Tetrahedron 64 (2008) 7782–7786

Contents lists available at ScienceDirect

Tetrahedron

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

Carexanes from *Carex distachya* Desf.: revised stereochemistry and characterization of four novel polyhydroxylated prenylstilbenes

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ARTICLE INFO

Article history:
Received 7 April 2008
Received in revised form 13 May 2008
Accepted 30 May 2008
Available online 5 June 2008

Keywords:
Carex distachya Desf.
Carexanes
Prenylstilbenes
NMR analysis
X-ray

ABSTRACT

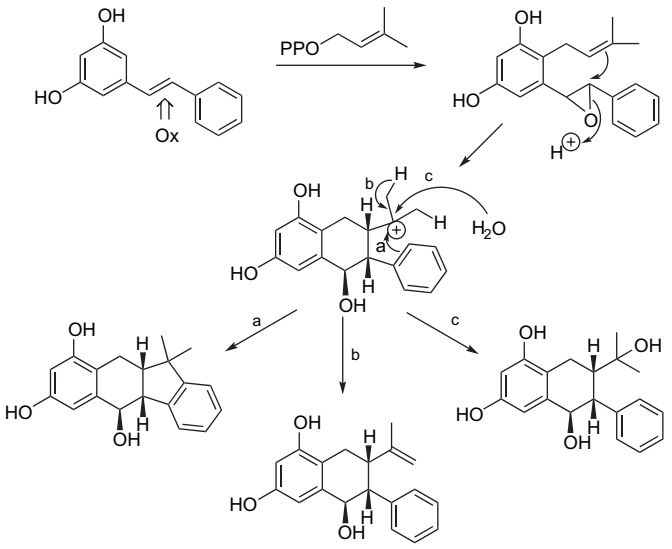
Carexanes are unusual secondary metabolites isolated from the herbaceous plant *Carex distachya*. Four new carexanes have been isolated and characterized from the methanolic leaf extract of the plant. All of the structures have been elucidated on the basis of spectroscopic data. The stereochemistry of all the carexane metabolites isolated from the plant, as well as their ^{1}H and ^{13}C NMR spectroscopic assignment, has been revised on the basis of X-ray analysis of carexane D, chemical correlations, and NOESY/ROESY experiments. All the data suggested a cis junction between B and C rings.

1. Introduction

Plants of the *Carex* genus produce oligostilbenes, comprising from two to four monomers of resveratrol (3,5,4-trihydroxystilbene), most of them showing antimicrobial activity.[1,2] Carexanes are new secondary metabolites isolated from *Carex distachya* Desf., characterized by a unusual tetracyclic skeleton arising from a cyclization of prenyl stilbenoid precursors (Scheme 1).[3] The isolation and the characterization of nine tetracyclic structures (**1**–**9**) have been reported,[4] together with a further three tricyclic derivatives[5] and distachyasin (**10**),[6] a carexane metabolite with high antioxidative properties, comparable to those showed by the ascorbic acid. In this work, we report on the revised stereochemistry of these compounds, determined on the basis of the X-ray data, the corrected NMR assignments, and the isolation and structural characterization of four new derivatives. The new structures **11**–**14** were elucidated by EIMS, 1D and 2D NMR spectroscopies (COSY, TOCSY, HSQC, HMBC, NOESY, and ROESY), energy-minimized models, and by correlations with the previously characterized structures.

2. Results and discussion

The structures of carexanes **1**–**9** have been elucidated on the basis of their spectroscopic features, especially 1D and 2D NMR



Scheme 1. Plausible biosynthetic pathway of carexane skeletons.

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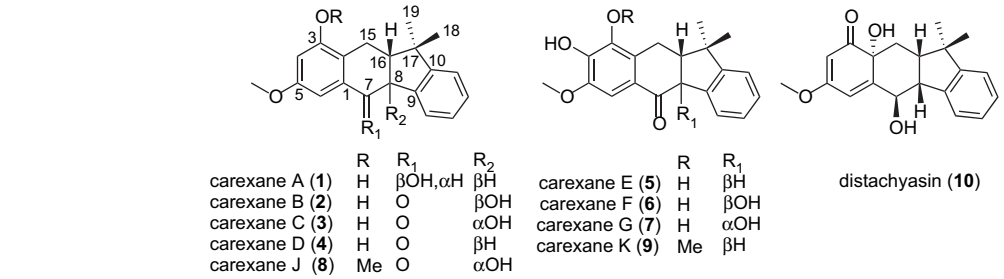
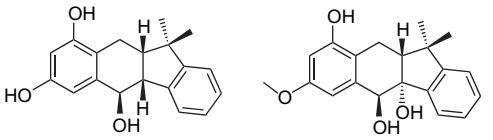
doi:10.1016/j.tet.2008.05.137



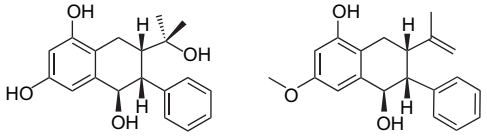
	R	R_1	R_2
carexane A (**1**)	H	βOH,αH	βH
carexane B (**2**)	H	O	βOH
carexane C (**3**)	H	O	αOH
carexane D (**4**)	H	O	βH
carexane J (**8**)	Me	O	αOH

	R	R_1
carexane E (**5**)	H	βH
carexane F (**6**)	H	βOH
carexane G (**7**)	H	αOH
carexane K (**9**)	Me	βH

distachyasin (**10**)



carexane M (**11**) carexane N (**12**)



carexane O (**13**) carexane P (**14**)

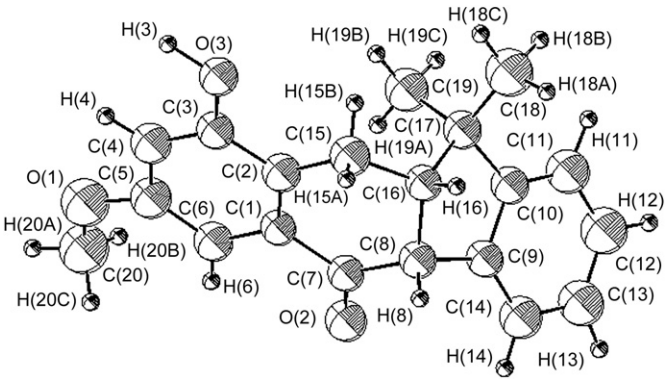


Figure 1. X-ray diffraction structure of carexane D (**4**), with the numbering of the atoms. Displacement ellipsoids are drawn at 50% probability level.

spectroscopic data.[3–6] In particular, the absolute configuration relative to the C-7 carbon of carexane A (**1**) was established by Mosher's method, while the configuration of the C-8 and C-16 chiral carbons was determined, in respect to the C-7 carbon, on the basis of the *J* values and NOE. While the *J* value of the H-7 proton (*J*=8.1 Hz) was in good accordance with a trans coupling with the H-8 double doublet, the *J* value of the latter, due to the coupling with the H-16 proton, was ambiguous (*J*=7.8 Hz) and a trans orientation was assigned on the basis of NOE showed by NOESY experiments.[3]

The complete stereochemistry of distachyasin **10**, having a tertiary hydroxyl at the C-2 carbon, required the application of X-ray diffractometric studies.[6] The high abundance of this metabolite in the plant material and its inclination to crystallize allowed unequivocal assignment of the relative configuration at all the stereogenic centers by X-ray and the absolute configuration by correlating them to the C-7 secondary carbinol, whose absolute stereochemistry was defined by Mosher's method. The crystallographic data confirmed a trans orientation among the H-7 and H-8 protons, but showed a cis orientation among the H-8 and H-16 protons, contrarily to what established for the carexanes. This data led us to hypothesize a mistake in the interpretation of previous data. In fact, by postulating an inversion of H-19 with the H-18 methyls and of H-15α with the H-15β protons, the NMR data matched this hypothesis. In order to solve this, crystallographic experiments were required, but each attempt to crystallize the most abundant carexanes failed because of their oily consistency.

By continuing the phytochemical investigation of *C. distachya*, the organic fraction obtained by shaking the MeOH extract with EtOAc and water was studied. The chromatographic processes led to the re-isolation of the previous carexanes and four new polyhydroxylated derivatives.

Among the others, carexane D was isolated in high amount from the EtOAc solution, and suitable crystals of this compound for X-ray crystallographic analysis were obtained by slow evaporation of a chloroform–methanol (1:2) solution at 4 °C. The solid state molecular structure of carexane D is shown in Figure 1.

The torsion angles H(8)–C(8)–C(16)–H(16) and H(8)–C(8)–C(7)–O(2) were 35.0(5)° and −80.3(6)°, respectively, showing synclinal (*sc*) conformation and a cis orientation between the H-8 and H-16 protons. The torsion angles H(16)–C(16)–C(17)–C(18) and H(16)–C(16)–C(17)–C(19) were, respectively, −30.6(5)° and −154.7(6)°, in accordance with the cis–trans orientation of the two methyl groups bonded at C(17). Furthermore, the angle 67(2)° between the plane identified by C(2)–C(3)–C(4) and C(9)–C(10)–C(11) indicates molecular folding.

In the crystal packing, the molecules are characterized by an intermolecular bond, two molecules are connected along the *b* axis by one hydrogen bond and the O(3) and O(2) oxygens [distance O(3)···O(2) 2.71(10) Å; angle O(3)···O(2)–C(7) 130.5(5)°]. This arrangement gives rise to a polymeric inclusion structure forming low rows. These rows are arranged in layers of parallel molecules along the ac plane. The layers pack in an antiparallel layout.

These data indicated a cis orientation for the H-8 and H-16 protons, which was confirmed by NOEs observed in NOESY and ROESY experiments, re-performed with a mixing time of 500 ms. In these experiments, NOEs between the H-8 and H-16 protons and the methyl at δ 1.27 were also evident, as well as those between the H-16 proton and the other methyl at δ 1.16 and with both the H-15 protons. These data allowed correction of the initial NMR assignment, and we have reported the definitive assignments in Supplementary data.

The conversion of carexane D into carexane A and its 7*S* epimer, by reduction with $NaBH_4$,[4] confirmed the cis orientation of the H-8 and H-16 protons for **1** and, consequently, to assign them the *R* and *S* configurations, respectively. Comparison of the NMR data of compounds **5** and **9** with those of previous compounds as well as the results of NOESY and ROESY experiments confirmed the same stereochemistry for these metabolites.

On this basis, we also revised the structure of the remaining carexanes, whose NMR data are reported in Supplementary data.

The new compounds **11**–**14** were isolated from the MeOH extract and structurally elucidated by spectroscopic techniques, mainly 1D and 2D NMR spectroscopy.

Compound **11** had a molecular formula $C_{19}H_{20}O_3$ in accordance with the EIMS and ^{13}C NMR data. The ^1H NMR spectrum showed six

aromatic protons as a multiplet at δ 7.61 and two doublets at δ 6.58 and 6.20, besides three overlapped signals ranging from 7.12 to 7.21 ppm. In the same spectrum, two methines as a doublet at δ 4.46 (J=8.0 Hz), a multiplet at δ 2.15, a methylene as two double doublets at δ 3.07 and 1.84, and two methyls as singlets at δ 1.34 and 1.20 were evident. In the DQ-COSY, the signal at δ 4.46 correlated with a signal at δ 3.35, obscured in the ^{1}H NMR spectrum by the solvent signal; the proton at δ 3.35 correlated with the methine at δ 2.15, which was, in turn, correlated with the methylene protons. The ^{13}C NMR spectrum showed 19 signals, identified, on the basis of a DEPT experiment, as two methyls, a methylene, three aliphatic and six aromatic methines, and seven tetrasubstituted carbons. The NMR values and the heterocorrelations showed by the HMBC experiment indicated the structure of a 3,5,7-trihydroxycarexane. 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.[7] The NOEs of the H-16 proton with the H-8, H-18 protons, as well as the *J* value of the H-7 proton agreed with the structure of (7*R*,8*R*,16*S*)-3,5,7-trihydroxycarexane for compound **11**.

Compound **12** had a molecular formula $C_{20}H_{22}O_4$ according to the NMR and EIMS data. The ^{1}H NMR spectrum showed the aromatic protons as three doublets at δ 7.68 (J=7.8 Hz), 6.69 (J=2.1 Hz), and 6.29 (J=2.1 Hz), and three overlapped protons centered at δ 7.17. In the upfield region of the spectrum, two methines as a singlet at δ 4.51, a multiplet at δ 2.34, a methylene as two signals at δ 3.09 and 2.35, and three methyl signals as singlets at δ 3.74, 1.39, and 1.23 were evident. The ^{13}C NMR and the DEPT experiments confirmed the presence of three methyls, a methylene, eight methines, and eight tetrasubstituted carbons. The 2D NMR experiment allowed the complete characterization of the molecule. In particular, the proton at δ 4.51 correlated to the carbon at δ 86.0 in the HSQC experiment and to the carbons at δ 144.9 (C-1), 117.6 (C-2), 101.7 (C-6), 96.0 (C-8), 140.8 (C-9), and 57.9 (C-16) in the HMBC experiment. The long range heterocorrelations between the carbon at δ 57.9 with the methyls at δ 1.23 and 1.39, as well as those between the proton at δ 6.69 (bonded to the carbon at δ 101.7) with the C-1, C-2, C-4, and C-5 aromatic carbons of the A ring, allowed the localization of the singlet proton at δ 4.51 to the C-7 carbon. Finally, the carbinol carbon at δ 96.0 correlated with the H-7 proton (δ 4.51), the H-15 methylenes, and H-16 methine, and with the H-14 proton at δ 7.68. These data were in good accordance with the structure of a carexane bringing a methoxyl at the C-5 carbon and three hydroxyls at the C-3, C-7, and C-8 carbons.

Compound **13** had a molecular formula $C_{19}H_{22}O_4$ according to the presence of 19 signals in the ^{13}C NMR and the presence of the molecular ion at *m*/*z* 314 in the EIMS spectra. The ^{1}H NMR spectrum showed signals due to the phenyl group as two overlapped signals at δ 7.13 (three protons) and 6.99 (two protons), besides a singlet at δ 6.31 integrated for two protons. In the same spectrum, a doublet at δ 4.38 and a broad singlet at δ 3.46 were also evident together to the H-16 methine at δ 2.47, the H-15 methylenes at δ 2.98 and 2.38, and the H-18 and H-19 methyls at δ 0.92 and 1.14. The ^{13}C NMR and DEPT experiments indicated the presence of 2 methyls, a methylene, 10 methines, and 6 tetrasubstituted carbons. The HSQC experiment indicated the connections between all the protons with the corresponding carbons of the molecule. Among the others, the doublet at δ 4.38 correlated to the carbon at δ 74.9, while the proton at δ 3.46 showed correlation with the carbon at δ 49.5. This carbon was heterocorrelated, in the HMBC experiment, with the H-15 and H-16 protons, with the signal at δ 4.38 and with the aromatic signal at δ 6.99. On the other hand, the proton at δ 3.46 correlated with both the carbinols at δ 74.0 and 74.9, with the aromatic carbons at δ 138.0 and 130.0, besides the C-15 and C-16 carbons. The carbon at δ 74.0 correlated with both the methyl groups, with the methylene protons, and with the methine at δ 2.47. The absolute configuration to the C-7 carbon has been established by Mosher's method as *R*. The relative configurations of the C-8 and C-16 carbons were established on the basis of the NOESY and ROESY experiments. The experimental data have been supported by the lowest-energy conformation of the 7*R*,8*R*,16*S* isomer, obtained by the MM$^+$ molecular mechanic method as implemented in HyperChem 8.0 (Fig. 2). In this model, the H-7α and H-8 protons distanced 2.55 Å and had a dihedral angle H(7)–C(7)–C(8)–H(8) of $-75.9(4)^\circ$. The dihedral angle H(8)–C(8)–C(16)–H(16) was found to be $-57.5(0)^\circ$ and, consequently, the distance among the H-8 and H-16 protons measured was 2.42 Å. Accordingly, the observed NOE in the NOESY and ROESY experiments of the H-8 proton with the H-7, H-16, and H-10/H-14 protons confirmed the hypothesis.

The last compound **14** had the molecular formula $C_{20}H_{22}O_3$ and showed NMR (Table 1) data comparable to those reported for the carexane I.[5] Differences were imputable to the substitution of a methoxyl at the C-3 carbon with a hydroxyl group. The 2D NMR and EIMS experiments confirmed this hypothesis.

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 Fourier transform NMR spectrometer in CD_3OD at 25 °C. The spectrum width was 2300 Hz. The initial matrix of 2 k×2 k data points was zero-filled to give a final matrix of 4 k×4 k points. The TOCSY experiments were performed in the phase-sensitive mode with a mixing time of 90 or 120 ms. The spectral width was 3000 Hz. The initial matrix of 512×512 data points was zero-filled to give a final matrix of 1 k×1 k points. The ROESY and NOESY experiments were performed in the phase-sensitive mode. The mixing time was 500 ms and the spectral width was 3000 Hz. The initial matrix of 512×512 data points was zero-filled to give a final matrix of 1 k×1 k points. Proton-detected heteronuclear correlations were measured using a gradient heteronuclear single-quantum coherence (HSQC), optimized for $^1J_{HC}$=140 Hz, a gradient heteronuclear multiple bond coherence (HMBC), optimized for $^nJ_{HC}$=8 Hz. The HSQC experiment was performed in the phase-sensitive mode with field gradient. The spectral width was 22,000 Hz in f_1 (^{13}C) and 3200 Hz in f_2 (^{1}H), and the matrix of 1 k×1 k data points was zero-filled to give a final matrix of 2 k×2 k points. The HMBC experiment was performed in the absolute value

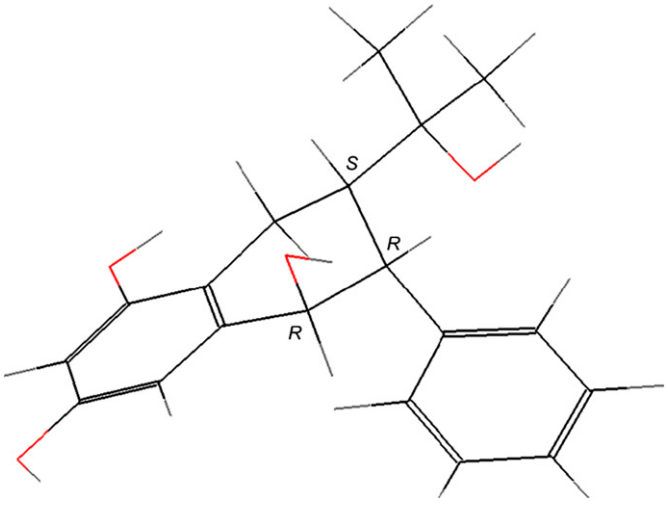


Figure 2. MM$^+$ geometry-optimized structure of 7*R*,8*R*,16*S* stereoisomer of carexane O (**13**).

Table 1
^1H and ^{13}C NMR data of carexanes M–P in CD_3OD

Position	Carexane M (**11**)		Carexane N (**12**)		Carexane O (**13**)		Carexane P (**14**)	
	δ ^1H	δ ^{13}C	δ ^1H	δ ^{13}C	δ ^1H	δ ^{13}C	δ ^1H	δ ^{13}C
1	—	145.0	—	144.9	—	138.0	—	140.0
2	—	117.6	—	117.6	—	117.5	—	120.2
3	—	155.1	—	155.1	—	157.2	—	156.1
4	6.20 d (2.4)	99.7	6.29 d (2.1)	100.5	6.31 d (0.9)	109.7	6.37 d (2.7)	98.0
5	—	156.2	—	159.9	—	157.2	—	160.5
6	6.58 d (2.4)	104.0	6.69 d (2.1)	101.7	6.31 d (0.9)	109.7	6.55 d (2.7)	106.5
7	4.46 d (8.0)	74.5	4.51 s	86.0	4.38 d (1.8)	74.9	4.73 d (2.7)	73.2
8	3.35 ob	51.8	—	96.0	3.46 br s	49.5	3.53 m	51.4
9	—	145.0	—	140.8	—	141.9	—	140.6
10	—	153.3	—	152.3	6.99 ov	130.0	6.85 ov	129.9
11	7.18 ov	123.0	7.15 ov	123.3	7.13 ov	128.8	7.11 ov	128.6
12	7.20 ov	128.2	7.19 ov	128.7	7.13 ov	127.5	7.11 ov	127.4
13	7.20 ov	127.9	7.16 ov	127.8	7.13 ov	128.8	7.11 ov	128.6
14	7.61 m	125.7	7.68 d (7.8)	125.8	6.99 ov	130.0	6.85 ov	129.9
15	3.07 dd (14.1, 5.4), 1.84 dd (14.1, 11.7)	21.7	3.09 dd (6.0, 15.7) 2.35 ob	20.6	2.98 dd (14.5, 5.8), 2.38 m	23.4	2.68 dd (17.4, 4.5), 2.22 dd (17.4, 12.2)	24.1
16	2.15 m	51.3	2.34 m	57.9	2.47 m	48.1	2.96 dt (12.2, 4.5)	40.5
17	—	46.4	—	44.1	—	74.0	—	147.9
18	1.20 s	32.7	1.39 s	32.4	0.92 s	27.0	4.57 s, 4.44 s	111.7
19	1.34 s	24.5	1.23 s	24.3	1.14 s	27.9	1.80 s	22.8
OMe	—	—	3.74 s	56.2	—	—	3.77 s	55.7

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

mode with field gradient. The spectral width was 20,000 Hz in f_1 (^{13}C) and 1100 Hz in f_2 (^1H), and the matrix of 1 k×1 k data points was zero-filled to give a final matrix of 4 k×4 k points. UV spectra were performed on UV-1700 Shimadzu spectrophotometer in MeOH solution. Optical rotations were measured on a Perkin–Elmer 141 in MeOH solution. IR spectra were determined on an FTIR Perkin–Elmer 1740 spectrophotometer in $CHCl_3$. Electron ionization mass spectra (EIMS) were obtained with an HP 6890 instrument equipped with an MS 5973N 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 the Nature Reserve of 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* (6.0 kg) were extracted with MeOH for 5 days at 4 °C in the dark chamber. After the removal of the solvent, the residual plant material was re-extracted first with EtOAc and then with hexane, each one for 5 days. The organic solutions were distilled under reduced pressure by a Rotavapor® to obtain three crude extracts. The MeOH extract (117.0 g) was dissolved in water and shaken with EtOAc. The organic solution was distilled under reduced pressure to give a crude EtOAc fraction (38.8 g).

The EtOAc fraction was chromatographed by SiO_2 CC using hexane–EtOAc solutions as eluent and affording fractions A–F.

Fraction A, eluted with hexane–EtOAc (5:1), was re-chromatographed on Sephadex LH-20 eluting with hexane–$CHCl_3$–MeOH (3:1:1). The eluate was purified by TLC eluting with hexane–EtOAc (4:1) to obtain pure carexane D (**4**, 40.0 mg).

Fraction B, eluted with hexane–EtOAc (4:1), contained carexanes A–C and E (**1**–**3** and **5**: 35.0 mg, 33.0 mg, 24.0 mg, and 22.0 mg, respectively).

Fraction C, eluted with hexane–EtOAc (3:1), was chromatographed on Sephadex LH-20 eluting with hexane–$CHCl_3$–MeOH (3:1:1) to obtain a fraction, which was purified by SiO_2 HPLC using $CHCl_3$–EtOAc–AcOH (169:30:1) as mobile phase to give pure carexane M (**11**, 4.8 mg).

Fraction D, eluted with hexane–EtOAc (7:3), was re-chromatographed on Sephadex LH-20 with hexane–$CHCl_3$–MeOH (3:1:1) to obtain two fractions; the first was purified by RP-8 HPLC eluting with MeOH–MeCN–H_2O (2:2:1) to obtain pure carexane N (**12**, 1.4 mg); the second fraction was first chromatographed by FCC ($CHCl_3$–EtOAc, 17:3), and the eluate purified by RP-8 HPLC (MeOH–MeCN–H_2O, 1:1:2) to give pure carexane P (**14**, 3.4 mg).

Fraction E, eluted with hexane–EtOAc (3:2) and purified on Sephadex LH-20 eluting with hexane–$CHCl_3$–MeOH (3:1:1), contained carexane F (**6**), carexane G (**7**), and the distachyasin (**10**) (102.0 mg, 64.0 mg, and 162.0 mg, respectively).

Fraction F, eluted with EtOAc, was chromatographed on Sephadex LH-20 eluting with hexane–$CHCl_3$–MeOH (2:1:1). One of the obtained fractions was re-chromatographed by RP-8 CC (MeCN-H_2O, 1:4) to furnish carexane O (**13**, 8.0 mg).

3.3. Characterization of the carexanes M–P

*Carexane M (**11**).* Colorless oil. Found: C, 76.7; H, 6.7. $C_{19}H_{20}O_3$ requires: C, 77.0; H, 6.8. $[\alpha]^{25}_D$ +6.9 (*c* 0.14, MeOH); UV (MeOH) λ_{max} nm (log ε): 298 (3.1), 264 (3.0), 251 (4.3); ν_{max} ($CHCl_3$) 3841, 1621; δ_H (300 MHz, CD_3OD) 7.61 (1H, m, H-14), 7.20–7.18 (3H, ov, H-11/H-13), 6.58 (1H, d, *J* 2.4 Hz, H-6), 6.20 (1H, d, *J* 2.4 Hz, H-4), 4.46 (1H, d, *J* 8.0 Hz, H-7), 3.35 (1H, ob, H-8), 3.07 (1H, dd, *J* 14.1, 5.4 Hz, H-15), 2.15 (1H, m, H-16),1.84 (1H, dd, *J* 14.1, 11.7 Hz, H-15), 1.34 (3H, s, H-19),1.20 (3H, s, H-18); δ_C (75 MHz, CD_3OD) 156.2, 155.1, 153.3, 145.0,

145.0, 128.2, 127.9, 125.7, 123.0, 117.6, 104.0, 99.7, 74.5, 51.8, 51.3, 46.4, 32.7, 24.5, 21.7; EIMS: m/z 296 $[M]^+$, 278 $[M-H_2O]^+$.

Carexane N (***12***). Amorphous powder. Found: C, 73.5; H, 6.8. $C_{20}H_{22}O_4$ requires: C, 73.6; H, 7.0. $[\alpha]^{25}_D$ −20.0 (c 0.01, MeOH); UV (MeOH) λ_{max} nm (log ε): 264 (5.2), 257 (5.3); ν_{max} ($CHCl_3$) 3835, 1641; δ_H (300 MHz, CD_3OD) 7.68 (1H, d, J 7.8 Hz, H-14), 7.19–7.16 (3H, ov, H-11/H-13), 6.69 (1H, d, J 2.1 Hz, H-6), 6.29 (1H, d, J 2.1 Hz, H-4), 4.51 (1H, s, H-7), 3.74 (3H, s, *OMe*), 3.09 (1H, dd, J 15.7, 6.0 Hz, H-15), 2.35 (1H, ob, H-15), 2.34 (1H, m, H16), 1.39 (3H, s, H-18),1.23 (3H, s, H-19); δ_C (75 MHz, CD_3OD) 159.9, 155.1, 152.3, 144.9, 140.8, 128.7, 127.8, 125.8, 123.3, 117.6, 101.7, 100.5, 96.0, 86.0, 57.9, 56.2, 44.1, 32.4, 24.3, 20.6; EIMS: m/z 326 $[M]^+$, 308 $[M-H_2O]^+$, 293 $[M-H_2O-CH_3]^+$.

Carexane O (***13***). Colorless oil. Found: C, 72.8; H, 6.7. $C_{19}H_{22}O_4$ requires: C, 72.6; H, 7.0. $[\alpha]^{25}_D$ +10.0 (c 0.04, MeOH); UV (MeOH) λ_{max} nm (log ε): 264 (5.1), 257 (5.3); ν_{max} ($CHCl_3$) 3837, 2852, 1606; δ_H (300 MHz, CD_3OD) 7.13–6.99 (5H, ov, H-10/H-14), 6.31 (1H, d, J 0.9 Hz, H-4), 6.31 (1H, d, J 0.9 Hz, H-6), 4.38 (1H, d, J 1.8 Hz, H-7), 3.46 (1H, br s, H-8), 2.98 (1H, dd, J 14.5, 5.8 Hz, H-15), 2.47 (1H, m, H-16), 2.38 (1H, m, H-15), 1.14 (3H, s, H-19),0.92 (3H, s, H-18); δ_C (75 MHz, CD_3OD) 157.2, 157.2, 141.9, 140.8, 130.0, 130.0, 128.8, 128.8, 127.5, 117.5, 109.7, 109.7, 74.9, 74.0, 49.5, 48.1, 27.9, 27.0, 23.4; EIMS: m/z 314 $[M]^+$, 296 $[M-H_2O]^+$.

Carexane P (***14***). Amorphous powder. Found C, 77.5; H, 6.9.$C_{20}H_{22}O_3$ requires: C, 77.4; H, 7.1. $[\alpha]^{25}_D$ −26.7 (c 0,02, MeOH); UV (MeOH) λ_{max} nm (log ε): 338 (3.7), 299 (3.5), 257 (5.4); ν_{max} ($CHCl_3$) 3851, 1621; δ_H (300 MHz, CD_3OD) 7.11–6.85 (5H, ov, H-10/H-14), 6.55 (1H, d, J 2.7 Hz, H-6), 6.37 (1H, d, J 2.7 Hz, H-4), 4.73 (1H, d, J 2.7 Hz, H-7), 4.57 (1H, s, H-18), 4.44 (1H, s, H-18), 3.77 (3H, s, *OMe*); 3.53 (1H, m, H-8), 2.96 (1H, dt, J 12.2, 4.5 Hz, H-16), 2.68 (1H, dd, J 17.4, 4.5 Hz, H-15), 2.22 (1H, dd, J 17.4, 12.2 Hz, H-15), 1.80 (3H, s, H-19),0.92 (3H, s, H-19); δ_C (75 MHz, CD_3OD) 160.5, 156.1, 147.9, 140.6, 140.0, 140.8, 129.9, 129.9, 128.6, 128.6, 120.2, 120.2, 111.7, 106.5, 98.0, 73.2, 55.7, 51.4, 40.5, 24.1, 22.8; EIMS: m/z 310 $[M]^+$, 292 $[M-H_2O]^+$.

3.3.1. Preparation of (S) and (R)-MTPA esters of compounds ***11*** and ***13***

(*R*)-(−)-MTPA chloride (5 μL, 26.0 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 (5.0 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 (3:2).

The (*S*)-MTPA diester of **11** had the ^{1}H NMR spectral data (300 MHz, CD_3OD) δ: 7.73–7.10 (4H, ov, H-11/H-14), 6.72 (1H, d, J=2.2 Hz, H-6), 6.58 (1H, d, J=2.2 Hz, H-4), 6.28 (1H, d, J=4.5 Hz, H-7), 4.00 (1H, m, H-8), 2.42 (1H, m, H-16), 2.25 (2H, m, H-15), 1.15 (3H, s, H-18), 0.95 (3H, s, H-19).

The (*R*)-MTPA diester of **11** had the ^{1}H NMR spectral data (300 MHz, CD_3OD) δ: 7.72–6.96 (4H, ov, H-11/H-14), 6.85 (1H, d, J=2.3 Hz, H-6), 6.60 (1H, d, J=2.3 Hz, H-4), 6.35 (1H, d, J=4.2 Hz, H-7), 3.95 (1H, m, H-8), 2.38 (1H, m, H-16), 2.30 (2H, m, H-15), 1.09 (3H, s, H-18), 0.84 (3H, s, H-19).

The (*S*)-MTPA diester of **13** had the ^{1}H NMR spectral data (300 MHz, CD_3OD) δ: 7.20 (3H, m, H-11/H-13), 6.88 (2H, m, H-10 and H-14), 6.23 (1H, d, J=0.9 Hz, H-6), 6.23 (1H, d, J=0.9 Hz, H-4), 6.06 (1H, d, J=3.7 Hz, H-7), 3.40 (1H, m, H-8), 2.31 (2H, m, H-15), 2.28 (1H, m, H-16), 1.19 (3H, s, H-19), 0.95 (3H, s, H-18).

The (*R*)-MTPA diester of **13** had the ^{1}H NMR spectral data (300 MHz, CD_3OD) δ: 7.18 (3H, m, H-11/H-13), 6.86 (2H, m, H-10 and H-14), 6.42 (1H, d, J=0.9 Hz, H-6), 6.42 (1H, d, J=0.9 Hz, H-4), 6.18 (1H, d, J=3.7 Hz, H-7), 3.32 (1H, m, H-8), 2.28 (2H, m, H-15), 2.20 (1H, m, H-16), 1.11 (3H, s, H-19), 0.90 (3H, s, H-18).

3.4. X-ray diffraction analyses of compound 4

Crystallography. Suitable crystals of *carexane D* for X-ray analysis were obtained by slow evaporation of MeOH–$CHCl_3$ at 4 °C. *Crystal Data for carexane D*: $C_{20}H_{20}O_3$, M=308.377, orthorhombic, space group $P2_12_12_1$, a=6.04(5) Å, b=13.82(5) Å, c=19.48(5) Å, V=1627(1) Å^3, Z=4, d=1.259 g/cm^3, crystal dimensions 0.70×0.20×0.23 mm were used for measurements on a Nonius MACH3 diffractometer with a graphite monochromator ($\omega-2\theta$ scans, $2\theta_{max}$) 50.0°, Mo Kα radiation (λ=0.70930 Å). Data analysis was performed with the maXus program.[8] The independent reflections were measured in the θ range 2–25°. Unit cell parameters were determined by least-squares refinement of the setting angles of 25 high-angle reflections ($5°<\theta<12°$). Three standard reflections were monitored periodically and showed no significant change during data collection. A total of 3820 independent reflections of which 1958 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: $R1$=0.0608, $wR2$=0.1437, S=1.132, $(\Delta/\sigma)_{max}$=0.001, $(\Delta/\Delta)_{min}$=−0.235 e/Å^3, $(\Delta/\Delta)_{max}$=0.169 e/Å^3. Using a prescan speed of 4.12 deg min, reflections with a net intensity $I<0.5\theta$ (I) were flagged as 'weak'; those with $I\geq 0.5\sigma$ (I) were measured at lower speed depending on the value of $\sigma(I)/I$. The structure was solved by direct methods using the SIR 92 program.[9] The best E maps revealed all the non-H atoms. Refinement by the full-matrix least-squares procedure on F^2 (all data) used the SHELXL97 program with anisotropic thermal factors for all non-hydrogen atoms.[10] Hydrogen atom positions were calculated and during the refinement was allowed to ride on their carrying atoms, with $U_{iso}=U_{eq}$ 1.2 of the attached atom. The scattering factors for all atomic species were calculated from Cromer and Waber.[11] Further details of crystal structure including final atomic parameters have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 682847). Copies of these data can be obtained free of charge via www.ccdc.cam. ac.uk www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK, fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.05.137.

References and notes

1. Kawabata, J.; Mishima, M.; Kurihara, H.; Mizutani, J. *Phytochemistry* **1995**, *40*, 1507–1510.
2. Meng, Y.; Bourne, P. C.; Whiting, P.; Šik, V.; Dinan, L. *Phytochemistry* **2001**, *57*, 393–400.
3. D'Abrosca, B.; Fiorentino, A.; Golino, A.; Monaco, P.; Oriano, P.; Pacifico, S. *Tetrahedron Lett.* **2005**, *46*, 5269–5272.
4. Fiorentino, A.; D'Abrosca, B.; Izzo, A.; Pacifico, S.; Monaco, P. *Tetrahedron* **2006**, *62*, 3259–3265.
5. Fiorentino, A.; D'Abrosca, B.; Pacifico, S.; Natale, A.; Monaco, P. *Phytochemistry* **2006**, *67*, 971–977.
6. Fiorentino, A.; D'Abrosca, B.; Pacifico, S.; Iacovino, R.; Mastellone, C.; Di Blasio, B.; Monaco, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6096–6101.
7. Ohtani, I.; Kusumi, T.; Kashman, Y.; Ka kisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
8. Mackay, S.; Gilmore, C. J.; Edwards, C.; Stewart, N.; Shankland, K. *maXus Computer Program for the Solution and Refinement of Crystal Structures*; Nonius, MacScience, The University of Glasgow: The Netherlands, Japan, 1999.
9. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435–436.
10. Sheldrich, G. M. *SHELXL97. Program for the refinement of crystal structures*; University of Gottingen: Gottingen, Germany, 1997.
11. Cromer, D. T.; Waber, J. T. *International Tables for X-Ray Crystallography*; Kynoch: Birmingham, 1974; Vol. IV, Table 2.2. B.