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# Quercetin Reduces Prooxidant Action of Organometallic Compounds on Liposome Membranes Irradiated with UV

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**Abstract**

We studied the effects of UV radiation on the degree of phosphatidylcholine (PC) liposome membrane oxidation in the presence of such toxic organometallic compounds (TOC) as diphenyltin dichloride (DPhT), triphenyltin chloride (TPhT), dibutyltin dichloride (DBT), tributyltin chloride (TBT), triphenyllead chloride (TPhL) and tributyllead chloride (TBL). PC liposome oxidation was also investigated in the presence of quercetin (Que) and equimolar mixtures of Que and TOC in order to determine the protective properties of this natural antioxidant with respect to liposome membrane oxidation induced by UV light. Concentration of the compounds studied was 10 μM**.** The degree of liposome oxidation was measured using the TBARS (Thiobarbitutric Reactive Substances) test. The sequence thus obtained of relative induction of PC oxidation due to TOC was as follows: TBT > TPhT ≅ DBT > DPhT ≅ TPhL > TBL. The results of studies with Que indicate its high efficacy in the antioxidative action of equimolar mixtures of Que and TOC.

From our study it follows that quercetin forms complexes both with phenyl- and butyl- tin and lead compounds. A high antiradical (towards DPPH radical) activity of the associates with respect to the activity of quercetin alone was also found. This result can partly explain the antioxidative properties of Que in the studied solution, connected with their antiradical action and chelating posibility towards the organometallic compounds studied.

A factor that differentiates the antioxidant activity of the complexes Que-TOC is probably their differentiated location in the liposome membrane bilayer.

**Keywords:** quercetin, UV radiation, prooxidant and antioxidant action, organometallic compounds, liposomes

## Introduction

The toxicity of organometallic compounds (TOC), which are often present in many regions of the human environment [1], is connected with the degree of their accumulation in cells of living organisms [2]. The hydrophobic properties of amphiphilic organometallic compounds, including the n-butyl and n-phenyl lead and tin compounds studied in the present work, indicate their large affinity to the lipid phase of membranes. Investigation of the interaction between TOC and model membranes may contribute to the explanation of their toxic effects on biological membranes. Reports on such studies are not numerous in literature [3-8]. It is thought that the harmful action of some TOC is the result, under certain conditions, of their free radical action [9,10], especially towards biological membrane lipids. It is probable that the radical forms of TOC, such as tributyl- or triphenyltin, are the cause of initiation and propagation of oxidation processes of lipids in biological membranes. There is no

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doubt that the low-molecular, exogenous, antioxidative substances such as, for instance, vitamin C, tocopherols, carotenoids or flavonoids can, especially under oxidative stress, effectively support the antioxidative system of the organism.

Studies on the antioxidative action of flavonoids - a group of compounds not yet well known in this respect - have been conducted very intensely in recent years [11-14]. The mechanism of the antioxidative action of those compounds have not yet been fully elucidated and are still a matter of considerable debate. As a conequence of their polyphenolic structure, those compounds are considered effective scavengers of peroxyl, hydroxyl and superoxide free radicals. This free radical scavenging mechanism has generally been described as the basis of their antioxidant activity [15-17]. Aditionally, flavonoids are known to chelate transition metal ions (Cu and Fe) [15,18] - another possible contributory mechanism toward their antioxidant action. Quercetin belongs to the most frequently studied and most active flavonoid compounds. It is commonly found in fruits and vegetables [ 19, 20 ]. It is known that quercetin is a potent antioxidant as assessed via the oxygen radical absorbance capacity (ORAC) assay [21]. It has been shown that quercetin is able to scavenge various reactive oxygen species [22], can prevent free radical-induced lipid peroxidation [21-24], reduce oxidative neuronal cells [25, 26] and DNA damage [27], inhibit oxidatively modified LDL [28] and participate in antioxidative defense in blood plasma [29]. It is just those potential antioxidant, chelating and antiradical properties of quercetin that decided its use in the present work. The aim was to compare it with the prooxidative behaviour of di- and triphenyltin, di- and tributyltin, tributyl- and triphenyllead towards PC liposomes and assess its protective action.

## Materials and Methods

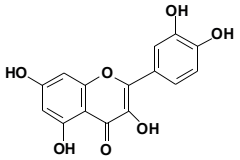
Phosphatidylcholine was extracted from egg, as described elsewhere [30]. The structures of compound studied are presented in Tab. 1.

Quercetin was purchased from Sigma Chemical Co. (St. Louis, MO). Organotin compounds were purchased from Alfa Products (Karlsruhe, Germany). The remaining chemicals were of analytical grade.

### Liposome Preparation and Induction of Peroxidation

A chloroform solution of egg yolk phosphatidylcholine was dried under vacuum in a nitrogen atmosphere. A 50 mM Tris:HCl [(hydroxymethyl) aminomethane] buffer of pH 7.4 was added and the sample was vortexed to obtain a milky suspension of multilamellar vesicles. The final concentration of lipid in the vesicle suspension was 1.5 mg ml $^{-1}$. Such a suspension was then sonicated for 10 min with a 20 kHz sonicator to obtain small vesicles. The quercetin or the equimolar mixtures of quercetin with TOC was then added from a concentrated methanol solution (2 $\cdot 10^{-3}$ M) to the stirred sample of suspended

Table 1. Chemical structure of the compounds studied.

| Chemical structure | Compound name |
|---|---|
| $Ph_3SnCl$ (TPhT) | - triphenyltin chloride |
| $Ph_2SnCl_2$ (DPhT) | - diphenyltin dichloride |
| $Ph_3PbCl$ (TPhL) | - triphenyllead chloride |
| $(C_4H_9)_3SnCl$ (TBT) | - tributyltin chloride |
| $(C_4H_9)_2SnCl_2$ (DBT) | - dibutyltin dichloride |
| $(C_4H_9)_3PbCl$ (TBL) | - tributyllead chloride |
|  | - quercetin (Que) |

vesicles. The concentration of the compounds studied was 10 μM. Lipid peroxidation in the egg phospholipid liposomes was induced by ultraviolet radiation from a bactericidal lamp (λ = 253.7 nm) at 3.5 mW/cm$^2$ intensity. The accumulation of phospholipid peroxidation products was estimated by determining 2-thiobarbituric acid reactive products (TBARS) in the incubation medium [31,32]. The amount of reaction products was determined by measuring the increase in absorbance at 535 nm. Percentage of PC liposome oxidation induction (by UV and TOC) and inhibition (by quercetin and their equimolar mixture with TOC) was calculated as follows: percentage of inhibition = $(1 - \Delta A_A / \Delta A_O)$ 100%, where $\Delta A_A$ – increase in absorption (for λ= 535 nm) after 30 min since the start of irradiation of liposome with antioxidant added, $\Delta A_O$ increase in absorption (for λ= 535 nm) after 30 min since the start of irradiation of liposomes without antioxidant added.

### Reduction of DPPH Free Radical

A methanol solution of the free radical 1,2-diphenyl-picrylhydrazil (DPPH) of absorption ca. 0.9 was mixed with a proper amount of methanol solution of an antioxidant (quercetin or equimolar mixture of quercetin with studied TOC). The mixture was then incubated for an hour at room temperature and darkness. During incubation a reduction occurred of a part of the free radical form DPPH. Then, absorption was measured at the characteristic for DPPH wavelength equal to 517 nm [33]. The measurements were done for 10 μM concentrations of antioxidant. The amount of DPPH reduction (expressed in percentage) by the antioxidant after one hour compared to the control sample (without antioxidant) was calculated.

### Complexation of TOC with Quercetin

The studies of complexation of TOC with quercetin were made using the spectrophotometric method. A constant amount of quercetin, was added to 2 ml of methanol, to obtain 48.8 μM concentration, and then an organometallic compound was titrated until its concentra-

tion / quercetin ratio reached **:** (1:4) for Que:DPhT; (1:10) for Que:DBT; (1:50) for Que:TPhT, (1:100) for Que:TBL, Que:TPhL and (1:200) for Que:TBT. Absorption of the stirred samples was measured at 250-600 nm and room temperature, in 1 cm cuvette with a UV-Vis spectrophotometer model 2401 (Shimadzu). Complexation of TOC with quercetin were presented by the hyperchromic effect (ΔA). In order to compare the chelating properties of the associates, we measured absorption increases ΔA maxima of the peaks coming from the complexes for the following ratios Que to TOC: (1:1) in the case of Que:DPhT and Que:DBT; (1:10) in the case of Que:TPhT, Que:TBT, Que:TPhL and Que:TBL [34, 35].

## Results and Discussion

Figure 1 shows time dependence of oxidation level of PC liposome membranes, induced by UV radiation (λ = 253.7 nm) in the presence of butyl (A) and phenyl (B) forms of tin and lead. The oxidation level was determined using the TBARS test. It consisted in measurement of absorption increase at a wavelength (λ = 535 nm) characteristic for malone dialdehyde produced when liposome oxidation products react with TBA acid. In Fig. 1A the respective plots are for butyltin and butyllead (TBT, DBT and TBL), while in Fig. 1B the plots refer to phenyltin and phenyllead (TPhT, DPhT and TPhL). The control (filled circles) shows time dependence of PC liposome oxidation when exposed to UV radiation and no TOC compounds are present. Concentration of all the applied TOC compounds was constant and equal to 10 μM. As seen from all the curves in Fig. 1, all TOC compounds cause a marked increase, relative to control, in liposome membrane oxidation. Apparently, this indicates that free-radical forms of TOC compounds arise when exposed to UV light. Induction of DPhT and TPhT free radicals as a result of photolysis was observed using the EPR method (our unpublished data). Under the conditions of our experiments with lipid membranes it is most probable that free radicals of TOC and those arising from UV interaction with the aqueous phase cooperate in initiation and propagation of lipid peroxidation. This is most pronounced with tributyl- and triphenyltin (TBT > TPhT) and least with the lead compounds (TBL < TPhL). Lipid oxidation induced by compound TBL was decisively weaker than that of TBT, DBT and DPhT. However, its intensity of action was comparable to that of DPhT. In order to have a quantitative comparison of the prooxidative behaviour of TOC under UV radiation, the relative lipid oxidation level was determined (in percentage). It was calculated by using the formula: $(\Delta A_{TOC} / \Delta A_{O} - 1) \cdot 100\%$, where $\Delta A_{TOC}$ is the increase in absorbance of the sample after 30 min of exposition to UV (of 3.5 mW/cm$^2$) in the presence of a TOC compound and $\Delta A_{O}$ is the increase in absorbance of the control sample (after same time of irradiation that is after 30 min). Values of that oxidation are compiled in Table 2. There are reported also percent values of liposome oxidation inhibition induced by quercetin and its equimolar

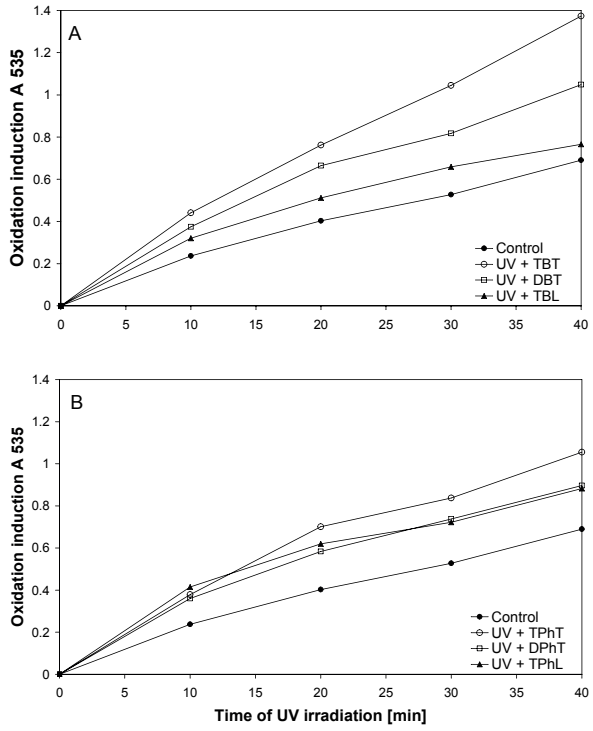



Fig. 1. Oxidation induction levels of PC membranes induced by butyl tin and lead compounds (A) and phenyl tin and lead compounds (B) as a function of UV irradiation time. Standard deviation was studied ≤ 0.04.

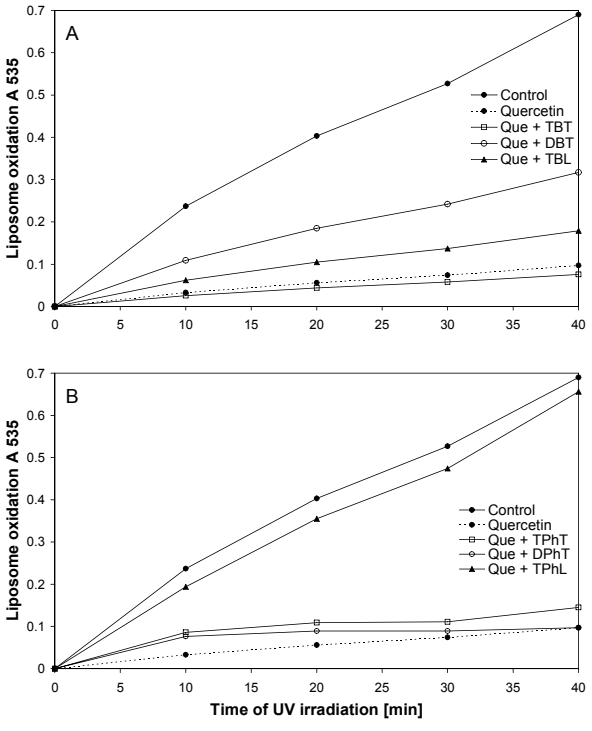



Fig. 2. Liposome oxidation levels of PC membranes induced by quercetin and its equimolar mixtures with butyl tin and lead compounds (A) and phenyl tin and lead compounds (B) as a function of UV irradiation time. Standard deviation was studied ≤ 0.04.

Table 2. Percent values for oxidation of liposomes by TOC compounds (10 μM) in the presence of UV radiation, oxidation inhibition by quercetin and equimolar mixtures of quercetin and TOC (10 μM) and reduction of the free radical DPPH (10 μM). The table also contains values of the hyperchromic effect (ΔA) in the process of Que titration with TOC compounds. The Que:TOC ratios are as follows: (1:1) for Que:DBT and Que:DPhT, (1:10) for Que:TBT, Que:TPhT, Que:TBL and Que:TPhL

| Relative liposome oxidation in the presence of UV and TOC [%] | | | Oxidation inhibition [%] | DPPH reduction [%] | Hyperchromic effect ΔA |
|---|---|---|---|---|---|
| TBT | 99 | Que:TBT | 89 | 73 | 0.063 (1:10) |
| DBT | 55 | Que:DBT | 54 | 60 | 0.228 (1:1) |
| TBL | 25 | Que:TBL | 74 | 62 | 0.061 (1:10) |
| TPhT | 59 | Que:TPhT | 79 | 66 | 0.195 (1:10) |
| DPhT | 40 | Que:DPhT | 82 | 67 | 0.216 (1:1) |
| TPhL | 37 | Que:TPhL | 10 | 50 | 0.189 (1:10) |
| | | **Quercetin** | **86** | **60** | - |

mixtures with TOC, calculated on the basis of measurements plotted in Fig. 2. This figure, like Fig. 1, presents lipid oxidation levels induced by UV in the presence of equimolar TOC-Que mixtures as a function of time. Both figures reveal a relatively high ability of quercetin to inhibit lipid peroxidation, and thus a protective role of Que against the effect of free radicals on membranes. To find out what is the mechanism of the protective role of Que, the complex-making ability of Que, reported in the literature with respect to metal ions, such as Fe, Cu, Co and Al [9, 12, 29], was investigated. The obtained electronic absorption spectra of Que taken during its titration with TOC compounds confirmed a complex formation by revealing a new absorption band characteristic for the complex. Fig. 3 shows some representative absorption spectra of Que titrated with DBT (Fig. 3A) and TBT (Fig. 3B). The peaks coming from quencetin ($A_{Que}$) and those from the complex Que:TOC ($A_{Que\text{-}TOC}$) are marked in the figure, the hyperchromic effect (ΔA) connected with that band being the assumed parameter that characterizes the association ability of Que towards TOC. Values of ΔA for various equimolar ratios Que:TOC are given in Tab. 2. On that basis it can be concluded that Que forms strongest complexes with ionic form of DBT and DPhT, weaker ones with TPhT and TPhL and the weakest with ions of TBT and TBL. Ligands of quercetin after complex formation with TOC seem to preserve their ability to scavenge free radicals. This can be confirmed by studies on the antiradical action of the complexes with respect to the free radical 1,2diphenyl-picrylhydrazil (DPPH), regarded as standard [24]. The degree of DPPH radical reduction (expressed in percentage) after its hour-long incubation with the complexes and with Que is given in Tab. 2. Except for the complex Que:TPhL all the remaining associates show a little higher ability to reduce the free radical of DPPH than the free quercetin does, the highest activity being that of Que with TBT. This may indicate that Que get stabilized in the associates molecules and thereby their reducing ability increases. The listing in Tab. 2 reveals a good correlation between the antiradical ability of the associates (with respect to DPPH) and their antioxidative properties. This suggests a one of the possible mechanisms of the antioxidative action of the complexes, i.e. free radical scavenging. There is, however, no (positive) correlation between the assumed parameter ΔA and antioxidative ability of the associates. The very fact of complex formation between Que and TOC can, however, have a positive significance in decreasing the destructive action of UV radiation on quercetin molecules. Our

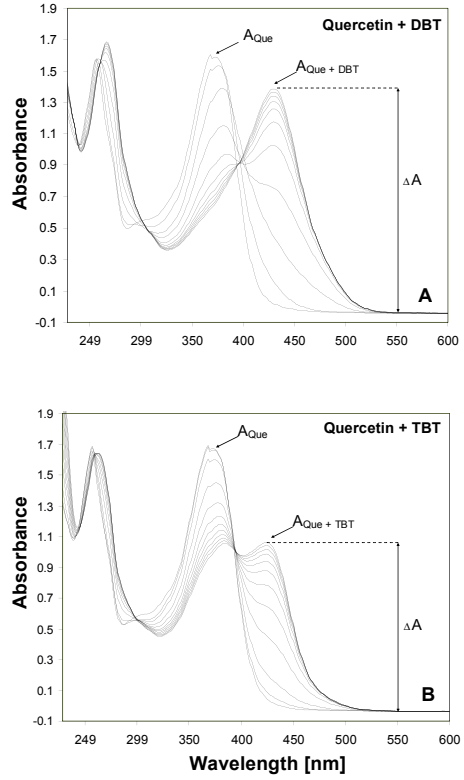



Fig. 3. Absorption spectra for quercetin titrated with DBT (A) and TBT (B). The initial concentration of quercetin was 48.8 μM. The final concentration ratio was: 1:10 for Que:DBT and 1: 200 for Que:TBT.

(unpublished) data indicate about a 20% decrease in Que destruction resulting from complex formation with DPhT compared to the degree of destruction of free Que (ca. 35% destruction after 1 hour exposition). One can thus conclude that under experimental conditions the pool of complexed, antioxidant Que molecules is larger than the pool of free quercetin. However, explaining the role and stability of the Que:TOC associates (and their stoichiometry) requires further investigation. Thus, for instance it is difficult to understand that to the high antioxidative activity of the complex Que:TBT (89%) corresponds to a low value of the parameter ΔA (equal to 0.063), while the low antioxidative ability of Que:TPhL (10%) corresponds to the relatively high value of ΔA (0.189) for that complex. It should be emphasized that the parameter discussed has been defined for complexes formed in the presence of methanol, and for the lipid membrane it can assume quite different values.

It seems that in order to explain the antioxidative abilities of the complexes one should also take into account the probable differentiation in the degree of adsorption in the liposome membrane. It cannot be excluded that for steric reasons, for instance, the complexes Que:TBT are localized more near the surface (compared with e.g. Que: DBT and DPhT) and thus able to catch free radicals coming to the membrane more effectively, with resulting high antioxidative activity.

In summary, the antioxidative efficacy of quercetin in the presence of TOC, under UV radiation, results partly from its chelating property towards TOC ions and from the antiradical property of quercetin and its complexes that are able to scavenge free radicals. When explaining the differences in the antioxidative action of the Que: TOC complex with respect to liposome membranes, one should take into account the possible differentiation of their localization in the membrane bilayer, which is to be explained in further investigation.

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