (3:1:1), to furnish pure carexane F (**3**, 70.0 mg).

3.3.2. Compounds characterization

Carexane I (***4***). Amorphous powder; $[\alpha]_D^{25} + 15.6°$ (MeOH, *c* 0.1500); UV (MeOH) λ_{max} nm (log ε): 287 (2.8); ^{1}H NMR (300 MHz, CD_3OD): see Table 1; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.14, (3H, *m*, H-11–H-13), 6.86 (2H, *m*, H-10 and H-14), 6.61 (1H, *d*, *J* = 2.4 Hz, H-6), 6.45 (1H, *d*, *J* = 2.4 Hz, H-4), 4.88 (1H, *d*, *J* = 2.4 Hz, H-8), 4.81 (1H, *s*, H-18), 4.47 (1H, *s*, H-18), 3.81 (3H, *s*, OMe), 3.80 (3H, *s*, OMe), 3.42 (1H, *t*, *J* = 3.0 Hz, H-8), 2.91 (1H, *dt*, *J* = 11.7 and 3.0 Hz, H-16), 2.78 (1H, *dd*, *J* = 17.7 and 4.8 Hz, H-15), 2.29 (1H, *dd*, *J* = 17.7 and 12.3 Hz, H-15), 1.79 (3H, *s*, H-19); ^{13}C NMR (75 MHz, CD_3OD): see Table 1; EI-MS: *m*/*z* 324 $[M]^+$, 306 $[M-H_2O]^+$; elemental analysis: Found: C, 78.3; H, 7.6. $C_{21}H_{24}O_3$ requires: C, 77.7; H, 7.4%.

Carexane J (***5***). Colourless oil; $[\alpha]_D^{25} - 79.7°$ (MeOH, *c* 0.0715); UV (MeOH) λ_{max} nm (log ε): 339 (3.0), 275 (3.6), 217 (4.1); ^{1}H NMR (300 MHz, CD_3OD) δ: 7.65, (1H, *m*, H-14), 7.28 (1H, *m*, H-12), 7.22 (1H, *m*, H-13), 7.17 (1H, *m*, H-11), 7.01 (1H, *d*, *J* = 3.0 Hz, H-6), 6.78 (1H, *d*, *J* = 3.0 Hz, H-4), 3.90 (3H, *s*, OMe), 3.79 (3H, *s*, OMe), 3.72 (1H, *t*, *J* = 5.1 Hz, H-16), 3.05 (2H, *d*, *J* = 5.1 Hz, H-15), 1.43 (3H, *s*, H-19), 0.72 (3H, *s*, H-18); ^{13}C NMR: see Table 2; EI-MS: *m*/*z* 338 $[M]^+$, 320 $[M-H_2O]^+$; elemen-

tal analysis: Found: C, 75.0; H, 6.6. $C_{21}H_{22}O_4$ requires: C, 74.5; H, 6.5%.

Carexane K (**6**). Colourless oil; $[\alpha]_D^{25} + 83.7°$ (MeOH, *c* 0.1305); UV (MeOH) λ_{max} nm (log ε): 291 (3.7), 212 (4.2); ^{1}H NMR (300 MHz, CD_3OD) δ: 7.38, (1H, *m*, H-14), 7.36 (1H, *s*, H-6), 7.23 (1H, *m*, H-12), 7.20 (1H, *m*, H-13), 7.16 (1H, *m*, H-11), 4.14 (1H, *d*, *J* = 6.9 Hz, H-8), 3.88 (3H, *s*, OMe), 3.78 (3H, *s*, OMe), 3.12 (1H, *dd*, *J* = 16.5, 6.0 Hz, H-15), 2.76 (1H, *ddd*, *J* = 9.0, 6.9, 6.0 Hz, H-16), 2.41 (1H, *dd*, *J* = 16.5, 9.0 Hz, H-15), 1.27 (3H, *s*, H-19), 1.22 (3H, *s*, H-18); ^{13}C NMR: see Table 2; EI-MS: *m/z* 338 $[M]^+$, 320 $[M-H_2O]^+$; elemental analysis: Found: C, 75.2; H, 6.8. $C_{21}H_{22}O_4$ requires: C, 74.5; H, 6.5%.

Carexane L (*7*). Colourless oil; $[\alpha]_D^{25}0°$ (MeOH, *c* 0.069); UV (MeOH) λ_{max} nm (log ε): 294 (4.3), 244 (4.8); ^{1}H NMR (300 MHz, CD_3OD) δ: 7.65, (1H, *m*, H-12), 7.63 (1H, *m*, H-14), 7.58 (1H, *m*, H-11), 7.38 (1H, *m*, H-13), 7.16 (1H, *m*, H-11), 6.88 (1H, *d*, *J* = 2.4 Hz, H-6), 6.57 (1H, *d*, *J* = 2.4 Hz, H-4), 3.81 (3H, *s*, OMe), 3.77 (3H, *s*, OMe), 3.38 (1H, *dd*, *J* = 13.8, 7.8 Hz, H-15), 3.17 (1H, *dd*, *J* = 13.8, 6.6 Hz, H-15), 2.84 (1H, *dd*, *J* = 7.8, 6.6 Hz, H-16), 3.72 (1H, *t*, *J* = 5.1 Hz, H-16), 1.32 (3H, *s*, H-19), 1.28 (3H, *s*, H-18); ^{13}C NMR: see Table 2; EI-MS: *m/z* 354 $[M]^+$, 336 $[M-H_2O]^+$; elemental analysis: Found: C, 71.9; H, 6.6. $C_{21}H_{22}O_5$ requires: C, 71.2; H, 6.2%.

3.3.3. Preparation of (S) and (R)-MTPA esters of carexane I

(*R*)-(−)-MTPA chloride (5 μl, 26 μmol) was added to a solution of pure compound (1.5 mg) 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 prep. TLC eluting with hexane–EtOAc (4:1). The (*S*)-MTPA ester of **4** had the ^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ: 7.16, (3H, *m*, H-11–H-13), 6.87 (2H, *m*, H-10 and H-14), 6.44 (1H, *d*, *J* = 2.4 Hz, H-6), 6.38 (1H, *d*, *J* = 2.4 Hz, H-4), 6.32 (1H, *d*, *J* = 2.9 Hz, H-7), 4.82 (1H, *s*, H-18), 4.46 (1H, *s*, H-18), 3.79 (3H, *s*, OMe), 3.70 (3H, *s*, OMe), 3.47 (1H, *t*, *J* = 2.9 Hz, H-8), 2.86 (1H, H-16, detected by DQ-COSY), 2.78 (1H, *dd*, *J* = 17.7 and 4.8 Hz, H-15), 2.29 (1H, *dd*, *J* = 17.7 and 12.3 Hz, H-15), 1.73 (3H, *s*, H-19). The (*R*)-MTPA ester of **4** had the ^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ: 7.15, (3H, *m*, H-11–H-13), 6.84 (2H, *m*, H-10 and H-14), 6.54 (1H, *d*, *J* = 2.4 Hz, H-6), 6.48 (1H, *d*, *J* = 2.4 Hz, H-4), 6.38 (1H, *d*, *J* = 2.4 Hz, H-7), 4.77 (1H, *s*, H-18), 4.40 (1H, *s*, H-18), 3.81 (3H, *s*, OMe), 3.75 (3H, *s*, OMe), 3.33 (1H, *m*, H-8), 2.75 (1H, H-16, detected by DQ-COSY), 2.75 (1H, H-15, detected by DQ-COSY), 2.25 (1H, *dd*, *J* = 18.3 and 13.5 Hz, H-15), 2.14 (3H, *s*, H-19).

3.4. 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. For bioassay Petri dishes (50 mm diameter) with one sheet of Whatman No. 1 filter paper as support were used. In four replicate experiments, germination and growth were conducted in aqueous solutions at controlled pH. Test solutions (10^{-3} M) were prepared using 2-[*N*-morpholino]ethanesulfonic acid (MES; 10 mM, pH 6) and the rest (10^{-4}–10^{-9} M) were obtained by dilution. Parallel controls were performed. After the addition of 25 seeds and 2.5 ml test solns, the 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 five 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

Callaway, R.M., Aschehoug, E.T., 2000. Invasive plants versus their new and old neighbours: a mechanism for exotic invasion. Science 290, 521–523.

D'Abrosca, B., Della Greca, M., Fiorentino, A., Monaco, P., Previtera, L., Simonet, A.M., Carrelli, A., 2001. Potential allelochemicals from *Sambucus nigra*. Phytochemistry 58, 1073–1081.

D'Abrosca, B., Della Greca, M., Fiorentino, A., Monaco, P., Natale, A., Oriano, P., Zarrelli, A., 2005a. Structural characterization of phytotoxic terpenoids from *Cestrum parqui*. Phytochemistry 66, 2681–2688.

D'Abrosca, B., Fiorentino, A., Golino, A., Monaco, P., Oriano, P., Pacifico, S., 2005b. Carexanes: prenyl stilbenoid derivatives from *Carex distachya*. Tetrahedron Lett. 46, 5269–5272.

D'Abrosca, B., De Maria, P., Della Greca, M., Fiorentino, A., Golino, A., Izzo, A., Monaco, P., 2006. Amarantholidols and amarantholidosides: new nerolidol derivatives from the weed *Amaranthus retroflexus*. Tetrahedron 62, 640–646.

Dayan, F.E., Romagni, J.G., Duke, S.O., 2000. Investigating the mode of action of natural phytotoxins. J. Chem. Ecol. 26, 2079–2094.

Fiorentino, A., D'Abrosca, B., Izzo, A., Pacifico, S., Monaco, P., 2006. Structural elucidation and bioactivity of novel secondary metabolites from *Carex distachya*. Tetrahedron 62, 3259–3265.

Fitter, A., 2003. Making allelopathy respectable. Science 301, 1337–1338.

Harborne, J.B., 1997. Recent advances in chemical ecology. Nat. Prod. Rep. 14, 83–98.

Ohtani, I., Kusumi, T., Kashman, Y., Kakisawa, H., 1991. High-field NMR application of Mosher's method. The absolute configurations of marine terpenoids. J. Am. Chem. Soc. 113, 4092–4096.

Rice, E.L., 1984. Allelopathy, 2nd ed. Academic Press, Orlando, FL.

Turk, M.A., Tawaha, A.M., 2003. Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). Crop Prot. 22, 673–677.