100. Electrochemical Behaviour and Antioxidant Activity of Some Natural Polyphenols

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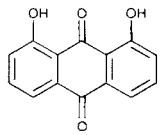
A number of natural polyphenols (chlorogenic acid (**9**), cordigol (**11**), cordigone (**12**), danthrone (**1**), 1,5-dihydroxy-3-methoxyxanthone (**2**), eriosematin (**7**), flemichin D (**8**), frutinone A (**6**), mangiferin (**4**), quercetin (**5**), 1,3,6,7-tetrahydroxyxanthone (**3**) and verbascoside (**10**)) were investigated for their redox properties using cyclic voltammetry. The antioxidant properties of these compounds were also examined in two models, namely lipid peroxidation in rat synaptosomes and AAPH-mediated oxidation of serum albumin. Compounds with a catechol group (**9**, **4**, **5**, **3** and **10**) were oxidized below 0.4 V and inhibited lipid peroxidation with IC_{50} values between 2 and 8 μM. Compounds having one or more isolated phenolic groups and showing an oxidation potential between 0.45 and 0.8 V (**11**, **12** and **8**) inhibited lipid peroxidation with IC_{50} between 7 and 9 μM, except **2** (0.45 V), danthrone (0.96 V) and eriosematin which showed no or modest antioxidant activity. Some of the investigated compounds also protected albumin from oxidation, but no structure-activity relationship was apparent, suggesting that other factors beside redox potential influence this activity.

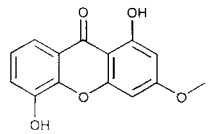
Introduction. – Phenolic compounds occur widely in plants and are of particular interest as protectors of biological systems against oxidative stress. Thus, flavonoids are known to be promising antioxidants [1–3] acting as free radical scavengers [2] [4–8] and metal ion chelators [9] [10] with activities influenced by their hydroxylation pattern on the phenyl and benzo-γ-pyrone rings. In this context, one flavonoid that has received much attention is quercetin (**5**) [11].

Another group of compounds with antioxidative properties are hydroxylated cinnamic acids such as caffeic acid and its derivatives chlorogenic acid (**9**) and verbascoside (**10**). It has been shown that caffeic acid can act as a pro-oxidant [12] or as an antioxidant [13] depending on experimental conditions. These activities are due to the chelation of metal ions such as iron and cupper [14] [15], and to the scavenging of free radicals [14] [16] [17]. The same properties have been reported for chlorogenic acid (**9**) [12] [13] [16] and verbascoside (**10**) [17] [18].

Danthrone (**1**), an anthraquinone derivative, is practically inactive in scavenging the 1,1-diphenyl-1-picrylhydrazyl radical (DPPH) and in other antioxidant activities [19] [20]. As far as xanthones are concerned, a few studies have been devoted to their antioxidant activity [21–24]. Thus, mangiferin (**4**) proved to be a free-radical scavenger and an inhibitor of lipid peroxidation in rat liver microsomes [22]. Some xanthones with a catechol functionality demonstrated activity as radical scavengers and antioxidants against lipid peroxidation, whereas analogues with a *para*-hydroquinone moiety had little or no activity [23].



1 (Danthrone)



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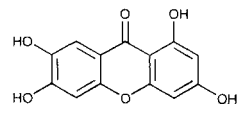
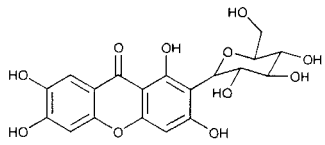
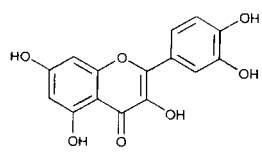


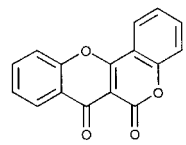
3 (1,3,6,7-Tetrahydroxyxanthone)



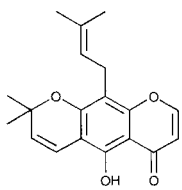
4 (Mangiferin)



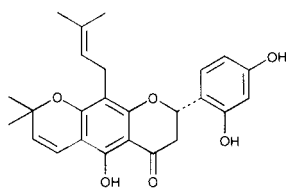
5 (Quercetin)



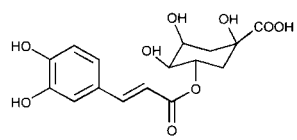
6 (Frutinone A)



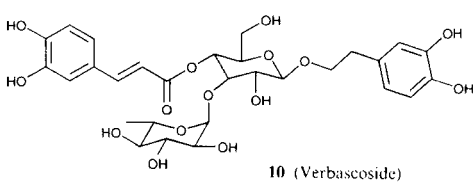
7 (Eriosematin)



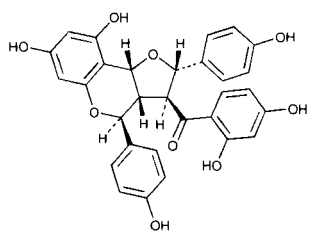
8 (Flemichin D)



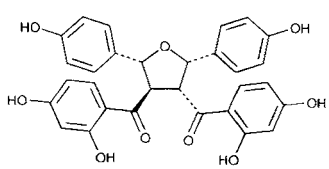
9 (Chlorogenic acid)



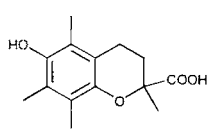
10 (Verbascoside)



11 (Cordigol)



12 (Cordigone)



13 (Trolox)

Considered globally, results in the literature afford only a very limited understanding of structure-property-activity relationships in antioxidant tests. One factor that appears to play a distinct role is the redox potential as measured by a variety of methods such as pulse radiolysis, flash photolysis and electrochemistry [5] [8] [25] [26]. To provide a contribution to our understanding of the structure-property-activity relationships of antioxidants, we have examined a number of natural polyphenols for their redox potential and antioxidant activities. Redox potential was measured by cyclic voltammetry, while antioxidant activities were assessed in two tests, namely lipid peroxidation in rat

synaptosomes and serum albumin oxidation mediated by 2,2-azabis(2-amidinopropane). Some known phenols (chlorogenic acid (**9**), danthrone (**1**), mangiferin (**4**), quercetin (**5**), trolox (**13**) and verbascoside (**10**)) were added to this study to complete the data available on their properties, to extend the structural variety of the compounds investigated, and/or for comparison purposes.

Results and Discussion. – 1. *Electrochemistry.* The compounds examined are: an anthraquinone (danthrone (**1**)), three xanthones (1,5-dihydroxy-3-methoxyxanthone (**2**), 1,3,6,7-tetrahydroxyxanthone (**3**), mangiferin (**4**)), one flavonoid (quercetin (**5**)), three other benzo-γ-pyrone derivatives (frutinone A (**6**), eriosematin (**7**), flemichin D (**8**)), two derivatives of caffeic acid (chlorogenic acid (**9**), verbascoside (**10**)), two furan derivatives (cordigol (**11**), cordigone (**12**)) and the soluble vitamin E analogue trolox (**13**) used as a reference compound. Based on their behaviour in cyclic voltammetry and on their chemical structure, they can be classified into three different groups. For comparison and reference purposes, we measured the first oxidation potential of catechol, resorcinol, phenol and 2,4-dihydroxybenzophenone under the standard conditions used here (*Table 1*).

First Group of Compounds. The first group is composed of quercetin (**5**), verbascoside (**10**), chlorogenic acid (**9**), 1,3,6,7-tetrahydroxyxanthone (**3**) and mangiferin (**4**). These compounds have in common a catechol moiety believed to be the electrochemically active group. When examined at a sweep rate of 100 mV s^{-1}, they showed a first oxidation peak at a potential E_{pa1} lower than 0.4 V. Caffeic acid (of which **9** and **10** are esters) was taken as a reference compound. Its cyclic voltammogram (CV) showed a redox couple at an E_{pa} of 0.23 V and on the reverse sweep at an E_{pc} of 0.02 V (*Fig. 1*). The same oxidation potential was measured for chlorogenic acid (**9**) at an E_{pa} of 0.23 V, but on the reverse sweep the reduction peak was registered at an E_{pc} of 0.11 V (*Fig. 2*). When the sweep rate was reduced to 5 mV s^{-1} (result not shown), the E_{pa} was identical for caffeic acid and **9** at 0.20 V. However, two reduction peaks appeared at 0.13 V and 0.03 V for **9**, while caffeic acid was reduced at $E_{pc} = 0.04$ V, suggesting that a slow reaction of rearrangement occurs for **9** following its oxidation, but not for caffeic acid.

Verbascoside (**10**) has two catechol moieties and showed a distinct CV (*Fig. 3*). One oxidation peak but two reduction peaks were detected. The two catechol moieties of verbascoside probably oxidize at the same potential ($E_{pa} = 0.19$ V) as confirmed at lower sweep rates (not shown) and by comparing the oxidation peak currents (*Table 2*). Indeed, the ratios of i_{pa}(**10**) to i_{pa}(caffeic acid) at different sweep rates were close to 1.5, while the ratios of i_{pa}(**9**) to i_{pa}(caffeic acid) were close to 1.0. Since caffeic acid undergoes a two-electron oxidation in aqueous solution [27] [28], we conclude that **10** must undergo a

Table 1. *First Oxidation Potentials Measured under the Standard Conditions of This Study*[a])

Compound	E_{pa1} [V]
Catechol	0.23
Resorcinol	0.61
Phenol	0.63
2,4-Dihydroxybenzophenone	0.81

[a]) 0.07M phosphate buffer (pH 7.4)/EtOH 1:1 (*v*/*v*); working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

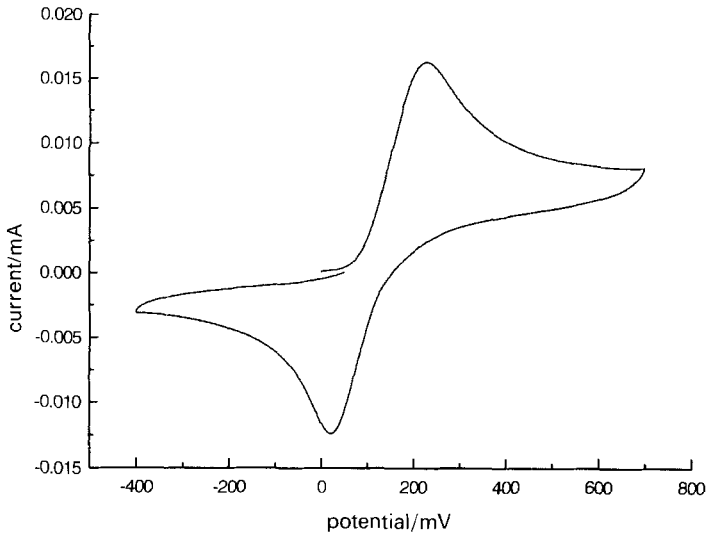


Fig. 1. *Cyclic voltammogram (CV) of 1 × 10^{-3}* M *caffeic acid in 0.07* M *phosphate buffer (pH 7.4)/EtOH 1:1 (v/v).* Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

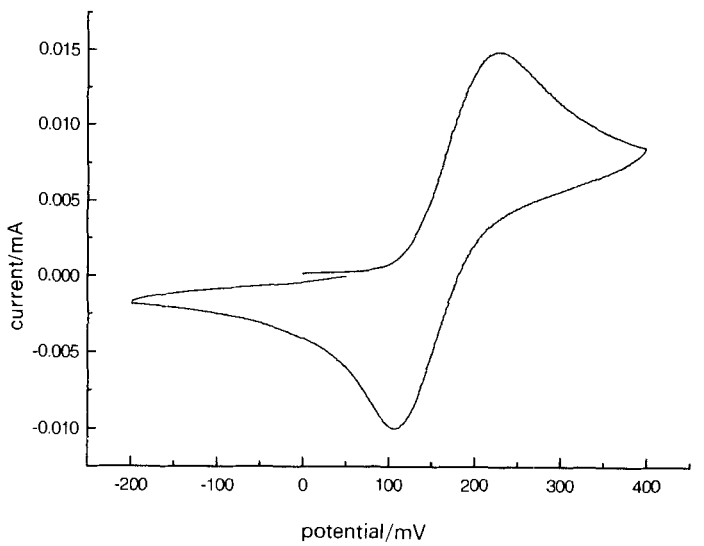


Fig. 2. *CV of 1 × 10^{-3}* M *chlorogenic acid* (**9**) *in 0.07* M *phosphate buffer (pH 7.4)/EtOH 1:1 (v/v).* Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

four-electron oxidation. On the reverse scan, two reduction peaks at $E_{pc1} = 0.13$ V and $E_{pc2} = 0.02$ V were observed (*Fig. 3*), again suggesting that a rearrangement reaction occurs for **10** following its oxidation.

The electrochemistry of a number of flavonoids has been already reported [29]. For quercetin (**5**), in particular, the pathways of oxidation were investigated [30], but the

Table 2. *Anodic Peak Current Ratios*[a])

Sweep rate [mV s^{-1}]	i_{pa} (**10**) / i_{pa} (caffeic acid)	i_{pa} (**9**) / i_{pa} (caffeic acid)
10	1.5	0.9
25	1.6	1.2
50	1.5	1.2
100	1.5	0.9
250	1.5	0.9
500	1.2	1.0
1000	2.1	1.0

[a]) The ratio equals the anodic peak current of **10** (i_{pa}(**10**)) or **9** (i_{pa}(**9**)) divided by the anodic peak current of caffeic acid (i_{pa}(caffeic acid)) that stands for a two-electron oxidation step [28].

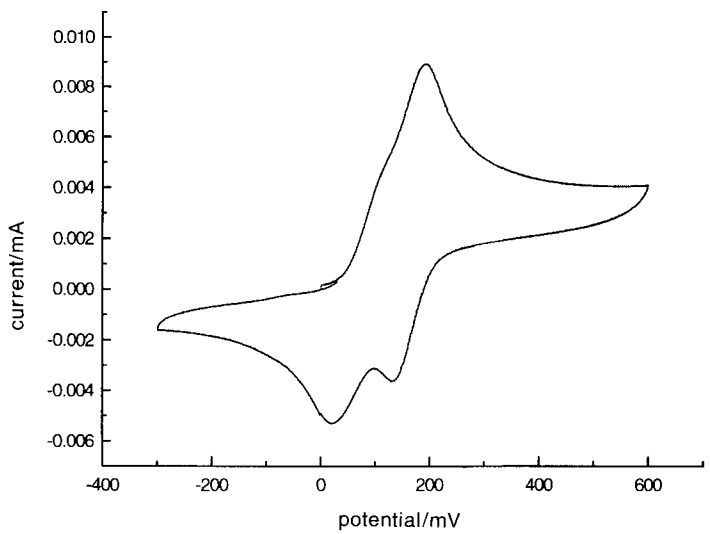


Fig. 3. *CV of 3.5 × 10^{-4} M verbascoside* (**10**) *in 0.07 M phosphate buffer (pH 7.4)/EtOH 1:1 (v/v)*. Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

products are not completely understood. Our results for **5** were in agreement with those in [30]. A redox couple corresponding to the catechol function was observed with an E_{pa1} at 0.11 V and an E_{pc1} at 0.04 V. A second and a third oxidation peak were observed at *ca.* 0.49 V and 0.9 V, probably corresponding to the oxidation of the OH group at C(4) and to the resorcinol moiety, respectively.

Comparable but not identical cyclic voltammograms were found for mangiferin (**4**) and 1,3,6,7-tetrahydroxyxanthone (**3**). At a sweep rate of 100 mV s^{-1}, two oxidation potentials were observed for **4** at E_{pa}s of 0.32 and *ca.* 0.86 V (*Fig. 4*), whereas for **3** the corresponding values were 0.28 and *ca.* 0.83 V (*Fig. 5*). When the sweep rate was decreased to 5 mV s^{-1}, three oxidation potentials occurred for **4** at 0.30, 0.48 and 0.83 V, and for **3** at 0.23, *ca.* 0.5 and 0.74 V. The oxidation potentials of **4** are thus slightly higher than those of **3**, presumably due to the influence of the substituent at C(2). The first oxidation

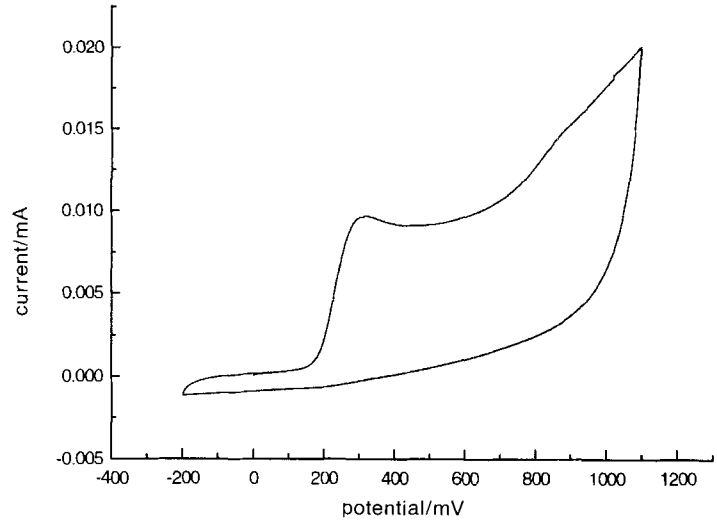


Fig. 4. *CV of* 5×10^{-4} M *mangiferin* (**4**) *in 0.07* M *phosphate buffer (pH 7.4)/EtOH 1:1 (v/v).* Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

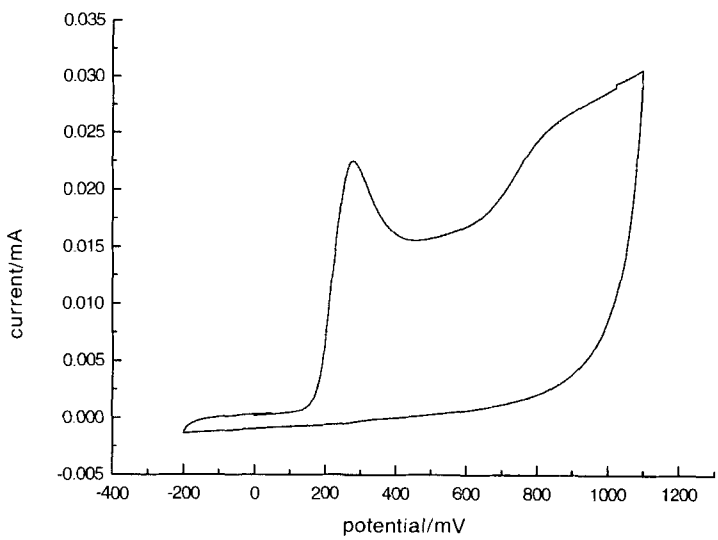


Fig. 5. *CV of* 1×10^{-3} M *1,3,6,7-tetrahydroxyxanthone* (**3**) *in 0.07* M *phosphate buffer (pH 7.4)/EtOH 1:1 (v/v).* Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

potential of these two xanthones can be assigned to the catechol moiety (see *Table 1*). In contrast, it is not clear whether the oxidation potential at higher values can be attributed to other moieties or to oxidation products. The third oxidation peak could be associated with the oxidation of the resorcinol moiety, since it corresponds to that seen for quercetin (**5**).

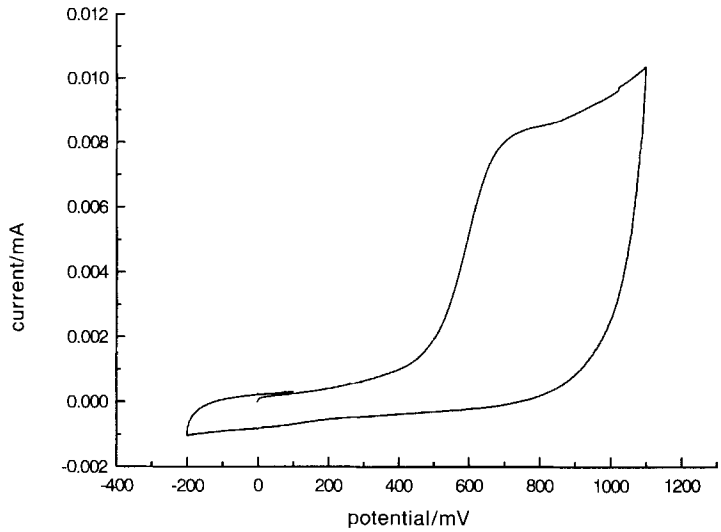


Fig. 6. *CV of* 5×10^{-4} M *cordigol* (**11**) *in 0.07* M *phosphate buffer (pH 7.4)/EtOH 1:1 (* v/v*)*. Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

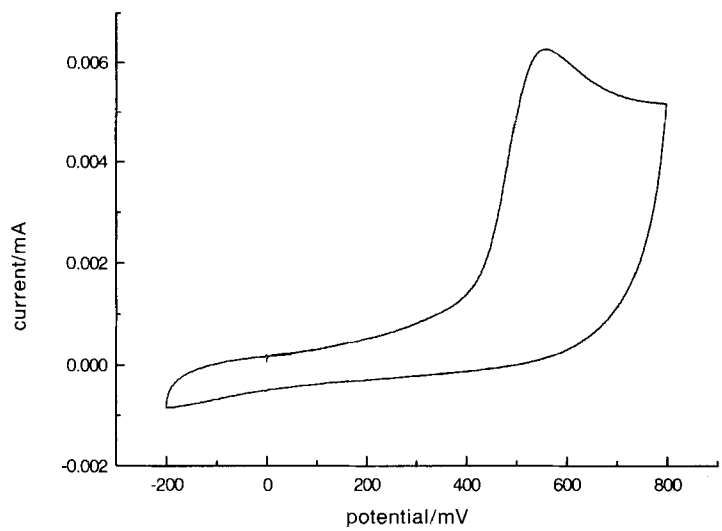


Fig. 7. *CV of* 4.5×10^{-4} M *flemichin D* (**8**) *in 0.07* M *phosphate buffer (pH 7.4)/EtOH 1:1 (* v/v*)*. Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 100 mV s^{-1}.

In summary, the first oxidation potential of compounds **9**, **10**, **3**, **4** and **5** is most likely due to the catechol moiety.

Second Group of Compounds. The second group comprises 1,5-dihydroxy-3-methoxyxanthone (**2**), flemichin D (**8**), cordigone (**12**), cordigol (**11**), danthrone (**1**; 1,8-dihydroxyanthraquinone) and eriosematin (**7**). These compounds have at least one phenol moiety

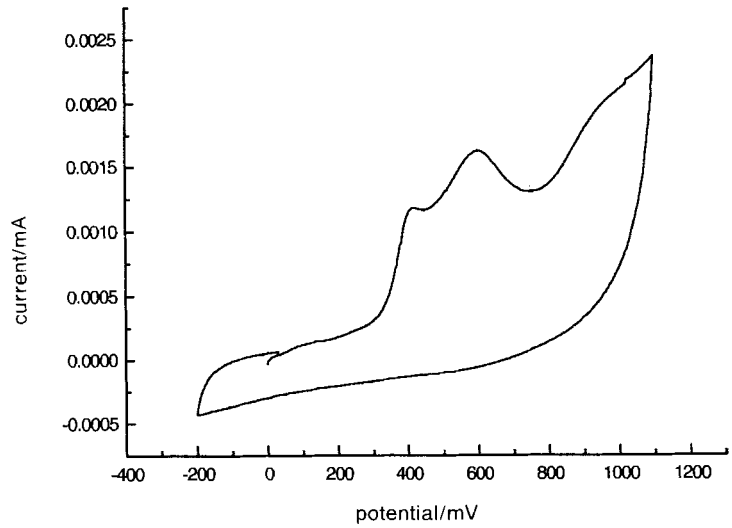


Fig. 8. *CV of 4.3 × 10^{-4} M 1,5-dihydroxy-3-methoxyxanthone* (**2**) *in 0.07 M phosphate buffer (pH 7.4)/EtOH 1:1* (v/v). Working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC; sweep rate: 25 mV s^{-1}.

and displayed E_{pa1} values between 0.4 and 1.0 V. Eriosematin (**7**) was not soluble enough to be investigated by electrochemistry. For **11** (*Fig. 6*) and **8** (*Fig. 7*), a single oxidation peak was found at *ca.* 0.71 and at 0.56 V, respectively. Cordigone (**12**) had two poorly defined oxidation peaks at *ca.* 0.64 and 0.84 V which stand probably for the phenolic and the 1,3-dihydroxy moieties. Although **11** incorporates principally the same phenolic groups like **12**, only one wave was observed for **11**. The oxidation currents of **11**, i_{pa}(**11**), were compared with the first oxidation currents of **12**, i_{pa}(**12**). The ratios i_{pa}(**11**)/i_{pa}(**12**) varied between 1.6 and 2.1 at sweep rates from 0.05 to 0.50 Vs^{-1}, indicating that the various phenolic groups of **11** are probably oxidized at the same potential.

1,5-Dihydroxy-3-methoxyxanthone (**2**) has a relatively low first E_{pa1} at 0.45 V compared with the other phenolic compounds. This may be due to the electron-donating ether O-atom *ortho* to the C(5)–OH group, which probably undergoes the first oxidation step. At a sweep rate of 25 mV s^{-1}, three oxidation steps could be seen at 0.41, 0.60 and *ca.* 0.98 V (shoulder) (*Fig. 8*). It is reasonable to postulate that the second oxidation peak is due to the phenolic moiety at C(1), while the shoulder is produced by products of oxidation.

Danthrone (**1**) was oxidized at a relatively high potential of 0.96 V. This is probably due to its two phenolic groups being strongly H-bound to the keto group, and/or being involved in keto-enol tautomeric equilibria.

In general, the compounds in the second group had E_{pa1} values comparable to those of other phenols (*Table 1*), indicating that their phenolic groups were indeed responsible for the first oxidation step. No reaction of reduction during reverse scans were detected, suggesting that the products of oxidation underwent further intramolecular or intermolecular reactions of an irreversible nature.

Third Group of Compounds. This group was composed of frutinone A (**6**) only. This compound could not be investigated by CV, being insufficiently soluble in the buffer/EtOH solvent used. The absence of a phenol or catechol moiety in **6** suggests inertness in the investigated range.

2. *Inhibition of Protein Oxidation.* The compounds **1**–**13** were examined for their activity and potency in inhibiting the oxidation of two representative biological targets, namely serum albumin and synaptosomal lipids. In the first test, a soluble radical-generating compound, 2,2-azobis(2-amidinopropane) (AAPH) was used to oxidize albumin [31]. The damage was quantified by the formation of carbonyl groups. Carbonyl content was determined spectrophotometrically using the method of *Keller et al.* [32] (*Table 3*).

Three compounds (**9**, **3** and **2**) showed modest but clear activities, while three other compounds (**4**, **11** and **1**) were marginally active and all others were inactive.

3. *Inhibition of Lipid Peroxidation.* The second test was based on the peroxidation of brain synaptosomal lipids by reactive oxygen species (mainly superoxide and hydroxyl radicals) generated from molecular oxygen by a $FeCl_2/FeCl_3$ system [33]. Aldehydes so produced were reacted with thiobarbituric acid and quantified photometrically. The results of the two biological tests are compared in *Table 3* together with the first oxidation potential measured by cyclic voltammetry. The biological value of **5** compares well with literature data [34] [35].

Of the twelve investigated compounds, nine showed good antioxidant activities by producing *ca.* 60–90% inhibition of lipid peroxidation at a test concentration of 0.1 mM

Table 3. *First Oxidation Potentials and Antioxidant Activities*

Compounds	E_{pa1} [V][a]	Lipid peroxidation		Protein oxidation	
		percent inhib.[b]	IC_{50} [μM][c]	percent inhib.[b]	IC_{50} [μM][c]
Group 1 (Catechols)					
Quercetin (**5**)	+0.11	86 ± 6	3.0 ± 0.2	0	n.d.[d]
Verbascoside (**10**)	+0.19	82 ± 6	3.2 ± 0.5	0	n.d.
Chlorogenic acid (**9**)	+0.23	78 ± 4	4.2 ± 0.2	43 ± 9	> 100
1,3,6,7-Tetrahydroxyxanthone (**3**)	+0.28	67 ± 9	2.1 ± 0.3	48 ± 5.4	> 100
Mangiferin (**4**)	+0.32	78 ± 7	8.0 ± 1.0	18 ± 0.7	> 100
Group 2 (Phenols)					
1,5-Dihydroxy-3-methoxyxanthone (**2**)	+0.45	28 ± 5	> 100	34 ± 8	> 100
Flemichin D (**8**)	+0.56	92 ± 6	7.2 ± 0.4	0	n.d.
Cordigone (**12**)	+0.64	69 ± 4	8.6 ± 0.4	0	n.d.
Cordigol (**11**)	+0.71	70 ± 3	8.3 ± 0.3	17 ± 0.1	> 100
Danthrone (**1**)	+0.96	0	–	11 ± 0.2	> 100
Eriosematin (**7**)	–[e]	64 ± 8	53 ± 8	0	n.d.
Group 3					
Frutinone A (**6**)	–[e]	0	n.d.	0	n.d.
Reference compound					
Trolox (**13**)	+0.11	87 ± 6	20 ± 3	71 ± 7.1	12 ± 2

[a] First oxidation potential determined by cyclic voltammetry at a sweep rate of 100 mV s^{-1} in 0.07M sodium phosphate buffer (pH 7.4)/EtOH 1:1 (*v*/*v*); working electrode: GC *vs.* Ag/AgCl/3M KCl; auxiliary electrode: GC. See text for the precision of electrochemical measurements.

[b] Percent inhibition at a concentration of 100 μM. Values are given as mean ± s.d. for triplicate experiments.

[c] Concentration inhibiting oxidation by 50%. Values are given as mean ± s.d. for triplicate experiments.

[d] Not determinable.

[e] Insufficiently soluble in the phosphate buffer/EtOH mixture.

(*Table 3*). For these compounds, IC_{50} values were then determined and in most cases were in the range 2–9 μM, but **7** was less active (IC_{50} *ca.* 0.05 mM). The most active compound was **3**.

One compound, **2**, was too weakly active for an IC_{50} value to be obtained. Two compounds were completely devoid of activity, namely **1** and **6**. Interestingly, they showed the highest oxidation potential (*ca.* 1 V), having either no phenolic group (**6**) or partly masked ones (**1**).

4. *Relations between Redox Potential and Antioxidant Properties.* For a variety of natural and synthetic compounds, good correlations were observed between antioxidant activities and oxidation potentials [25] [36] [37]. Radical scavengers in particular are oxidized at relatively low oxidation potentials. The reference compound trolox (**13**) confirmed its very low oxidation potential and good antioxidant properties (*Table 3*) [38].

The compounds examined here show a qualitative relationship between molecular structure, first oxidation potential and antioxidant activity in the lipid peroxidation assay. Indeed, their chemical classification into catechols, phenols and non-phenols is related not only to their oxidation potential measured by cyclic voltammetry, but also to their activity as inhibitors of lipid peroxidation.

The first group examined is that of catechols, which showed an oxidation potential lower than 0.4 V and appeared to undergo a reversible two-electron oxidation. These compounds were also the most active in the antioxidant test, with IC_{50} values in the range 2.1–8 μM. No inactive compound was found in this group. The second group is that of phenols. Their oxidation potential was in the range 0.45 to 1 V, and the compounds appeared to undergo follow-up reactions such as dimerization, hydroxylation or polymerization [39]. The antioxidant activities in this group were more scattered, with three compounds having IC_{50} values close to 8 μM, two others being modestly-to-weakly active, and one completely inactive. The third group contains a single compound devoid of any OH substituent and inactive in antioxidant tests.

An additional finding emerges from *Table 3*, that compounds active as inhibitors of lipid peroxidation have an oxidation potential lower than 0.8 V while the oxidation potential of inactive compounds is higher than 0.9 V. Eriosematin (**7**) was not soluble enough to be examined by cyclic voltammetry, and thus remains as a open question. The threshold value of 0.8–0.9 V that discriminates between active and inactive compounds can only have an indicative value and is far from being a first step towards quantitative relationships between anti-lipid peroxidation activity and oxidation potential. No such statistical relation emerges from the data in *Table 3*, but it is interesting that compounds such as 1,5-dihydroxy-3-methoxyxanthone (**2**) with its low oxidation potential yet poor antioxidant activity suggest that other properties besides oxidation potential must influence antioxidant activity.

At this stage, one can only conjecture about the nature of such additional properties. Perhaps inhibitors of lipid peroxidation act not only by scavenging oxygen radicals formed in the *Fenton* reaction, but also by chelating ferrous and ferric ions [35]. Such a mechanism could also be possible for the compounds of this series, since all compounds that were active in the lipid peroxidation assay have a chelating group (catechol or β-hydroxycarbonyl moiety). Chelating capacity alone should not be sufficient to obtain antioxidant activity, as suggested here by danthrone (**1**).

When it comes to the protection of albumin against radical-mediated oxidation, the data in *Table 3* are difficult to interpret. While the three most active compounds also have

low oxidation potentials (< 0.5 V), other compounds with low oxidation potentials are fully inactive in this test. Compounds with higher oxidation potentials (> 0.5 V) have either low or no activity. Thus, a low oxidation potential appears as a necessary but not sufficient condition for activity in this test, again implying that other properties beside oxidation potential must condition this antioxidant activity. In as much as scavenging of the alkyl radical formed from AAPH is related to the oxidation potential, this activity appears as an unlikely mechanism to explain the inhibition of albumin oxidation. Perhaps reversible binding to albumin is a prerequisite for its protection against radical-mediated damage, a hypothesis currently under investigation.

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Experimental Part

Compounds and Reagents. Chlorogenic acid (**9**), danthrone (**1**) and quercetin (**5**) were obtained from *Roth AG* (Karlsruhe, Germany). The other natural products were isolated as previously described: mangiferin (**4**) and 1,3,6,7-tetrahydroxyxanthone (**3**) from *Gentiana rhodentha* (Gentianaceae) whole plant [40]; 1,5-dihydroxy-3-methoxyxanthone (**2**) from the roots of *Chironia krebsii* (Gentianaceae) [41]; verbascoside (**10**) from the root bark of *Sesamum angolense* (Pedaliaceae) [42]; cordigol (**11**) and cordigone (**12**) from *Cordia goetzei* (Boraginaceae) stem bark [43]; eriosematin (**7**) and flemichin D (**8**) from the roots of *Eriosema tuberosum* (Leguminosae) [44]; frutinone A (**6**) from the leaves of *Polygala fruticosa* (Polygalaceae) [45]. All reagents and solvents were obtained from *Fluka* (Buchs, CH) in *pro analysi* grade.

Cyclic Voltammetry. Cyclic voltammetry was carried out with an electrochemical interface *IMT 101* from *Radiometer Analytical* (Villeurbanne, France). A three-electrode system with a working and auxiliary electrode of glassy carbon (GC) and an Ag/AgCl/3M KCl reference electrode was used.

The compounds were dissolved to concentrations of 0.3–1 mM in a solvent made of EtOH and 0.07M sodium-phosphate buffer of pH 7.4 (50:50 *v/v*). The solns. were purged with N_2 and held under constant N_2 flux during the electrochemical experiments. The cyclic voltammograms (CV) were recorded at 22 ± 2°. Peak potentials were measured at a scan rate of 100 mV s^{-1} unless otherwise indicated.

The working electrode was polished intensively with aluminium oxide prior to each electrochemical measurement, because formation of a polymeric film inactivated the electrode after each scan. Since the surface cannot be reproduced identically each time, the peak potentials depended on each pretreatment and for a given compound varied up to 0.1 V between measurements. For each compound, several cyclic voltammograms were recorded, and the oxidation peak potential reported here was taken from the CV having the lowest peak potential.

Free-Radical Damage to Albumin. 2,2-Azobis(2-amidinopropane)·2 HCl (AAPH) was obtained from *Wako Pure Chemical Ind.* (Neuss, Germany). Human serum albumin (HSA), fatty acid-free, was purchased from *Sigma* (Saint Quentin Fallavier, France). Radical attack on HSA was performed under the following conditions [31]: HSA 15 μM in 10 mM phosphate buffer of pH 7.2 was pre-incubated with the test antioxidant 10–300 μM at 37° for 30 min. AAPH 20 mM was then added, and the mixture incubated for 60 min. Samples were subsequently diluted 10 times in the phosphate buffer. Protein damage was quantified by the carbonyl-groups formation determined spectrophotometrically at 367 nm according to the method of *Keller et al.* [32].

Lipid Peroxidation. A rat cerebral cortex was homogenized in a *Potter* instrument in 0.32M saccharose [33]. After centrifugation at 1000 g for 15 min, the supernatant was centrifuged at 50000 g for 15 min. The synaptosomal pellet was washed twice in 0.9% NaCl and centrifuged at 50000 g for 15 min. All these operations were conducted at 4°.

Synaptosomal suspensions (0.4 ml) were incubated with 0.1 ml $FeCl_2$ (50 μM)/$FeCl_3$ (150 μM) and 5 μl of a soln. of the test compound for 10 min at 37°. The reaction was stopped by 1 ml of CF_3COOH (30%) and 0.5 ml of desferroxamine (0.2 mM). Thiobarbituric acid (46 mM in 1.5 ml) was then added. The samples were boiled for 1 h, cooled and centrifuged at 1500 g for 10 min at 4°. The absorbance of the supernatant was read at 530 nm, quantification being based on a molar extinction coefficient of 1.56×10^5. The IC_{50} values were determined from at least 6 concentrations.

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