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Carexanes: prenyl stilbenoid derivatives from *Carex distachya*

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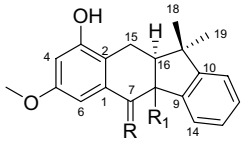
Abstract—Metabolites with a new molecular skeleton, named carexane, have been isolated from the leaves of *Carex distachya*. The structures have been determined on the basis of the spectroscopic characteristics of the compounds. Bidimensional NMR has furnished important data useful for the characterization and the stereochemistry of the molecules. The compounds have a tetracyclic skeleton derived from the coupling of the prenyl moiety on a stilbenoid structure.

Carex species produce oligostilbenes,[1,2] constituted by two to four monomers of resveratrol (3,5,4′-trihydroxystilbene), most of them showing antimicrobial activity.

In studying the allelopathic effects of natural products, isolated from Mediterranean plants, on aquatic[3] and agronomic[4] ecosystems, we investigated *Carex distachya*,[5] a herbaceous plant, growing in the Mediterranean bush.

The hexane extract of the plant was chromatographed successively on silica gel, Sephadex LH-20 and RP-8 HPLC to give three new compounds (**1**–**3**, 0.03% w/w) originating from 2-prenylstilbene precursors and named carexanes A–C, respectively (Fig. 1).



1 R = βOH,αH R_1 = βH
2 R = O R_1 = αOH
3 R = O R_1 = βOH

Figure 1. Structures of carexanes A–C.

The elemental analysis of carexane A[6] and the presence of 20 carbon signals in the ^{13}C NMR spectrum (Table 1) justified the molecular formula $C_{20}H_{22}O_3$. The EIMS spectrum showed the molecular peak at *m/z* 310 (42) confirming the presence of 10 unsaturations in the molecule. In the aromatic region, the ^1H NMR spectrum showed two meta coupled doublets at δ 6.28 and 6.69, a proton at δ 7.61 and a signal, integrated for three protons, centred at δ 7.18. In the aliphatic region of the spectrum were evident a doublet at δ 4.49, a methine proton as a double doublet at δ 3.35, a diastereotopic methylene as two double doublets at δ 3.09 and 1.86, a methine double double doublet at δ 2.16 and two methyl singlets at δ 1.34 and 1.21. The ^{13}C NMR spectrum and the DEPT experiment indicated the presence of a carbinol carbon, seven quaternary carbons, eight methines, a methylene and three methyls.

The value and the multiplicity of the protons at δ 6.28 and 6.69 suggested the presence of two *meta* orientated oxygenated functional groups on an aromatic ring. This hypothesis was supported by an HMBC experiment (Table 1) showing correlations between both the protons and the mutual methine carbon. The upfield resonating proton showed heterocorrelations with the carbon at δ 117.6 and with two tetrasubstituted oxygenated carbons at δ 155.1 and 160.0. This latter signal was also correlated with the proton at δ 6.69 and with the methoxyl at δ 3.75.

The DQ-COSY experiment showed cross peaks between the proton at δ 4.49, bonded to the carbon at δ 74.6 and the proton at δ 3.35 and between this latter and

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Table 1. NMR data of carexanes A (**1**)

Position	δ ^{1}H	DQ-COSY	δ ^{13}C	DEPT	HMBC	NOESY
1	—	—	144.9	C	—	—
2	—	—	117.6	C	—	—
3	—	—	155.1	C	—	—
4	6.28 d (2.9)	H-6	100.5	CH	C-2, C-3, C-5, C-6	OMe
5	—	—	160.0	C	—	—
6	6.69 d (2.9)	H-4	101.7	CH	C-2, C-4, C-5, C-7	OMe
7	4.49 d (8.1)	H-8	74.6	CH	C-1, C-2, C-5, C-6, C-8, C-16	
8	3.35 dd (8.1, 7.8)	H-7, H-16	51.8	CH	C-7, C-9, C-10, C-14, C-15, C-16, C-17	
9	—	—	145.0	C	—	—
10	—	—	153.3	C	—	—
11	7.18 m	H-12	123.0	CH	C-9, C-10, C-13, C-17	
12	7.20 m	H-11	128.2	CH	C-11, C-14	
13	7.20 m	H-14	127.9	CH	C-11, C-14	
14	7.61 m	H-13	125.8	CH	C-10, C-12	
15α	3.09 dd (14.4, 5.4)	H-15β, H-16	21.7	CH_2	C-1, C-2, C-3, C-8, C-16, C-17	H-18
15β	1.86 dd (14.4, 11.4)	H-15α, H-16			C-1, C-2, C-3, C-8, C-16, C-17	H-18
16	2.16 ddd (11.4, 7.8, 5.4)	H-8, H-15α, H-15β	51.3	CH	C-2, C-8, C-9, C-10, C-15, C-17, C-18, C-19	H-19
17	—	—	46.4	C	—	—
18	1.34 s	—	24.5	CH_3	C-10, C-16, C-17, C-19	15α, 15β
19	1.21 s	—	32.7	CH_3	C-10, C-16, C-17, C-18	15α, H-16
OMe	3.75	—	55.6	CH_3	C-5	H-4, H-6

s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, m = multiplet; the couplings (Hz) are reported in brackets.

the proton at δ 2.16 which was correlated with the methylene protons at δ 1.86 and 3.09. Both protons were heterocorrelated with the carbons at δ 117.6, 144.9, 51.3, 51.8, 46.4. This last carbon showed interactions with the methyls at δ 1.21, 1.34 and with an aromatic proton at δ 7.18. The carbinolic carbon at δ 74.6 was also correlated with the methine at δ 3.35 which in turn was correlated with the carbons at δ 153.3, 145.0, 51.3, 21.7. These correlations were in accordance with a pentahydrobenzo[*b*]fluorene ring system with a methoxyl group at the carbon C-5 and two hydroxyl groups at the carbons C-3 and C-7. The complete interpretation of the NMR data was based on the results of DQ-COSY, HSQC and HMBC experiments (Table 1). The coupling constants of the H-7 (d, 8.1 Hz), H-8 (dd, 8.1 and 7.8 Hz) and H-16 (ddd, 11.4, 7.8 and 5.4 Hz), suggested a trans orientation between the H-7/H-8 and H-8/H-16 protons. Furthermore the coupling constants of the diastereotopic protons H-15α (dd, 14.4 and 5.4 Hz) and 15β (dd, 14.4 and 11.4 Hz) indicated

Table 2. NMR data of carexanes B (**2**) and C (**3**)

Position	Carexane B				Carexane C			
	δ ^{1}H	δ ^{13}C	DEPT	HMBC	δ ^{1}H	δ ^{13}C	DEPT	HMBC
1	—	134.2	C	—	—	135.5	C	—
2	—	125.8	C	—	—	125.8	C	—
3	—	157.1	C	—	—	157.4	C	—
4	6.63 d (2.4)	108.7	C	C-2, C-3, C-5	6.62 d (2.4)	107.3	C	C-2, C-3, C-5, C-6
5	—	160.3	C	—	—	160.3	C	—
6	6.93 d (2.4)	102.0	CH	C-2, C-4, C-7	7.11 d (2.4)	103.6	CH	C-2, C-4
7	—	198.6	C	—	—	196.7	C	—
8	—	82.2	C	—	—	80.3	C	—
9	—	143.1	C	—	—	141.7	C	—
10	—	153.2	C	—	—	155.5	C	—
11	7.18 m	123.1	CH	C-9, C-13	7.31 m	123.6	CH	C-9, C-13
12	7.28 m	129.9	CH	C-14	7.35 m	130.1	CH	C-14
13	7.23 m	127.9	CH	C-9, C-11, C-14	7.26 m	127.5	CH	C-9, C-11
14	7.65 m	127.0	CH	C-8, C-10, C-12	7.90 m	127.4	CH	C-10, C-12
15	3.04 d (4.8)	18.9	CH_2	C-1, C-2, C-3, C-4, C-6, C-8, C-16, C-17	3.09 dd (16.5, 10.8)	20.7	CH_2	C-1, C-2, C-3, C-8, C-16
					2.94 dd (16.5, 5.4)			C-1, C-2, C-3, C-8, C-16
16	2.72 t (4.8)	57.1	CH	C-2, C-7, C-8, C-15, C-17, C-18, C-19	2.22 dd (10.8, 5.4)	58.6	CH	C-7, C-17, C-18, C-19
17	—	46.1	C	—	—	44.9	C	—
18	0.75 s	26.5	CH_3	C-10, C-16, C-17, C-19	1.39 s	27.7	CH_3	C-10, C-16, C-17, C-19
19	1.43 s	29.4	CH_3	C-10, C-16, C-17, C-18	1.42 s	27.0	CH_3	C-10, C-16, C-17, C-18
OMe	3.74	55.7	CH_3	C-5	3.74	58.6	CH_3	C-5

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

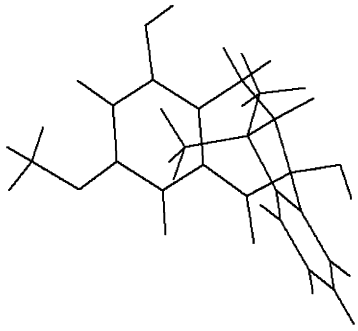

Figure 2. Geometry-optimized structure of MM+ low-energy conformations of carexane B.

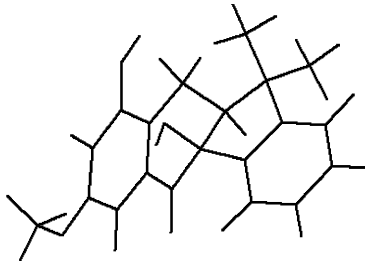

Figure 3. Geometry-optimized structure of MM+ low-energy conformations of carexane C.

a trans orientation between the H-15β and H-16 protons and a cis configuration between the H-15α and H-16 protons. The absolute configuration of the C-7 carbon was assigned using a modified Mosher method.[7] The positive and the negative $\Delta\delta_{R-S}$ values for H-8, the H-6 and H-4 protons were found, respectively, on the right and the left sides of the MTPA plane indicating an *R* configuration for C-7 carbon and, consequently, an *R* configuration for both C-8 and C-16 carbons. A NOESY experiment confirmed the proposed structures and allowed the α and β orientation of the H-15 protons to be established. In fact NOE were evident between the H-18 methyl and both the H-15 protons, while the H-19 methyl gave NOE with the H-16 methine and the H-15α proton.

Carexane B[8] showed a molecular formula $C_{20}H_{20}O_4$, calculated on the basis of the elemental analysis and the EIMS spectrum that showed the molecular peak at *m/z* 324. NMR data (Table 2) were similar to those registered for carexane A, with differences due to the presence of a C-7 carbonyl carbon and a C-8 hydroxylated carbon. In fact, the ^{1}H NMR spectrum showed two doublets at δ 6.63 and 6.93, downshifted due to the presence of oxygenated groups on the aromatic ring and four protons as multiplets at δ 7.65, 7.28, 7.23 and 7.18 due to the H-11–H-14 protons. The ^{13}C NMR spectrum and the DEPT experiment indicated the presence of a carbonyl carbon, a carbinol carbon, eight quaternary carbons, six methines, a methylene and three methyls.

The DQ-COSY experiment showed cross peaks between the methylene and the methine at δ 2.72. In the HMBC experiment, the latter was correlated, with the methylene carbon, the methyl groups at δ 0.75 and 1.43, the quaternary carbon at δ 46.1 and the carbinol at δ 82.2. The correlations between the carbon at δ 108.7 and the proton at δ 6.93 and between the carbinol carbon and the methine at δ 2.72, the methylene at δ 3.04 and the H-14 proton at δ 7.65 suggested the location of the hydroxyl group at C-8 carbon and a double bond with the oxygen on the C-7 carbon. The NOESY experiment showed NOE between the both the methyl groups and the H-15 methylene protons and between the H-16 methine and the methyl at δ 1.43.

Carexane C[9] had the same molecular formula as the previous compound. The EIMS spectrum showed an identical fragmentation pattern. NMR data (Table 2) were very similar to those of carexane B. The ^{1}H NMR showed the six aromatic protons of the A and D aromatic rings, a methylene as two double doublets at δ 3.09 and 2.94, a methine as double doublet at δ 2.22 and the H-18 and H-19 methyls at δ 1.39 and 1.42. The DQ-COSY, HSQC and HMBC data were consistent with the same structure proposed for carexane B. The NOESY showed NOE between the H-15 at δ 3.09 and the methyl at δ 1.42 and between the H-16 proton at δ 2.22 and the methyl at δ 1.42. These differences could be justified by hypothesizing two epimeric structures for carexane B and C.

To establish the configurations at C-8 and C-16 carbons, the lowest-energy conformations of the two cis and trans isomers were determined using the MM+ molecular mechanics method as implemented in Hyperchem 6.0. The NMR data of the carexane B were in good agreement with the cis geometry for the hydroxyl at C-8 and the hydrogen at C-16 (Fig. 2). In fact, the cis geometry causes a bending of the molecule that exposes the β-oriented H-18 methyl at the inner ring current. Indeed, in the ^{1}H NMR of carexane B, we observed the upshield of the methyl at 0.75 ppm. The distance measured between the H-16 and the H-19 was 2.36 Å, while the H-15β was at 2.49 and 2.50 Å from the H-18 and H-19 methyls, respectively, in good accordance with the NOE observed for the carexane B.

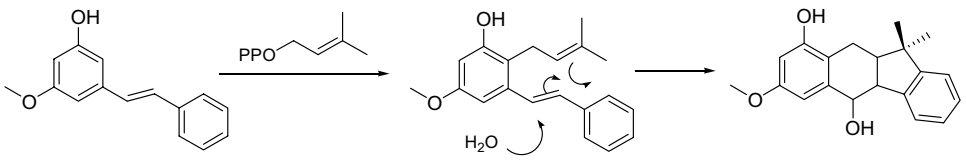

Figure 4. Plausible biogenetic pathway proposed for the formation of carexanes.

The minimized structure with a trans geometry for the hydroxyl at C-8 and the hydrogen at C-16 (Fig. 3) was consistent with that of carexane C. In fact, the distance measured between the H-15β and H-18 methyl was 2.62 Å, while the distance between the H-16 and H-19 methyl was 2.36 Å. These data were in accordance with the NOE observed for the carexane C.

Prenylated stilbenes are unusual natural products.[10] These compounds are reported as cytotoxic against ovarian cancer cell lines.[11] In our study tetracyclic prenylated structures have been reported for the first time. They originated by the prenylation and successive cyclization of stilbene precursors (Fig. 4).

Acknowledgements

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References and notes

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5. Plants of *Carex distachya* Desf. (Cyperaceae) was collected in June 2004 in Castelvolturno near Caserta (Campania, Italy) and identified by Dr. Assunta Esposito of the Department of Scienze della Vita of Second University of Naples (SUN). A voucher specimen (CE278) has been deposited at the herbarium of the Dipartimento di Scienze della Vita of SUN.
6. Carexane A: (4 mg) amorphous solid, $[\alpha]_D^{25}$ +76.7 (*c* 0.04, MeOH); UV λ_{max} (log ε) (MeOH) 273 (3.47) nm; EIMS *m/z* 310 $[M]^{+\cdot}$ (42), 292 $[M-H_2O]^{+\cdot}$ (21), 277 $[M-H_2O-CH_3]^{+\cdot}$ (79). Anal. Calcd for $C_{20}H_{22}O_3$: C, 77.39; H, 7.14. Found: C, 77.93; H, 7.27. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1. The (*S*)-MTPA ester had the ^{1}H NMR spectral data (300 MHz, CD_3OD): δ 7.70–7.00 (4H, overlapped H-11–H-14), 6.71 (1H, d, *J* = 2.7 Hz, H-6), 6.60 (1H, d, *J* = 2.7 Hz, H-4), 6.30 (1H, d, *J* = 4.2 Hz, H-7), 3.97 (1H, dd, *J* = 12.9 and 4.2 Hz, H-8), 3.67 (3H, s, OMe), 2.43 (1H, m, H-16), 2.27 (2H, m, H-15), 1.13 (1H, s, H-19), 0.97 (3H, s, H-18). The (*R*)-MTPA ester had the ^{1}H NMR spectral data (300 MHz, CD_3OD): δ 7.70–6.95 (4H, overlapped H-11–H-14), 6.84 (1H, d, *J* = 2.4 Hz, H-6), 6.62 (1H, d, *J* = 2.4 Hz, H-4), 6.37 (1H, d, *J* = 3.8 Hz, H-7), 3.93 (1H, m, H-8), 3.72 (3H, s, OMe), 2.40 (1H, m, H-16), 2.32 (2H, m, H-15), 1.07 (1H, s, H-19), 0.80 (3H, s, H-18).
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8. Carexane B: (3 mg) amorphous solid, $[\alpha]_D^{25}$ −174.3 (*c* 0.03, MeOH); UV λ_{max} (log ε) (MeOH) 278 (3.94), 344 (3.46) nm; EIMS *m/z* 324 $[M]^{+\cdot}$ (15), 306 $[M-H_2O]^{+\cdot}$ (31), 291 $[M-H_2O-CH_3]^{+\cdot}$ (100). Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21. Found: C, 74.22; H, 6.27. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 2.
9. Carexane C: (2 mg) amorphous solid, $[\alpha]_D^{25}$ +139.3 (*c* 0.02, MeOH); UV λ_{max} (log ε) (MeOH) 273 (3.47) nm; EIMS *m/z* 324 $[M]^{+\cdot}$ (12), 306 $[M-H_2O]^{+\cdot}$ (27), 291 $[M-H_2O-CH_3]^{+\cdot}$ (100). Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21. Found: C, 74.32; H, 6.45. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 2.
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