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Determination of the free radical scavenging activity of dihydropyran-2,4-diones

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Abstract—The antioxidant activities of four synthetic dihydropyran-2,4-diones have been established through the determination of their abilities to inhibit free radicals using DPPH· as the stable radical. Whilst all of the compounds exhibited high inhibition percentages, the most active member of the group was 6-phenyl-dihydropyran-2,4-dione. The antioxidant activity of the dihydropyran-2,4-diones is reported here for the first time and extends our knowledge of the range of valuable biological activities associated with this group of compounds.
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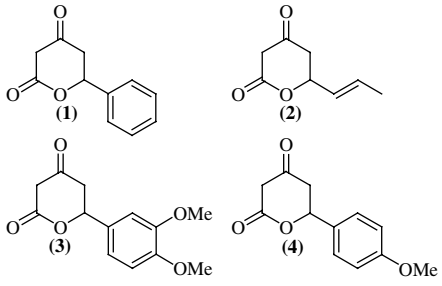
1. Introduction

Recent evidence[1] suggests that free radicals, which are generated in many bioorganic redox processes, may induce oxidative damage in various components of the body (e.g., lipids, proteins and nucleic acids) and may also be involved in processes leading to the formation of mutations. Furthermore, radical reactions play a significant role in the development of life-limiting chronic diseases such as cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, cataracts and others.[2]

A key factor in the induction of oxidative stress appears to be the overproduction of free radicals typically caused by avitaminosis A, C and E, and reduced levels of specific enzymes such as superoxide dismutase, catalase and glutathione peroxidase. One important way to protect the body against such stress is to increase the levels of antioxidants.[3] Such compounds may play a significant role in the prevention or alleviation of the above-mentioned diseases by reducing oxidative damage to cellular components caused by reactive oxidant species.[4]

Antioxidants may be classified according to their mode of action as being free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen in closed systems.[5] A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) has received most attention owing to its ease of use and its convenience.[6] This assay is the most widely used in vitro test through which to assess free radical scavenger capacities.[7] In the DPPH· assay, the antioxidant activity of a compound is evaluated spectrophotometrically by monitoring the decrease in absorbance at 515 nm as DPPH· (purple) is transformed to the reduced form DPPH-H (yellow).

The dihydropyran-2,4-diones exhibit structural features present in many biologically active natural products possessing important pharmacological activities.[8] As part of our continuing studies aimed at ascertaining



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the biological activity of this class of compound, 6-phenyl-dihydropyran-2,4-dione (**1**), 6-propenyl-dihydropyran-2,4-dione (**2**), 6-(3,4-dimethoxyphenyl)-dihydropyran-2,4-dione (**3**) and 6-(4-methoxyphenyl)-dihydropyran-2,4-dione (**4**) were synthesized[9] and their antioxidant activities analyzed in vitro.

The assay to determine radical scavenging activity involved measuring the decrease in absorbance at 515 nm that occurred when the DPPH radical was reduced by the antioxidant. Various initial concentrations of DPPH$^{\cdot}$ (from 0.025 to 0.415 g/L) and reaction times (30 min or the time to reach steady state) have been used previously by different authors.[6] In the present study, a 0.1 mM ethanolic solution of DPPH$^{\cdot}$ (from Aldrich) was employed and the dihydropyran-2,4-diones **1**–**4** were assayed at concentrations of 2.0, 4.0, 8.0, 10.0, 12.0, 16.0 and 20.0 mM in ethanol. Ethanolic solutions of 2,6-di-*tert*-butyl-4-methylphenol (BHT, from Fluka)[10] at the concentrations mentioned above were used as positive controls, while ethanol was the negative control.

An aliquot (1.0 mL) of test solution was added to 1.0 mL of a 0.1 mM solution of the DPPH radical in absolute ethanol and incubated at 25 °C. The absorbance of the reaction mixture at 515 nm was determined immediately and then every 10 min for 60 min. Ethanol was used to zero the spectrophotometer. Absorbencies were measured using a Perkin–Elmer model Lambda 2 UV–vis spectrophotometer.

The radical scavenging capacities of **1**–**4** were determined as the percentage reduction of the initial absorption of DPPH$^{\cdot}$ by the tested compound. The inhibition percentage (IP) values with respect to the DPPH radical were calculated using the following equation:

$$\mathrm{IP} = \frac{\mathrm{Abs}_{t_0} - \mathrm{Abs}_{t_{60\,\mathrm{min}}}}{\mathrm{Abs}_{t_0}} \times 100$$

All four dihydropyran-2,4-diones exhibited free radical scavenging activities, comparable with that of BHT, in the in vitro test system. At concentrations greater than 8.0 mM, all dihydropyran-2,4-diones showed IP values at 60 min in excess of 50%, whilst that of the negative control was only 28.7%. Compounds **1**–**4** exhibited similar IP value versus concentration profiles (Fig. 1) in which IP was not strongly affected by changes in concentration.

All of the dihydropyran-2,4-diones exhibited analogous kinetics for the reaction with the DPPH radical; in each case, complete inhibition was not achieved within 60 min even at the highest concentration tested. Among the three different types of kinetics that have been previously described,[7] the dihydropyran-2,4-diones appear to exhibit a slow reaction mechanism type requiring more than 30 min to reach a steady state.

The concentration of DPPH radical in the reaction medium was calculated from the calibration curve: $A_{515\,\mathrm{nm}} = 8.91\ [\mathrm{DPPH}^{\cdot}]_t + 0.0083$ ($R = 0.99968$) determined by linear regression using triplicate measurements, where $[\mathrm{DPPH}^{\cdot}]_t$ is expressed in mmol/L. The percentage of DPPH radical remaining (% $\mathrm{DPPH}^{\cdot}_{\mathrm{rem}}$) after 60 min could then be evaluated from the equation:

$$\%\mathrm{DPPH}^{\cdot}_{\mathrm{rem}} = \frac{[\mathrm{DPPH}]_{t_{60\,\mathrm{min}}}}{[\mathrm{DPPH}]_{t_0}} \times 100$$

The results are shown in Figure 2.

The antioxidant ability of a compound may also be defined by the parameter antiradical power (ARP), which is related to the factor EC_{50} (the concentration of antioxidant necessary to decrease the initial concentration of DPPH$^{\cdot}$ by 50% after 60 min) by the relationship below. The EC_{50} values were obtained by plotting the percentage of remaining DPPH$^{\cdot}$ against the concentration of antioxidant.

$$\mathrm{ARP}_{60\,\mathrm{min}} = \frac{1}{\mathrm{EC}_{50}}$$

From this it can be seen that the more efficient the antioxidant, the smaller the EC_{50} will be and hence the larger the value of ARP. Table 1 below summarizes the parameters obtained from the DPPH assay.

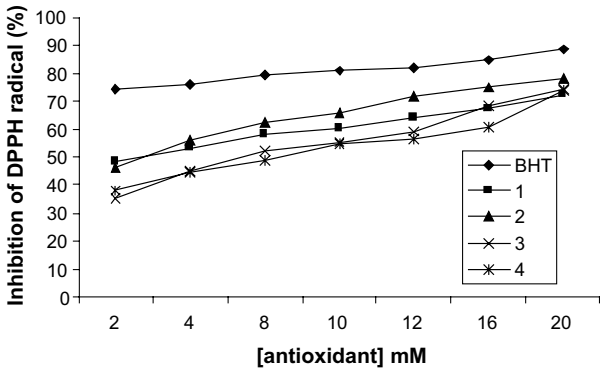

Figure 1. Antioxidant activity as determined by the inhibition percentage of DPPH$^{\cdot}$ at 60 min produced by various concentrations of dihydropyran-2,4-diones **1**–**4** (BHT was assayed as the positive control; data shown are means of three replicates).

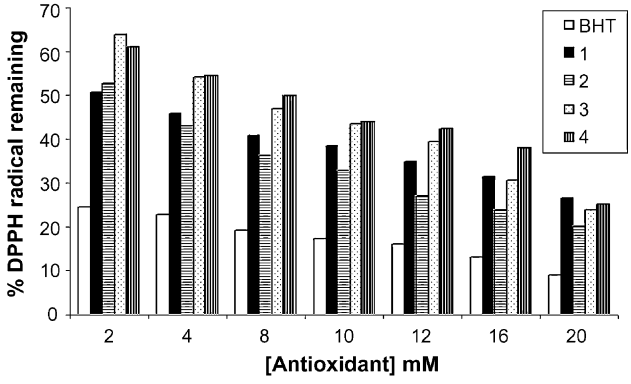

Figure 2. The percentage of DPPH radical remaining in the reaction mixture as a function of the concentration of antioxidant applied.

Table 1. Antioxidant abilities for compounds **1**–**4** evaluated from DPPH assays

Compounds	EC_{50}[a] (mM)	ARP (mM^{-1})
1	1.40 ± 1.05	0.71
2	1.44 ± 3.26	0.70
3	7.13 ± 2.04	0.14
4	7.61 ± 2.24	0.13

[a] Mean values standard error ($n = 3$).

With respect to the establishment of a relationship between structural characteristics and antioxidant potential, an exact correlation of activity with molecular structure cannot currently be presented. It does appear, however, that substituents on C-6 have importance in the reaction between dihydropyran-2,4-diones and the DPPH radical since pyrones with different substituents at this position show different ARP values. In order to confirm this, and to determine the mechanism of transfer of hydrogen atoms to DPPH$^{\cdot}$, it will be necessary to identify the reaction products and to determine the antioxidant activities of dihydropyran-2,4-diones with substituents at other positions.

2. Conclusions

This work describes for the first time the in vitro antioxidant activities of 6-substituted dihydropyran-2,4-diones, a new class of potential antioxidants. All of the compounds assayed showed high inhibition percentages, although 6-phenyl-dihydropyran-2,4-dione (**1**) and 6-propenyl-dihydropyran-2,4-dione (**2**) were the most active. The introduction of electron-withdrawing groups on the aromatic ring of **1** reduced considerably the antioxidant activity. In order to elucidate the mechanism of the reaction between the dihydropyran-2,4-diones and the DPPH radical, other dihydropyran-2,4-diones with substituents in different positions will be prepared and assayed. The reactivities of the tested pyrones reflect the antioxidant activity of this class of compound and demonstrate that dihydropyran-2,4-diones are promising antioxidants.

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