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Comparative Studies of the Redox Behaviour and the Antioxidative Activity of Arylnaphthoquinones

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Summary. Comparative studies of redox behaviour and antioxidative activity (AOA) were carried out with two series of arylnaphthoquinone derivatives. For the electrochemical investigations, a voltammetric procedure combined with a glassy carbon electrode was applied. The AOA was examined using the photo-chemiluminometric method with the Photochem® measuring device according to *Popov et al.* and the chemical procedure with the radical quencher *DPPH* (diphenylpicrylhydrazyl). Both the redox potential and the antioxidative activity of the investigated compounds are influenced by their substituents. Compounds with the OH-group at position 3 are easiest to oxidize ($E_p = 0.18$ V; *pH* 7.4). The lag phases (AOA) of these compounds are five to ten times greater than those of the strong antioxidants ascorbic acid and Trolox®. As suggested for other natural compounds, we also found a good correlation between the oxidation potential and the AOA. Therefore, it seems that a good antioxidative activity requires a low redox potential.

Keywords. Antioxidative activity; Arylnaphthoquinones; Cyclic voltammetry; Photochemistry.

Introduction

1,4-Naphthoquinones are a group of compounds widespread in nature. Due to their antibacterial properties, they are able to protect plants from invaders. Traditional medicine has used different naphthoquinone derivatives for centuries in the form of plant extracts because of their anti-inflammatory activity and improving wound healing. Some compounds have also anti-thrombotic and anti-tumour effects [1].

Antioxidants are of particular importance, for organisms with aerobic physiology as they are constantly subject to attack by reactive oxygen derived species (ROS: $^1O_2$, $O_2^{-\bullet}/HO_2^{\bullet}$, H_2O_2, $HO^{\bullet}$, $ONOO^-$). Endogenous protective mechanisms

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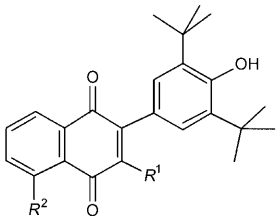
	R^1	R^2
1	H	H
2	Cl	H
3	Br	H
4	OMe	H
5	OH	H



	R^1	R^2
6	H	OH
7	Cl	OH
8	Br	OH
9	OMe	OH
10	OH	OH

Fig. 1. 1,4-Naphthoquinone derivatives arylated with 2,6-di-*tert*-butylphenol and examined with respect to their AOA using electrochemical and photochemical methods

prevent cells and *DNA* from damage, such as vitamins C and E representing low-molecular antioxidants, or antioxidative enzymes. Qualitative or quantitative dysfunctions of the antioxidative network in advanced age may cause pathological changes, such as neurodegenerative diseases, arteriosclerosis, inflammation of the bowel, diabetes, and cancer [2].

In this paper we report on two series of analogous aryl naphthoquinones – 1,4-naphthoquinone and 5-hydroxy-1,4-naphthoquinone (juglon), arylated in position 2 with the radical quencher 2,6-di-*tert*-butylphenol [3, 4] (Fig. 1) – and examined how the substituents influence the electrochemical properties and the antioxidative activity (AOA).

Results and Discussion

The determination of the antioxidative activity (AOA) of new compounds has become routine, for example to determine AOA as the desired or unwanted effect during the development of active substances. It is, however, often impossible to draw comparisons between such data because different methods are applied. In addition, most of the routine procedures for the measurement of AOA are not able to quantify highly potent antioxidants with sufficient accuracy, and no precise statements about the extent of AOA of such compounds can be made.

We applied a voltammetric procedure using a glassy carbon electrode, a photochemiluminometric method with the Photochem® measuring device according to *Popov et al.* [5], and a chemical procedure with the radical quencher *DPPH* (diphenylpicrylhydrazyl).

Electrochemical investigation

The examination of the electron affinity of the 2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1,4-naphthoquinones substituted in position 3 was carried out at a glassy carbon electrode by means of cyclic voltammetry. The naphthoquinones examined displayed two voltammetric signals (E_{pa1} and E_{pa2}) in the potential range from 0.00 to 1.30 V *vs.* Ag/AgCl, with the exception of compounds **1** and **5**. The potential of the first peak, which is a reliable indicator of the oxidation potential, depends first on the *pH* value of the solution and second on type, number, and position of the substituents. The E_{pa2} values (all at approx. + 0.90 V) are, on the other hand, only slightly influenced by the substituent pattern.

Table 1. Oxidation potentials (E_{pa1}) of compounds **1–10** and the standard antioxidants ascorbic acid and Trolox® depending on the *pH* value and compared to the photo-chemiluminometrically determined AOA (lag phases)

E_{pa1}/V					lag phases/s	
	$pH = 1.1$	$pH = 5.4$	$pH = 7.4$	$pH = 10.0$	$c = 10^{-6} M$	$c = 10^{-7} M$
1	0.635	0.392	0.270	0.093	64±2	
2	0.776	0.507	0.404	0.224	45±7	
3	0.780	0.506	0.382	0.234	56±3	
4		0.467	0.352	0.185		3.5±1
5	0.420	0.200	0.185	0.204		152±10
6	0.632	0.390	0.278	0.141	101±5	6.7±2
7	0.771	0.506	0.389	0.239		4.9±1
8	0.779	0.511	0.360	0.234		3.5±1
9		0.478	0.345	0.179	62±5	9.2±2
10	0.424	0.175	0.176	0.204		79±4
Ascorbic acid			0.289			15±4
Trolox®			0.110			33±3

Table 1 shows a summary of the oxidation potentials of the examined naphthoquinones with reference to the first peak. It can be seen that the oxidation potential is compromized by a Cl or Br atom inserted at position 3. The difference in potential is approximately $+100$ mV compared to the unsubstituted compound **1**. The introduction of a methoxyl group also leads to a shift of E_{pa1} towards more positive values ($E_{diff} = 70$ mV).

Compounds **5** and **10** with the OH group at position 3 are easiest to oxidize under physiological conditions (phosphate buffer, *pH* 7.4) at the glassy carbon (GC) electrode. In contrast, the hydroxyl group at position 5 has hardly any influence on the electron affinity.

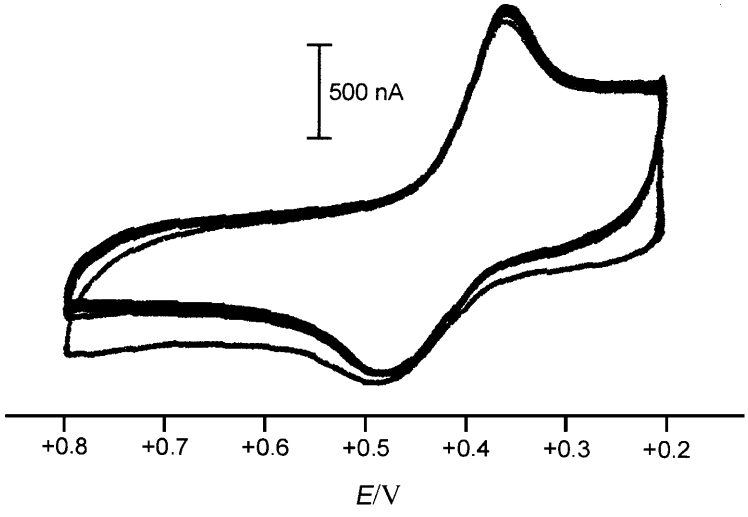


Fig. 2. Cyclic voltammogram of compound **10** in H_2SO_4/MeOH = 1:1 (v/v)

The oxidation potential of all compounds decreases with increasing *pH* value. The 3-hydroxy derivatives **5** and **10**, vinylogous hydroquinone derivatives, are an exception. At *pH* values of 1–7.4 they are the strongest reducing agents but, contrary to all other compounds, their oxidation potentials increase considerably at $pH = 10$, *i.e.* the *pH* range of the photo-chemiluminometric procedure. This effect is due to the deprotonation of the OH groups in position 3, because 3-hydroxy-1,4-naphthoquinones are vinylogous carboxylic acid derivatives.

The strongest reducing agents in the physiological *pH* range ($pH = 7.4$) are compounds **1** and **6** as well as, in particular, **5** and **10**. Their oxidation potentials are between the values of ascorbic acid and the tocopherol analogon Trolox® [6].

Photochemical investigation

The photochemical properties were determined using the measurement principle according to *Popov et al.* [5] by means of a Photochem® device. With this method, reactive oxygen derived species (ROS, *e.g.* superoxide radicals) are formed by exposure of a luminol solution to UV radiation in the presence of oxygen ($^3O_2$). These ROS oxygenate the luminol molecule with generation of chemiluminescence. If radical quenchers are present (such as ascorbic acid or tocopherols), light emission is prevented, an effect that can be used for measuring and quantifying antioxidative activities. The device consists of two chambers connected by a short passage. In the first chamber, $O_2^{\bullet -}$ is generated through exposure of an aqueous luminol solution to UV radiation at $pH = 10.8$ (ACW kit); the $O_2^{\bullet -}$ is then pumped into the second, dark chamber and engages in a chemical reaction with luminol with an associated emission of chemiluminescence. The chemiluminescence signal is amplified in a photomultiplier and recorded.

If there is an $O_2^{\bullet -}$ quencher in the system, luminescence is suppressed, and lag phases are formed that are registered in the region of seconds. Weak inhibitors are measured at $c = 10^{-6}$, strong ones at $10^{-7}\,\mathrm{mol \cdot dm^{-3}}$ (Table 1).

The results listed in Table 1 show that – with the exception of the vinylogous hydroquinones **5** and **10** – none of the aryl naphthoquinones reaches the antioxidative activity of ascorbic acid. The 3-hydroxyl derivatives **5** and **10**, on the other hand, surpass even Trolox® as $O_2^{\bullet -}$ quenchers. Compound **5** is one of the most active antioxidants.

Comparisons

A comparison of the electrochemical and photochemical methods used to determine the reducing and/or ROS quenching properties of aryl naphthoquinones **1**–**10** shows roughly comparable activities. The 3-halogen compounds are characterized by both the most positive oxidation potentials and the smallest lag phases. In both measurement procedures, the 3-hydroxy compounds **5** and **10** proved to be the strongest antioxidants, whereas the data of the 3-methoxy- and 3-unsubstituted quinones **1** and **6** as well as those of **4** and **9** lay between the extreme values. Nevertheless, the results produced by the two methods for the strong antioxidants **5** and **10** differ considerably. Voltammetric measurement is possible even at a physiological *pH* value of 7.4. The chemiluminometric procedure [5] can, however, only be used at *pH* values above 10 where the oxidation potentials of **5** and **10** increase compared (see above) and these compounds are no longer the strongest reducing agents. Whereas compounds **2**, **3**, **4**, **7**, **8**, and **9** show considerably higher

oxidation potentials at $pH = 7.4$ than the antioxidants ascorbic acid and Trolox®, the oxidation potentials of **1** and **6** are comparable to those of ascorbic acid. The oxidation potentials of **5** and **10** range exactly between those of ascorbic acid and Trolox®.

As photochemical properties are concerned, the situation is far more differentiated, **5** and **10** exhibiting dramatically larger lag phases compared to **1**–**4** and **6**–**9** as $O_2^{\bullet -}$ quenchers. These lag phases are five to ten times lager than the lag phases of the strong antioxidants ascorbic acid and Trolox®. The results show that the procedure according to *Popov et al.* [5] facilitates recording of complex redox properties in addition to oxidation potentials; these redox properties could also be defined as antioxidative capacity [7], a term which describes the sum of all antioxidative and prooxidative reactivities of a compound with ROS. In an earlier paper [8] we were able to demonstrate that in an $O_2^{\bullet -}$ generating system (O_2–*DMSO*–OH^-) **5** is irreversibly decomposed in a cascade of radical reactions. The difference between the procedures probably lies in the sensitivity of the chemiluminometry to such irreversible processes compared with that of voltammetry. Both procedures suggest that compounds **5** and **10** have the strongest antioxidative properties. It must, however, not be forgotten that the redox potentials at $pH = 7.4$ were compared with the AOA at $pH > 10$.

In further experiments, an examination was undertaken as to whether the AOA of compounds **5** and **10** in acidic conditions is similar to that at $pH > 10$. For this purpose, the routine procedure with the *N*-centred radical *DPPH* in neutral ethanol or acetone solution was chosen. This procedure is a suitable quick test for the differentiation between strong and weak antioxidants. The decrease in colour intensity of the *DPPH* is photometrically evaluated. Mixing of identical volumes of equimolar solutions of strong antioxidants (ascorbic acid, Trolox®, **5**, **10**) and *DPPH* causes sudden decoloration. The reaction is so fast that photometric quantification was impossible. With weak antioxidants, *e.g.* aryl naphthoquinones **1**–**4** and **6**–**9**, decoloration is slow (from minutes to hours) and can be observed photometrically.

To date, good correlations of AOA and oxidation potential have been proved for a number of natural agents and/or synthetic compounds [9–11]. A comparable correlation can also be derived for the aryl naphthoquinones examined by us. Good AOA seems to require a low oxidation potential [6], as in the case of the reference substances Trolox® and ascorbic acid. In addition, further physico-chemical properties are indicative of the degree of AOA. As far as the determination of the antioxidative capacity of highly active antioxidants is concerned, the procedure according to *Popov et al.* [5] supplies additional information, but with the restriction that measurements can only be carried out in highly alkaline solutions.

Experimental

Voltammetry

The electrochemical investigations were carried out with the polarographic/voltammetric analyzer model 64 A (EG & G, PARC, New Jersey, USA) in combination with a house made electrode stand [12]. A glassy carbon electrode was used as the working electrode, an Ag/AgCl electrode (3 *M* KCl) as

the reference electrode, and a Pt wire as the auxiliary electrode. An *XY*-plotter RE 0150 (EG & G, PARC) was used for recording the cyclic voltammograms. The *pH* values were measured with an Orion glass electrode, model 91-0, and an Orion *pH* meter, model 520 A.

The depolarizer parent solutions ($c = 10^{-3}\,M$) were prepared with EtOH (Merck). HAc/NaAc buffer (0.1 *M*), phosphate buffer (0.066 *M*, $pH = 6.88$), H_2SO_4 (0.05 *M*), and *Britton-Robinson* buffer (9.5) mixed 1:1 (v/v) with MeOH were used as basic electrolyte solutions. The chemicals used were of suprapure (Merck) and the H_2O of nanopure quality.

10 cm^3 of the basic solution were voltammetrically analyzed after mixing with 200 mm^3 stock solution and 8 min of purging with N_2 under setting of the following device parameters: mode, CV; diameter of the electrode, 3 mm; potential range, 0.00 V–1.30 V; scan speed, 100–1000 mV/s; sensitivity, 50 μA.

Determination of the antioxidative capacity ($O_2^{\bullet -}$ quenching)

a) Blank value: A mixture of 25 mm^3 luminol standard solution (ACW-Kit, F.A.T. GmbH, D-10559 Berlin, Germany) and 2.5 cm^3 carbonate–hydrogen carbonate buffer ($pH = 10.8$, 0.1 *M*) is added to the measuring device from the start receptacle under addition of 0.1 $mmol \cdot dm^{-3}$ Na_2-*EDTA*. The measurement of five blank values results in a lag phase mean value (lag_0) of 50 s.
b) Measured value: The solution mixture for the determination of the blank value is adjusted to measurement concentrations ($c = 10^{-6}$, 10^{-7}, and $10^{-8}\,mol \cdot dm^{-3}$) with the MeOH parent solutions of the examined substances. Measurement results in the total lag phase of the sample (lag 1).

The effective lag phase of the sample is

$$L = lag_1 - lag_0$$

Differentiation of strong and weak antioxidants with DPPH

1.5 cm^3 *DPPH* solution (1 μM in EtOH or acetone) and 1.5 cm^3 test solution (1 μmol test substance per dm^{-3} EtOH or acetone) are mixed rapidly in a test tube. The strong antioxidants (ascorbic acid, Trolox®, **5**, and **10**) cause immediate (1–3 s) decoloration of the deep-blue solution.

References

[1] Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC (1999) Angew Chem **111**: 280
[2] Aruoma O (1999) Free Radical Research **30**: 419
[3] Wurm G (1991) Arch Pharm (Weinheim) **324**: 491
[4] Wurm G (1992) Arch Pharm (Weinheim) **325**: 365
[5] Popov I, Lewin GI (1994) Phys Chem Biol and Med **1**: 75
[6] Born M, Carrupt P-A, Zini R, Bree F, Tillement J-P, Hostettmann K, Test B (1996) Helv Chim Act **79**: 1147
[7] Bombardelli E, Morazzoni P (1993) Chim Oggi **11**: 25
[8] Wurm G, Damerau W, Duchstein H-J (1995) Arch Pharm (Weinheim) **328**: 451
[9] Buettner GR (1993) Arch Biochem Biophys **300**: 535
[10] Van Acker SABE, Koymans LMH, Bast A (1993) Free Radic Biol Med **15**: 311
[11] Dryhurst G, Kadish K, Scheller F, Renneber R (1982) Biol Electrochem **1**: 256
[12] Kalcher K (1986) Fresenius Z Anal Chemie **323**: 238