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# Natural dibenzoxazepinones from leaves of *Carex distachya*: Structural elucidation and radical scavenging activity

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**Abstract**—Two new dibenzoxazepinones have been isolated from the leaves of *Carex distachya*, an herbaceous plant growing in the Mediterranean area. The structures have been elucidated on the basis of their spectroscopic properties. Bidimensional NMR (DQ-COSY, TOCSY, NOESY, ROESY, HSQC, and HMBC) furnished important data useful for the characterization of the molecules. The compounds have been assayed, for the antioxidant activity, by measuring its capacity to scavenge the DPPH, the superoxide anion, and nitric oxide radicals.


Many diseases are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance.[1–4]

Vitamins A, C, and E are the antioxidant food vitamins. They inhibit the oxidation process, or chemical reactions, that occurs during the development of coronary heart disease and some cancers, which means these vitamins may also help with the prevention of these diseases.

In the search for new antioxidant metabolites from natural sources we studied some plants living in the Mediterranean area, and many new bioactive metabolites have been isolated and characterized.[5] Recently[6] we reported the structural elucidation and the antioxidant activity of the distachyasin, a novel antioxidant prenylated stilbenoid isolated from the leaves of *Carex distachya*.[7]

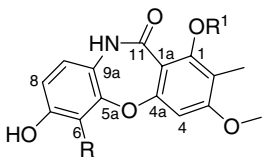
In this article, we report the isolation and the structural elucidation of two new dibenzoxazepinones.[8]

The EI mass spectrum of compound **1**[9] showed a molecular ion at *m/z* 315 which, together with the elemental analysis, defined the molecular formula as $C_{17}H_{17}NO_5$. The IR spectrum showed an intense band at 1619 $cm^{-1}$, besides other medium bands at 3702, 3601, and 1582 (see Fig. 1).

The $^1H$ NMR spectrum (Table 1) showed three aromatic protons as a singlet at $\delta$ 6.93 and two doublets at $\delta$ 6.83 and 6.42, two methoxyl groups at $\delta$ 3.97 and 3.81, two methyls at $\delta$ 2.19 and 2.12, and a singlet at $\delta$ 8.07.

All the proton signals were assigned to the corresponding carbons through direct $^1H$–$^{13}C$ correlations in the HSQC (Table 1) spectrum, with exception of the singlet at $\delta$ 8.07, which value was in good accordance with a proton of an amidic group.

The $^{13}C$ NMR spectrum showed 17 carbon signals, identified on the basis of the DEPT experiment as four




**1** R = $R^1$ = Me
**2** R = $R^1$ =H

**Figure 1.** Chemical structures of dibenzoxazepinones from *C. distachya*.

*Keywords*: Dibenzoxazepinones; Carex distachya; Spectroscopic analysis; Radical scavenging activity.
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**Table 1.** NMR data of dibenzoxazepinones **1** and **2**[a]

| No | 1 | | | | 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | $\delta$ $^1$H | $\delta$ $^{13}$C | DEPT | HMBC | $\delta$ $^1$H | $\delta$ $^{13}$C | DEPT | HMBC |
| 1 | — | 159.2 | C | — | — | 159.3 | C | — |
| 2 | — | 119.2 | C | — | — | 106.5 | C | — |
| 3 | — | 164.7 | C | — | — | 165.0 | C | — |
| 4 | 6.93 s | 96.1 | CH | C2, C3, C4a, C5a | 6.64 s | 90.5 | CH | C2, C3, C4a, C1a, C11 |
| 6 | — | 113.2 | C | — | 6.40 d (2.1 Hz) | 104.2 | CH | C5a, C8, C9a |
| 7 | — | 156.9 | C | — | — | 160.1 | C | — |
| 8 | 6.42 d (8.4 Hz) | 108.4 | CH | C6, C9 | 6.38 dd (8.1, 2.1 Hz) | 108.0 | CH | C5a, C7, C8, C9a |
| 9 | 6.83 d (8.4 Hz) | 128.9 | CH | C5a, C7, C8, C9a | 7.05 d (8.1 Hz) | 132.9 | CH | |
| NH | 8.03 s | — | — | C1, C1a, C9a, C11 | 8.08 s | — | — | C1a, C4a, C5a, C9, C9a, C11 |
| 11 | — | 178.0 | C | — | — | 182.4 | C | |
| 1a | — | 113.1 | C | — | — | 109.6 | C | |
| 4a | — | 159.3 | C | — | — | 157.6 | C | |
| 5a | — | 155.2 | C | — | — | 157.7 | C | |
| 9a | — | 126.4 | C | — | — | 122.6 | C | |
| (C2)–Me | 2.19 s | 8.5 | $CH_3$ | C1, C2, C3 | 2.08 s | 8.2 | $CH_3$ | C1, C2, C3 |
| (C6)–Me | 2.12 s | 8.9 | $CH_3$ | C5a, C6, C7 | — | — | — | |
| (C1)–OMe | 3.81 s | 62.1 | $CH_3$ | C1 | — | — | — | |
| (C3)–OMe | 3.98 s | 56.8 | $CH_3$ | C3 | 3.94 s | 56.5 | $CH_3$ | C3 |

[a] Data were recorded in $CD_3OD$ on Varian Mercury 300 MHz ($^1$H, $^{13}$C) spectrometer (DQ-COSY, TOCSY, HSQC, HMBC, ROESY, and NOESY); chemical shifts ($\delta$) were expressed in parts per million with reference to the signal of $CD_3OD$ ($\delta$ 3.31 ppm) for $^1$H, and to the center peak of the signal of $CD_3OD$ ($\delta$ 49.0 ppm) for $^{13}$C, respectively.

methyls, three methines, and 10 tetrasubstituted carbons, including one carbonyl at $\delta$ 178.0.

The HMBC experiment (Fig. 2) showed correlations between the proton at $\delta$ 8.07 and the carbons at $\delta$ 113.1, 126.4, and 159.2, which was correlated with the methoxyl at $\delta$ 3.81; between the singlet at $\delta$ 6.93 and the carbons at $\delta$ 113.1, 119.2, 155.2, 159.2, and 164.7. The methoxyl at $\delta$ 3.98 also had an interaction with the carbon at $\delta$ 164.7. In the same HMBC experiment were also evident correlations between the proton at $\delta$ 6.42 and the carbons at $\delta$ 113.2 and 128.9. The proton at $\delta$ 6.83 and the carbons at $\delta$ 108.4, 126.4, 155.2, and 156.9 were also correlated. These latter two signals, together with the carbon at $\delta$ 113.2, showed a correlation with the methyl at $\delta$ 2.12. These results suggested the presence of two aromatic rings linked across an amidic bond and an oxygen bridge which formed an [1,4]oxepin-5-one ring. The NOE observed in the NOESY and ROESY experiments (Fig. 2) confirmed the hypothesized structure.

Compound **2**[10] had a molecular formula $C_{15}H_{13}NO_5$ on the basis of the elemental analysis and the EI mass spectrum, showing a molecular peak at *m*/*z* 287.

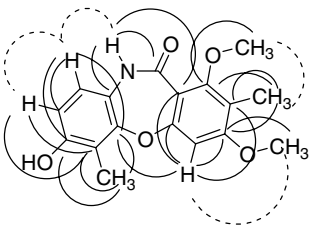


**Figure 2.** Selected correlation showed in the HMBC (continuous curves) and NOESY (dashed curves) experiments of the compound **1**.

The $^1$H NMR showed a singlet at $\delta$ 6.64 and three protons as two doublets at $\delta$ 7.05 ($J$ = 8.1 Hz), 6.40 ($J$ = 2.1 Hz) and a double doublet at $\delta$ 6.38 of a 1,2,4-trisubstituted aromatic unit. The amidic proton at $\delta$ 8.08, a methoxyl at $\delta$ 3.94, and a methyl group at $\delta$ 2.08 were also evident in the $^1$H NMR. The $^{13}$C NMR spectrum showed 15 signals identified as two methyls, four methines, and nine tetrasubstituted carbons.

These differences were in good accordance with the presence of a hydroxyl group on the C-1 carbon and the lack of the methyl group on the C-6 carbon. This hypothesis was supported by the observed heterocorrelations in the HMBC experiment from H-4 ($\delta$ 6.64) to C-1a, C-2, C-3, C-4a; from H-6 ($\delta$ 6.40) to C-5a, C-8, C-9a; from H-8 ($\delta$ 6.38) to C-7, C-9; from H-9 ($\delta$ 7.05) to C-5a, C-7, C-9a; and from the NH ($\delta$ 8.08) to C-5a, C-9, C-9a, C-11.

The NOE observed in the NOESY experiment confirmed the proposed structure for **2**.

The isolated molecules were the object of antioxidant assays that tended to evaluate their radical scavenging capacity.

The methods used estimated the scavenging activities of the investigated compounds against three different radicals that included the DPPH[11] radical and the two biological radicals superoxide anion[12] radical and NO[13]. The metabolites were assayed at a concentration of 0.1 mg/ml and their activities were compared with those of α-tocopherol and ascorbic acid, two known natural antioxidant molecules. The results are reported in Figure 3.

Although all the substances showed a weak activity against the free commercial radical DPPH, metabolite

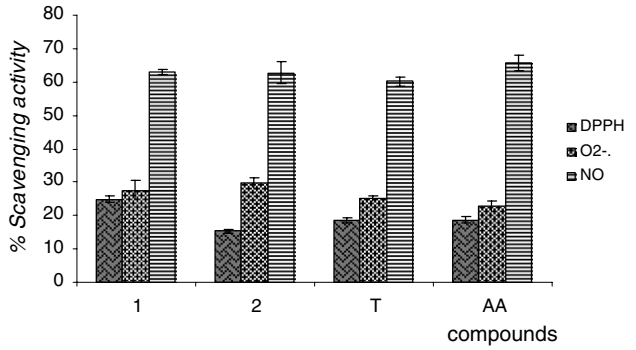


**Figure 3.** Radical scavenging activity of the dibenzoxazepinones from *C. distachya*. T, α-tocoferol; AA, ascorbic acid.

**1** was more active than both used standards exercising an activity equal to 25%.

The two compounds exhibited strong NO scavenging activity leading to the reduction of the nitrite concentration in the assay medium of 63% and 59%, respectively. The NO scavenging capacity of the two compounds was slightly lower to that of ascorbic acid (66%).

The results of the assay of superoxide radical scavenging activity, based on the capacity of the isolated metabolites to inhibit the photochemical reduction of nitroblue tetrazolium, allowed us to observe that the substances were responsible for a good reductive power. The inhibiting activities were estimated similar to that of α-tocopherol.

Although compound **2** bears two phenolic groups on molecular skeleton, the observed comparable activities of the investigated substances seem to be due to the possible formation in compound **2** of a blocking intramolecular hydrogen bond between the hydroxyl group on C-7 and carbonylic oxygen on C-11.

The research into natural products as health protecting factors against oxidative damage is an interesting field. Recently we reported the chemical and biological characterization of distachyasin.[6] Comparing the obtained results to that of distachyasin we are able to assess the potential bioactivity of the two dibenzoxazepinones. In fact at the tested concentration the new molecules are more active than distachyasin.

Diverse studies have shown that natural products have a large range of biological activities such as antitumor and antiviral activities.

The discovery of compounds that are capable of inhibiting wild-type viral replication in low nanomolar concentrations showed that some benzoxazepinone derivatives inhibit HIV-1 replication by interacting with the NNRTI binding pocket.[14]

Furthermore, several members of the benzoxazepinone class have been reported as monoanionic inhibitors of squalene synthetase in HMG-CoA reductase regulation.[15] Other studies allowed these substances to be pharmaceutically used as γ-secretase inhibitors for the treatment of Alzheimer's disease.[16]

Antioxidant analysis of isolated metabolites suggests an important role of these substances as scavenging factors and supports the research of antioxidative secondary metabolites from non-edible plants.

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7. Plants of *Carex distachya* Desf. (Cyperaceae) were collected in June 2004 in Castel Volturno, 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.
8. Fresh leaves of *Carex distachya* (6.0 kg) were extracted with EtOAc for 5 days, to obtain 67.0 g of residual material. The EtOAc extract was chromatographed on silica gel, with hexane and EtOAc solutions. The fraction eluted with hexane–EtOAc (7:3) was rechromatographed on Sephadex LH-20® eluting with hexane–$CHCl_3$–MeOH (3:1:1) to obtain two fractions. The first, purified initially by TLC with $CHCl_3$–EtOAc (93:7) and then by HPLC using an RP-8 preparative column eluting with MeOH–MeCN–$H_2O$ (2:2:1), gave pure dibenzoxazepinone **1** (6 mg). The second fraction was purified by HPLC using an RP-8 preparative column eluting with MeOH–MeCN–$H_2O$ (2:2:1) to give pure **2** (3 mg).
9. 7-hydroxy-2,6-dimethyl-1,3-dimethoxy-dibenz[*b*,*f*][1,4]oxazepin-11-(10*H*)-one (**1**): colorless powder UV $\lambda_{max}$ (log varepsilon) (MeOH) 266 (2.02), 204 (2.35) nm; IR $\nu_{max}$ ($CHCl_3$) 3702, 3600, 2929, 1619, 1582 $cm^{-1}$; EIMS *m*/*z* 315 $[M]^+$ (100), 298 $[M–OH]^+$ (59), 284 $[M–OMe]^+$ (32). Anal. Calcd for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.43. Found C, 64.69; H, 5.51. HRESIMS *m*/*z* 316.1186 $[M+H]^+$ (Calcd for $C_{17}H_{17}NO_5$, 316.1185). $^1H$ NMR (300 MHz, $CD_3OD$) and $^{13}C$ NMR (75 MHz, $CD_3OD$): Table 1. NMR experiments were recorded at 300 MHz for $^1H$ and 75 MHz for $^{13}C$ on a Varian Mercury 300 spectrometer Fourier trans-

form NMR. Electronic impact mass spectra (EI-MS) were obtained with an HP 6890 instrument equipped with a MS 5973 N detector. HR mass spectra were obtained with a ESI-MS Waters 2690-ZMD instrument.

10. 1,7-dihydroxy-2-methyl-3-methoxy-dibenz[*b*,*f*][1,4]oxazepin-11-(10*H*)-one (**2**): colorless powder UV $\lambda_{max}$ (log $\varepsilon$) (MeOH) 267 (2.23), 203 (2.30) nm; IR $\nu_{max}$ ($CHCl_3$) 3702, 3600, 2929, 1619, 1582 $cm^{-1}$; EIMS *m*/*z* 287 $[M]^+$ (100), 270 $[M–OH]^+$ (72), 256 $[M–OMe]^+$ (43). HRESIMS *m*/*z* 288.0874 $[M+H]^+$ (Calcd for $C_{15}H_{13}NO_5$, 288.0872). Anal. Calcd for $C_{15}H_{13}NO_5$: C, 62.72; H, 4.56. Found C, 62.76; H, 4.51. $^1H$ NMR (300 MHz, $CD_3OD$) and $^{13}C$ NMR (75 MHz, $CD_3OD$): Table 1.
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