Determination of the sequential order of acidity in a polyhydroxylated benzophenone series. Consequence on the oxidation reaction in relation to hepatotoxicity

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The pK_a values of successive acid–base equilibria involved in the pyrogallol ring ionization of exifone, 2,3,4-trihydroxybenzophenone 1, and related methoxy derivatives were determined by UV–VIS absorption spectrometry and potentiometric titration. Due to strong intramolecular hydrogen bonding, the sequential order of acidity of the three hydroxy groups of 1 was found to be 4-OH > 3-OH > 2-OH. The polyhydroxylated benzophenones were oxidized to 3,4-quinone only in a narrow range of acidity in which the monoanionic 4-olate species predominated in solution. The attachment of an amino-alcohol residue resulted in the trapping of the transient 3,4-quinone and provided a convenient route to novel 1,4-benzoxazine derivatives. Cytotoxicity experiments in rat hepatocytes indicated that some of these compounds were significantly less toxic than the parent exifone.

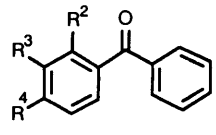
Exifone (*Adlone*®)[1] belongs to the benzophenone series (**2**) which was launched in France in 1988, for the treatment of cognitive problems in the elderly.[2,3] Unfortunately, in 1990, the registration was revoked and exifone was withdrawn from the market because of reports of hepatotoxicity associated with the product.

In the toxicological field, it is well known that paracetamol is metabolized by the cytochrome P-450 system to a reactive *para*-quinone-imine that can combine with glutathione (GSH), so causing severe GSH depletion in the liver.[4] Similarly, the previous studies concerning the toxicity of 6-hydroxydopamine for catecholamine neurons were consistent with this approach. It was postulated that this toxicity resulted from covalent binding of the oxidized electrophilic *para*-quinone of 6-hydroxydopamine with several groups (especially R–SH and R–NH_2) within the cellular proteins.[5–7]

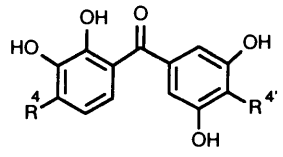
Oxidation of aromatic rings is an important metabolic pathway by which xenobiotics may be activated prior to conjugation with proteins. In this context, it seemed reasonable to suggest that a potential toxic metabolite of exifone could be the transient reactive *ortho*-quinone species. Hence we embarked upon a program aimed at the oxidation chemistry of exifone and its possible role in the hepatotoxic effects of the drug. Our objective was to scavenge the transient electrophilic *ortho*-quinone by the formation of an adduct blocking the electrophilic sites generally attacked by membrane nucleophiles.

We reported in a preceding paper[8] the results of a study concerning the oxidation chemistry of exifone and its model compound **1**, in methanol, in presence of amino-alcohols. These nucleophiles were intended as models for amino-acids which have been strongly implicated in protein coupling reactions.[9–11] We have found that the attachment of an amino-alcohol residue such as tris(hydroxymethyl)-aminomethane (Tris) resulted in the trapping of the transient unstable 3,4-quinone, electrochemically or chemically generated, and provided a convenient route to novel 1,4-benzoxazine derivatives which are significantly more active[12] and about twenty fold less toxic than the parent exifone.[13] However, the scope and limitations of this reaction had not been fully defined.

In this context, we decided to investigate the electrochemical oxidation of exifone and its model compound **1**, in the presence of various amino-alcohols. From the results obtained, it appeared that increasing basicity of the amino-alcohols led to a lower yield of benzoxazine derivatives (see Table 4). To justify this observation, the hypothesis was initially made that, when the phenolic group at the 2-position was ionized, the substitution reaction of the transient 3,4-quinone with the amino-alcohol yielding the benzoxazine derivatives could no longer occur, so that the transient 3,4-quinone degraded by a parallel consecutive polymerization reaction. So, in order to hinder the ionization of the phenolic group at the 2-position, we were interested in the synthesis of the corresponding 2-methoxy derivatives.



1: $R^2 = R^3 = R^4 = OH$
3: $R^2 = R^3 = OH$; $R^4 = OMe$
4: $R^2 = OH$; $R^3 = R^4 = OMe$
5: $R^2 = OMe$; $R^3 = R^4 = OH$



2: $R^4 = R^{4'} = OH$ (exifone)
6: $R^4 = OH$; $R^{4'} = OMe$
7: $R^4 = OMe$; $R^{4'} = OH$
8: $R^4 = R^{4'} = OMe$

Therefore, a detailed study of the acid–base properties of **1** and exifone **2** was undertaken to realize the selective methylation of **1** at the 2-position and to determine the ionization sequence. The pK_a values of the successive acid–base equilibria involved in the ionization of exifone **2**, its model compound **1** and their methoxy derivatives were deduced from spectrometric and potentiometric titrations.

Furthermore, to bring additional mechanistic information, particular attention was paid to the electrochemical oxidation reaction of the 2-methoxy derivative **5**, in methanol, in the presence of various amino-alcohols.

Results and discussion

Acid–base properties of exifone and its model compound 1

Model compound 1. *Regioselective methylation of* 2,3,4-*trihydroxybenzophenone* **1**. At the onset, we were fully aware of

the problems associated with the selective methylation of **1** at the 2-position. As commonly admitted, it was reasonable to assume that the monoanion of **1** would alkylate preferentially at the more acidic site. In this connection, previous works on dihydroxyacetophenones,[14] close analogues of **1**, had demonstrated that phenolic groups whose pK_a value is lower than 8 could be alkylated selectively with an alkyl halide in DMF in the presence of lithium carbonate. Accordingly, treatment of dihydroxyacetophenones with methyl iodide resulted in the selective methylation of the hydroxy groups at the *ortho* and *para* positions.

Under these experimental conditions, compound **1** was converted into the corresponding 4-methyl ether **3** (30% yield), whilst the 3,4-dimethyl ether **4** was obtained in 17% yield, along with 25% of starting material (see Experimental section). No trace of the expected 2-methyl ether was detected. Alternatively, we designed a more elaborate procedure involving protection of the hydroxy groups at the 3- and 4-positions. For this purpose, the use of the acetyl protecting group produced a mixture (70:30) of the 3,4- and 2,3-diacetyl compounds that could not be separated. As the acetyl groups were easily removed in basic media, we chose to methylate the diacetyl compound mixture with diazomethane. Finally, the removal of the two acetyl protecting groups was achieved by basic hydrolysis in carbonate buffered solution. The expected 2-methyl ether derivative **5** was obtained in 50% yield (see Experimental section).

Surprisingly, the hydroxy group at the 2-position behaved as the least acidic phenol function of the molecule. This result could be further substantiated when considering the acid–base properties of **1** in buffered aqueous solutions.

pK_a Determination of the successive equilibria involved in the ionization of **1** *and its methoxy derivatives* **3–5**. The pK_a values were determined spectrometrically. UV–VIS absorption spectra were used to gain information about the extent of delocalization in the anionic species. The UV–VIS spectral characteristics of **1** and its methoxy derivatives **3–5** in buffered aqueous solutions containing 10% (*v*/*v*) methanol are collected in Table 1.

In the pH range 5–10 (see Experimental section), with increasing pH, a decrease in the UV absorption band shown by the starting material **5** at 292 nm was observed, while a new band at 356 nm developed. Spectral changes included two isosbestic points at 280 and 316 nm, indicating that a simple acid–base equilibrium was shifted (Fig. 1).

The pK_{a_2} value was determined from graphs of $\log\{[A - A(H_2A)]/[A(HA^-) - A]\}$ *vs.* pH, at 356 nm, where $A(H_2A)$ and $A(HA^-)$ are the absorbances of the neutral and monoanionic species respectively, and A is the absorbance of their mixture at a fixed pH, according to the following equation: $pH = pK_{a_2} + \log\{[A - A(H_2A)]/[A(HA^-) - A]\}$. The pK_{a_2} value was found to be 7.70 ± 0.05 at 20 °C, and was attributed to the ionization of the phenolic group at the 4-position. This low pK_{a_2} value could be explained by the intramolecular migration of the doublet of electrons from the phenolate anion at the 4-position towards the carbonyl group of the benzophenone.

In the pH range 10–14 (see Experimental section), a hypochromic effect and a hypsochromic shift of the UV–VIS absorption band shown by the monoanion HA^- at 356 nm were observed with increasing pH, while a new intense band developed at 402 nm. The UV–VIS spectrum showed three isosbestic points at 274, 306 and 384 nm, indicating that a simple acid–base equilibrium was shifted (Fig. 2). To ensure that the oxidative reaction of the pyrogallol ring was not fast enough to interfere with the pK_a determination, the UV–VIS spectra in deoxygenated solutions were used. Moreover, it was verified that rapid acidification of the alkaline solutions provoked a return to the spectrum specific of the neutral species H_2A, arguing in favour of a reversible reaction. The pK_{a_1} value was determined from graphs of $\log\{[A - A(HA^-)]/[A(A^{2-}) - A]\}$ *vs.* pH, at 402 nm, where $A(HA^-)$ and $A(A^{2-})$ are the respective absorbances of the monoanionic and bianionic species, and A is the absorbance of their mixture at a fixed pH, according to the equation: $pH = pK_{a_1} + \log\{[A - A(HA^-)]/[(A(A^{2-}) - A]\}$.

Table 1 UV Absorption spectra of compounds **1**, **3–5**, in buffered aqueous solutions containing 10% (*v*/*v*) methanol

Compound	Species	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}
1	H_2A	240	9 500
		305	16 400
	HA^-	250	10 600
		349	22 500
	A^{2-}	330	12 000
		406	11 600
3	H_2A	250	8 150
		305	16 000
	HA^-	253	13 500
		316	10 400
		400 (sh)	4 000
4	HA	296	16 500
	A^-	235	17 500
		285	8 500
		370	4 000
5	H_2A	254	12 750
		292	8 500
	HA^-	252	14 600
		356	12 000
	A^{2-}	275 (sh)	12 000
		340 (sh)	6 000
		402	9 000

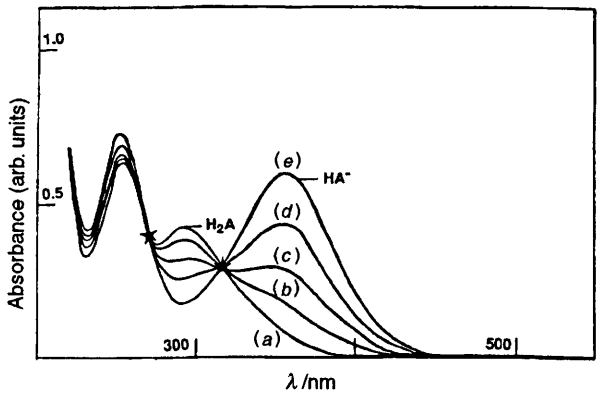


Fig. 1 Acid–base reactivity of **5** in buffered aqueous solutions containing 10% (*v*/*v*) methanol. The pK_{a_2} value was spectrometrically determined. The following pH were used for curves (*a*)–(*e*): (*a*) 5.0; (*b*) 7.0; (*c*) 7.5; (*d*) 8.0; (*e*) 10.0. Concentration, 0.1 mmol dm^{-3}; cell thickness, 0.5 cm; 20 °C; * isosbestic point.

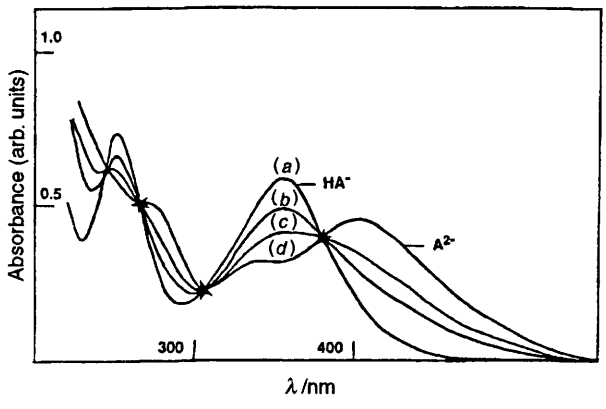


Fig. 2 Acid–base reactivity of **5** in buffered aqueous solutions containing 10% (*v*/*v*) methanol. The pK_{a_1} value was spectrometrically determined. The following pH were used for curves (*a*)–(*d*): (*a*) 10.0; (*b*) 12.0; (*c*) 12.8; (*d*) 14.0. Concentration, 0.1 mmol dm^{-3}; cell thickness, 0.5 cm; 20 °C; * isosbestic point.

Table 2 pK_a Values of the successive equilibria given by compound **1** and its methoxy derivatives **3–5**, in buffered aqueous solutions containing 10% (*v*/*v*) methanol, at 20 °C

Compound	pK_{a_2}	OH-position	pK_{a_1}	OH-position
1	7.2	4	11.0	3
3	9.4	3	*a*	—
4	9.4	2	—	—
5	7.7	4	12.5	3

[a] Not measured.

Table 3 pK_a Values of the successive acid–base equilibria given by exifone **2** and its methoxy derivatives **6** and **7** in buffered aqueous solutions containing 10% (*v*/*v*) methanol, at 20 °C

Compound	pK_{a_2}	OH-position	pK_{a_1}	OH-position
2	7.2	4	9.0	4′
6	7.2	4	9.3	3′
7	8.0	4′	10.0	3

This gives a pK_{a_1} value of 12.50 ± 0.05 at 20 °C, attributed to the ionization of the phenolic group at the 3-position.

Similarly, the pK_a values of the acid–base equilibria involved in the ionization of compound **1** and its methoxy derivatives **3** and **4** were determined using the method performed on **5**. The experimental data are listed in Table 2 and allowed the following deductions.

(*a*) The roughly identical UV–VIS absorption spectra exhibited by the monoanionic species HA^- of **1** and **5** (Table 1) allowed us to conclude that the first ionization occurred at the 4-position and, moreover, corroborated the above findings concerning the methylation sequence of **1**. Likewise, the absorption spectrum of the bianionic species A^{2-} of **1** and **5** showed an additional absorption band at about 405 nm, consecutive to the ionization of the phenolate anion at the 3-position.

(*b*) Both 4-olate and 2-olate anions are believed to be more involved in the resonance of the chromophoric group owing to the electron withdrawing effect exerted by the carbonyl group of the benzophenone. Accordingly, a low pK_a value was found for the 4-hydroxy group. However, an unexpected weaker acidity was found for the 2-hydroxy group. Consequently, there was little doubt left that hydrogen bonding between the hydroxy group at the 2-position and the oxygen atom of the carbonyl group of the benzophenone enhanced the stability of both the neutral species H_2A and the monoanionic species HA^-. Thus, in the case of compound **1**, surprisingly the second ionization concerned the hydroxy group at the 3-position. It is worth mentioning that strong hydrogen bonding has been previously reported in the case of 2-hydroxy-4-substituted benzophenones.[15,16]

(*c*) Compound **1** exhibited lower pK_a values than its methoxy derivative **5**: 7.2 *vs.* 7.7 and 11.0 *vs.* 12.5. A likely explanation is that, in the case of **1**, the strong hydrogen bonding would result in the stabilization of the anionic species HA^- and A^{2-}.

(*d*) The 3-hydroxy group of compound **3** exhibited a lower pK_a value than that shown by **1** (9.4 *vs.* 11.0), in agreement with ar easier first ionization of a neutral species H_2A compared with a second ionization of a monoanion HA^-. Accordingly, the 3,4-dimethoxy compound **4** was easily produced in the course of the methylation procedure used with **1**.

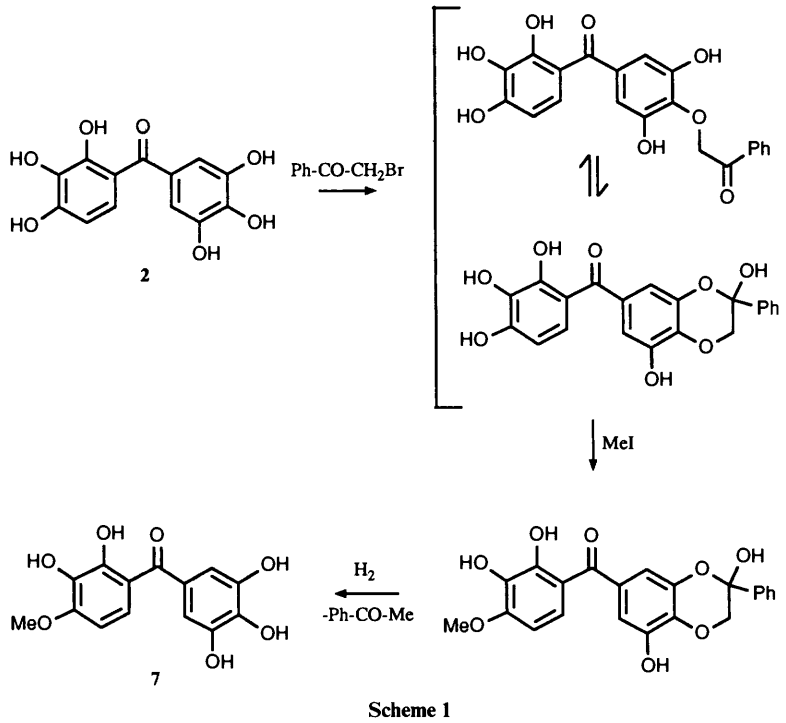
Finally, from this acid–base study and the preparation of the methoxy derivatives, a sequential order of acidity can be proposed for the three hydroxy groups of compound **1**: 4-OH > 3-OH > 2-OH. This finding is in good agreement with the data obtained from the monohydroxybenzophenones: 4-OH, pK_a = 8.0; 3-OH, pK_a = 9.1; 2-OH, pK_a = 9.5.

Exifone 2. The introduction of a second pyrogallol ring induced noticeable changes in the acid–base properties of exifone **2**, compared with those of compound **1**. Thus, it was not *a priori* possible to decide which of the pyrogallol rings was at first prone to ionization. However, a distinction could be made on the basis of whether or not, the carbonyl group of the benzophenone skeleton extended the electronic conjugation of the phenolate anion groups at the 4- or 4′-position. In this context, we wondered whether the intramolecular bridge between the hydrogen of the hydroxy group at the 2-position and the oxygen of the benzophenone carbonyl function could specifically enhance the electronic conjugation of the phenolate anion at the 4-position, in preference to the phenolate anion at the 4′-position.

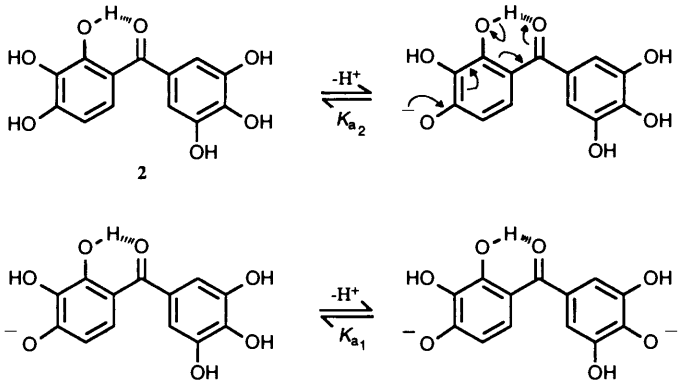
Regioselective methylation of exifone **2**. The results of the regioselective methylation reaction of exifone could not provide unambiguous discrimination between the reactivity of the phenolate groups at the 4- and 4′-positions. Indeed, upon treatment with methyl iodide in DMF, in the presence of lithium carbonate (see Experimental section), exifone **2** was converted into the 4′-methyl ether **6** in poor yields ranging from 10 to 15%, whereas the 4,4′-dimethyl ether **8** was obtained in 30% yield, along with 25% of non reacted exifone. We were unable to detect the 4-methyl ether **7**. Therefore, we decided to synthesize this compound using a more elaborate method (Scheme 1). The procedure required preliminary protection of the 4′-position with a phenacyl group yielding the 4′-phenacyl ether,[8] this was methylated at the 4-position, using the experimental conditions above. Finally, removal of the phenacyl protecting group was achieved using zinc in methanolic acetic acid solution. As the 4-methyl ether **7** isolated was found to be poorly soluble in a dichloromethane–acetone mixture, it was not surprising that it was not isolated during the direct methylation procedure. Indeed, this procedure was achieved by silica gel chromatography using dichloromethane–acetone as the eluent (see Experimental section).

pK_a *Determination of the successive equilibria involved in the ionization of exifone* **2** *and its methoxy derivatives* **6–8**. The results of the acid–base study of exifone **2** and its methoxy derivatives **6–8** were more conclusive. The potentiometric titration of **2** exhibited two badly defined inflexion points. The corresponding pK_{a_2} and pK_{a_1} values were found to be 7.2 ± 0.1 and 9.0 ± 0.1, at 20 °C, respectively. It should be noted that (*a*) the pH of the solution was measured quickly, with vigorous nitrogen bubbling, to minimize errors due to changes in the chemical species by oxidative reaction; (*b*) under these experimental conditions, a rapid acidification of the reaction mixture provoked a return to the initial stage, arguing in favour of a reversible reaction; (*c*) addition of *n* hydroxide ion equivalents ($n > 2$) did not permit to show the occurrence of weaker acidities as the polyanionic species followed parallel consecutive oxidative–polymerization reactions, while the solution turned dark.

Comparison of the pK_{a_2} values obtained from exifone with those given by the corresponding monomethoxy derivatives **6** and **7** constituted a convenient method to provide an unambiguous attribution. Indeed, examination of data reported in Table 3 revealed that exifone and the 4′-methoxy derivative **6** showed identical pK_{a_2} values, whereas the 4-methoxy derivative **7** exhibited a higher pK_{a_2} value (ΔpK_{a_2} = +0.8). This result allowed us to conclude that (*a*) in the case of exifone, the first ionization concerned the hydroxy group at the 4-position (Scheme 2). As it might be expected, the hydrogen bonding promoted the electronic conjugation of the negative charge of the phenolate anion at the 4-position with the carbonyl function of the benzophenone skeleton, in preference to the phenolate anion at the 4′-position, according to Scheme 2. (*b*) At physiological pH, exifone existed predominantly (60%) as the 4-phenolate anion. (*c*) The mean pK_a value showed by **8** (pK_a = 9.4) is in good agreement with what is expected in the



Scheme 1



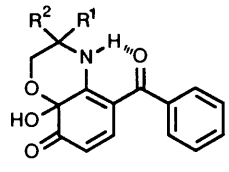
Scheme 2

case of phenolic substituents at the *meta* position (see compound **3**).

Electrochemical oxidation of compound 5

The cyclic voltammogram of **5**, in methanol containing tetraethylammonium perchlorate (TEAP) as the supporting electrolyte, and an excess of tris(hydroxymethyl) aminomethane (Tris), at a stationary platinum electrode, showed an oxidation peak Pa around +400 mV (*vs.* SCE), the sweep rate being 0.5 V s^{-1}. After sweep reversal, no cathodic peak was recorded, showing the irreversibility of the anodic process.

When the controlled potential of the platinum working electrode was fixed at +450 mV (*vs.* SCE), *i.e.* at a potential immediately following the peak Pa, a coulometric value of 2.0 ± 0.1 was found for the number of electrons involved in the oxidation of one molecule of **5**. As the electrolysis proceeded, a decrease in the Pa intensity was observed and no more anodic peak was recorded in the cyclic voltammogram exhibited by the exhaustively oxidized solution of compound **5**. Finally, preparative scale electrolyses allowed the isolation of the 1,4-benzoxazin-8-one derivative **9**, a product (see ref. 8 for detailed consideration of structure) previously obtained in the course of the electrochemical oxidation of compound **1**. The results of the preparative scale electrolyses obtained with a series of amino-alcohol derivatives are summarized in Table 4.



9: $R^1 = R^2 = CH_2OH$

10: $R^1 = CH_2OH$; $R^2 = Me$

11: $R^1 = R^2 = Me$

Mechanistic deductions

First, when using Tris as nucleophilic species, from the UV–VIS spectra analysis, it was apparent that the monoanionic species of compounds **1**, **2**, **5** and **6** predominated under our experimental conditions. By contrast, when using 2-amino-2-methylpropanol, the bulk solution basicity increased markedly, as it could be substantiated when considering the UV–VIS spectra analysis, so that the monoanionic species no longer constituted the sole form present in methanolic solution. Simultaneously, the yield of 1,4-benzoxazine derivatives decreased (Table 4). So, the hypothesis was initially made that the prerequisite to the formation of 1,4-benzoxazine derivatives concerns the phenolic group at the 2-position of the electrogenerated quinone. Data reported in Table 4 reveal that compounds **1** and **5** afforded the 1,4-benzoxazin-8-one

Table 4 Products and yields of controlled potential electrolyses of **1** and **5** in methanol containing an excess of amino-alcohol CH_2OH-$C(R^1,R^2)$-NH_2

				Yield (%)	
R^1	R^2	pK_a	Product	From **1**	From **5**
CH_2OH	CH_2OH	8.20	**9**	70	66
CH_2OH	Me	8.75	**10**	60	70
Me	Me	9.75	**11**	35	30

derivatives **9**, **10** and **11** in comparable yields, only depending on the nature of the amino-alcohol. Thus, the absence of any change in the anodic behaviour of **1** and **5** rules out this hypothesis. Therefore, in view of the information available, increasing basicity of amino-alcohols was speculated to provoke the ionization of a phenolic group not at the 2-position and related to a transient species formed in a subsequent step to the oxidative step (see ref. 8 for the electrochemical pathway).

Pharmacological deductions

In the case of exifone, at physiological pH, the monoanionic species HA^- is presumed to be easily oxidized by molecular oxygen, in the presence of Fe^{2+} ions, both intracellular constituents, to a transient 3,4-quinone that would bind irreversibly to the NH_2 and SH residues of cellular proteins to form conjugates.[17,18] Formation of these conjugates provides strong evidence that the hepatotoxic effects exhibited by exifone could be regarded as a consequence of reaction of an oxidative metabolite with cellular proteins. Indeed, alkylation and cross-linking of cellular proteins by highly electrophilic oxidation products (quinones) are widely believed to express their degenerative effects.[4–7]

Accordingly, the attachment of an amino-alcohol residue was identified as an easy method to scavenge the transient *ortho*-quinone by blocking the electrophilic sites and consequently to prevent toxicity. Reflecting these objectives, our studies have culminated in the selection of three novel molecules derived from 1,4-benzoxazine,[8] that were identified as significantly more active and about twenty fold less toxic than the parent exifone.[13] Consequently, they have been protected by a patent.[12]

Experimental

Materials

UV–VIS Spectra were recorded on a Varian Cary 13E spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300 MHz for ^1H observations. Chemical shifts are relative to internal tetramethylsilane. *J* Values are given in Hz. The measurements were carried out using the standard pulse sequences. The carbon type (methyl, methylene, methine or quaternary) was determined by DEPT experiments. Mass spectra were recorded on a Nermag R 10-10 C spectrometer equipped with desorption chemical ionization (DCI) mode. Samples were introduced by means of a direct insertion probe. Ammonia was used as the reagent gas. A Radiometer-Tacussel PHN 850 electronic pH meter was used for the potentiometric titrations. Melting points were determined on a Köfler block and are uncorrected.

Electrochemical measurements were made with a Radiometer-Tacussel PRG 5 multipurpose polarograph that was used only as a rapid-response potentiostat. For cyclic voltammetry, triangular waveforms were supplied by a Tacussel GSTP 4 function generator. Current-potential curves were recorded on a Sefram SI 8312 instrument. The cell was a Tacussel CPRA water-jacketed cell working at a temperature of 20 °C. The reference electrode was an aqueous saturated calomel electrode (SCE) (Metrohm EA 441-1). The aqueous saturated calomel solution was enclosed in the first compartment which was put inside the second compartment containing a 0.02 mol dm^{-3} solution of tetraethylammonium perchlorate in methanol. The compartments were separated by sintered porous glass disks. The counter electrode was a platinum electrode Tacussel Pt 11. The working electrode was the platinum disk (effective area 0.030 cm^2) of a Tacussel EDI rotating electrode connected to a Tacussel controvit servo-control electronic amplifier. Controlled potential electrolyses were carried out using a three compartment water jacketed cell protected from light, whose counter and reference compartments were filled with the background solution. A Tacussel PJT 120–1 potentiostat and a Tacussel IG6-N electronic integrator were included in the circuit. The counter electrode was a platinum foil. The solid working electrode was a platinum grid (*d* 6 cm).

Analytical TLC were performed on Merck Silica Gel 60 F 254 (lot 5714). Column chromatography was conducted on open glass columns packed with Merck Silica Gel 60 (lot 9385).

Exifone (*Adlone*®) was supplied by the Pharmascience Laboratories. Tetraethylammonium perchlorate (TEAP) was obtained from Fluka (purum-grade purity). Methanol was obtained from Prolabo (analysis-grade purity).

The methoxy derivatives **3–8** were synthesized as follows.

(2,3-Dihydroxy-4-methoxyphenyl)(phenyl)methanone 3 and (3,4-dimethoxy-2-hydroxyphenyl)(phenyl)methanone 4

To a reaction mixture of compound **1** (5.0 g, 21.7 mmol) and lithium carbonate (4.0 g, 55.0 mmol) in DMF (15 cm^3) was added, at 30 °C, 7.8 g of methyl iodide (55.0 mmol). The reaction mixture was stirred for 15 h at 30 °C under nitrogen. Then, the resulting mixture was poured into a molar acetic acid buffered solution (pH *ca.* 4.5) (300 cm^3) and extracted with ethyl acetate (150 cm^3). The organic phase was washed vigorously with water and dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure at 35 °C. Flash chromatography on silica gel with toluene–acetone (95:5) as the eluent afforded compounds **3** (1.6 g, 30%), mp 172–174 °C, and **4** (0.9 g, 17%), mp 185–187 °C, as yellow solids along with 25% of starting material (1.2 g).

3 (Found: C, 68.79; H, 5.00. $C_{14}H_{12}O_4$ requires C, 68.85; H, 4.92%); δ_H[300 MHz; $(CD_3)_2SO$] 3.95 (3 H, s, OMe), 6.65 (1 H, d, *J* 9, 5-H), 7.00 (1 H, d, *J* 9, 6-H), 7.60 (5 H, m, Ph), 10.0 (1 H, br s, 3-OH, D_2O exchanged) and 11.5 [1 H, br s, 2-OH, D_2O exchanged]; δ_C[75 MHz; $(CD_3)_2SO$] 56.2 (OMe), 103.6 (C-5), 115.4 (C-1), 124.3 (C-6), 128.6, 129.1 and 132.1 (CH, Ph), 134.2 (C-3), 138.2 (C_Q, Ph), 150.4 and 153.2 (C-2 and C-4) and 199.4 (CO, methanone); *m/z* 245 (MH^+).

4 (Found: C, 69.73; H, 5.54. $C_{15}H_{14}O_4$ requires C, 69.77; H, 5.43%); δ_H[300 MHz; $(CD_3)_2SO$] 3.75 (3 H, s, 3-OMe or 4-OMe), 3.95 (3 H, s, 3-OMe or 4-OMe), 6.70 (1 H, d, *J* 9, 5-H), 7.25 (1 H, d, *J* 9, 6-H), 7.50–7.70 (5 H, m, Ph) and 11.45 (1 H, br s, 2-OH, D_2O exchanged); δ_C(75 MHz; $(CD_3)_2SO$] 56.2 and 60.2 (3-OMe and 4-OMe), 103.9 (C-5), 116.0 (C-1), 128.6, 129.1 and 132.2 (CH, Ph), 128.9 (C-6), 136.2 (C-3), 138.0 (C_Q, Ph), 155.0 and 158.0 (C-2 and C-4) and 198.9 (CO, methanone); *m/z* 259 (MH^+).

(3,4-Dihydroxy-2-methoxyphenyl)(phenyl)methanone 5

The reaction sequence resulting in the production of **5** required three steps as follows.

Acylation of 1 at the 3- and 4-positions. To a reaction mixture of compound **1** (6.8 g, 29.7 mmol) and lithium carbonate (6.6 g, 89.1 mmol), in DMF (15 cm^3), was added, at room temp., 6.1 g of acetic anhydride (59.4 mmol). The reaction mixture was stirred for 15 h, at room temp., under nitrogen. Then, the resulting mixture was poured into a molar acetic acid-buffered solution (pH *ca.* 4.5) (300 cm^3) and extracted with ethyl acetate (150 cm^3). The organic phase was washed vigorously with water

and dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure at 35 °C. Flash chromatography on silica gel with toluene–acetone (98:2) as the eluent afforded a mixture of 3,4-diacetoxy-2-hydroxybenzophenone and 2,3-diacetoxy-4-hydroxybenzophenone (70:30) that could not be separated (40%). The ^{1}H NMR spectrum of the mixture supported the proposed assignment.

Methylation of 3,4-diacetoxy-2-hydroxybenzophenone and 2,3-diacetoxy-4-hydroxybenzophenone mixture at the 2-position. A solution of 3,4-diacetoxy 2-hydroxybenzophenone and 2,3-diacetoxy-4-hydroxybenzophenone mixture synthesized as indicated above (3.8 g, 12 mmol) in dichloromethane (50 cm^3) was stirred for 2 min at 10 °C. A solution of diazomethane (0.8 g, 19 mmol) in diethyl ether (50 cm^3) was added dropwise. After addition, stirring was continued for 2 h at 10 °C, and for an additional 15 h at room temp. The reaction mixture was quenched with 4 drops of concentrated acetic acid and was poured into water (30 cm^3). The solvents were evaporated under reduced pressure. Then, the aqueous solution was extracted with diethyl ether (100 cm^3). The organic phase was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure at 35 °C to give 3.9 g (100%) of a pale yellow oil. Analysis of this material by NMR showed a mixture (70:30) of 3,4-diacetoxy-2-methoxybenzophenone and 2,3-diacetoxy-4-methoxy benzophenone.

Removal of the acetoxy groups. A solution of 3,4-diacetoxy-2-methoxybenzophenone and 2,3-diacetoxy-4-methoxybenzophenone mixture synthesized as mentioned above (3.9 g, 12 mmol) in methanol (50 cm^3) was treated with 50 cm^3 of a 5% sodium hydrogen carbonate solution and stirred at 30 °C for 2 h under nitrogen. The solution was acidified with 10% hydrochloric acid and the solvent evaporated under reduced pressure to produce an aqueous solution that was extracted with diethyl ether (100 cm^3). The organic phase was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure at 35 °C. Flash chromatography on silica gel with toluene–acetone (95:5) as the eluent afforded **5** as an amorphous colourless solid (1.45 g, 50%), mp 130–132 °C along with **3** (0.75 g, 25%), mp 172–174 °C.

5 (Found: C, 68.63; H, 5.03 $C_{14}H_{12}O_4$ requires C, 68.85; H, 4.92%); δ_H[300 MHz; $(CD_3)_2SO$] 3.55 (3 H, s, OMe), 6.65 (2 H, m, 5-H and 6-H), 7.50–7.70 (5 H, m, Ph) and 9.40 (2 H, br s, 3-OH and 4-OH, D_2O exchanged); δ_C[75 MHz; $(CD_3)_2SO$] 61.8 (OMe), 111.6 (C-5), 121.2 (C-6), 125.1 (C-1), 129.4, 130.3 and 133.6 (CH, Ph), 139.2 and 139.5 (C_Q, phenyl and C-3), 148.4 and 151.0 (C-2 and C-4) and 195.0 (CO, methanone); *m/z* 245 (MH^+).

(2,3,4-Trihydroxyphenyl)(3′,5′-dihydroxy-4′-methoxyphenyl)-methanone 6 and (2,3-dihydroxy 4-methoxyphenyl)(3′,5′-dihydroxy-4′-methoxyphenyl)methanone 8

To a reaction mixture of exifone **2** (5.0 g, 18 mmol) and lithium carbonate (3.3 g, 45 mmol) in DMF (15 cm^3) was added, at 30 °C, 6.4 g of methyl iodide (45 mmol). The reaction mixture was stirred for 15 h, at 30 °C, under nitrogen. Then, the resulting mixture was poured into a molar acetic acid-buffered solution (pH *ca.* 4.5) (300 cm^3) and extracted with ethyl acetate (150 cm^3). The organic phase was washed vigorously with water and dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure at 35 °C. Flash chromatography on silica gel with dichloromethane–acetone (90:10) as the eluent afforded compounds **6** (0.79 g, 15%), mp 202–204 °C, and **8** (1.7 g, 30%), mp 216–218 °C, as yellow solids, along with 25% of starting material (1.25 g).

6 (Found: C, 57.46; H, 4.18. $C_{14}H_{12}O_7$ requires C, 57.53; H, 4.11%); δ_H[300 MHz; $(CD_3)_2SO$] 3.75 (3 H, s, OMe), 6.40 (1 H, d, *J* 9, 5-H), 6.60 (2 H, s, 2′-H and 6′-H), 7.00 (1 H, d, *J* 9, 6-H), 8.70 (1 H, br s, 3-OH, D_2O exchanged), 9.50 (2 H, br s, 3′-OH and 5′-OH, D_2O exchanged), 10.15 (1 H, br s, 4-OH, D_2O exchanged) and 12.05 (1 H, br s, 2-OH, D_2O exchanged); δ_C[75 MHz; $(CD_3)_2SO$] 60.8 (OMe), 108.5 (C-5), 109.6 (C-2′ and C-6′), 113.8 (C-1), 125.9 (C-6), 133.7 and 134.2 (C-3 and C-1′), 139.6 (C-4′), 151.5 (C-3′ and C-5′), 153.2 and 153.4 (C-2 and C-4) and 200.0 (CO, methanone); *m/z* 293 (MH^+).

8 (Found: C, 61.60; H, 4.82. $C_{15}H_{14}O_7$ requires C, 61.64; H, 4.79%); δ_H[300 MHz, $(CD_3)_2SO$] 3.75 (3 H, s, 4-OMe or 4′-OMe), 3.90 (3 H, s, 4-OMe or 4′-OMe), 6.60 (1 H, d, *J* 9, 5-H), 6.65 (2 H, s, 2′-H and 6′-H), 7.05 (1 H, d, *J* 9, 6-H), 8.80 (1 H, br s, 3-OH, D_2O exchanged), 9.50 (2 H, br s, 3′-OH and 5′-OH, D_2O exchanged) and 11.20 (1 H, br s, 2-OH, D_2O exchanged); δ_C[75 MHz; $(CD_3)_2SO$] 57.0 and 60.8 (4-OMe and 4′-OMe), 104.2 (C-5), 109.8 (C-2′ and C-6′), 116.6 (C-1), 124.6 (C-6), 134.0 and 135.0 (C-3 and C-1′), 140.0 (C-4′), 150.8 (C-2), 151.6 (C-3′ and C-5′), 153.6 (C-4) and 199.0 (CO, methanone); *m/z* 307 (MH^+).

(2,3-Dihydroxy-4-methoxyphenyl)(3′,4′,5′-trihydroxyphenyl)-methanone 7

The synthesis of **7** required three steps as follows.

Phenacylation of 2 at the 4′-position. The procedure leading to the 4′-phenacyl ether of exifone is described in ref. 8.

Methylation of the 4′-phenacyl ether of exifone at the 4-position. To a reaction mixture of the 4′-phenacyl ether of exifone (3.0 g, 7.5 mmol) and lithium carbonate (1.4 g, 19 mmol) in DMF (15 cm^3) was added, at 30 °C, 3.0 g of methyl iodide (21.0 mmol). The reaction mixture was stirred for 15 h, at 30 °C, under nitrogen. Then, the resulting mixture was poured into a molar acetic acid-buffered solution (pH *ca.* 4.5) (300 cm^3) and extracted with ethyl acetate (150 cm^3). The organic phase was washed vigorously with water and dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure at 35 °C. Chromatography on silica gel with dichloromethane–acetone (95:5) as the eluent afforded the 4-methoxy-4′-phenacyl exifone derivative (1.0 g, 30%). Analysis of this material by ^{1}H NMR and ^{13}C NMR supported the structure we have assigned.

Removal of the phenacyl protecting group. To a solution of 4-methoxy-4′-phenacyl exifone derivative synthesized as indicated above (1.0 g, 2.4 mmol) in 30 cm^3 of methanol, was added at room temp., 10 cm^3 of glacial acetic acid. Then, zinc dust (1.5 g, 24 mmol) was added and the resulting mixture was stirred for 2 min at room temp. The reaction mixture was filtered and water (20 cm^3) was then added. The resulting solution was concentrated to 10 cm^3 under reduced pressure at 35 °C and extracted with ethyl acetate (50 cm^3). The organic phase was dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure at 35 °C. The residue was then triturated with acetone to yield compound **7** (0.35 g, 50%), mp 260–262 °C;

7 (Found: C, 57.48; H, 4.15. $C_{14}H_{12}O_7$ requires C, 57.53; H, 4.11%); δ_H[300 MHz; $(CD_3)_2SO$] 3.85 (3 H, s, OMe), 6.60 (1 H, d, *J* 9, 5-H), 6.70 (2 H, s, 2′-H and 6′-H), 7.05 (1 H, d, *J* 9, 6-H), 9.50 (4 H, br s, 3-OH, 3′-OH, 4′-OH and 5′-OH, D_2O exchanged), and 11.6 (1 H, br s, 2-OH, D_2O exchanged); δ_C[75 MHz; $(CD_3)_2SO$] 57.0 (OMe), 104.0 (C-5), 110.2 (C-2′ and C-6′), 117.1 (C-1), 124.2 (C-6), 130.0 and 135.0 (C-3 and C-1′), 139.2 (C-4′), 146.5 (C-3′ and C-5′), 150.5 and 153.1 (C-2 and C-4) and 198.0 (CO, methanone); *m/z* 293 (MH^+).

Spectrometric measurements

1 mmol dm^{-3} Stock solutions of compounds **1**, **3**–**5** were prepared in methanol and diluted tenfold with the appropriate buffered aqueous solution. For pH 4–7, citric buffer (0.2 mol dm^{-3}), for pH 7–9, tris(hydroxymethyl)aminomethane buffer (0.2 mol dm^{-3}), and for pH 10–11, carbonate buffer (0.2 mol dm^{-3}) were used. For pH ≥ 12, solutions of sodium hydroxide were used and the ionic strength *I* was kept constant (*I* = 1 mol dm^{-3}) by the addition of sodium chloride.

Potentiometric titrations

A 10 cm^3 aliquot of a stock solution of exifone **2** or **6**–**8** (25

mmol dm^{-3}) in DMF was diluted 2:8 in water. The resulting solution was titrated with a 0.1 mol dm^{-3} NaOH solution.

Isolation of products 9–11

The assignment of structures reported below has been established by NMR spectra comparison with an authentic sample. Spectroscopic data and elemental analyses for products **9**–**11** have been earlier reported in ref. 8.

(*RS*)-5-Benzoyl-3,3-bis(hydroxymethyl)-8*a*-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazin-8(8a*H*)-one 9

A solution of compound **5** (0.122 g, 0.5 mmol), TEAP (1.150 g, 5.0 mmol) and Tris (3.020 g, 25.0 mmol) in methanol (250 cm^3) was oxidized under nitrogen, at 25 °C, at a platinum electrode (E = +450 mV *vs.* SCE). After exhaustive electrolysis, *i.e.* when a steady state minimum value of the current was recorded, the resulting solution was evaporated to dryness under reduced pressure at 35 °C. The residue was poured into a molar acetic acid-buffered aqueous solution of pH *ca.* 4 (100 cm^3) and extracted with ethyl acetate (200 cm^3). The organic phase was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure, at 35 °C. Chromatography on silica gel with ethyl acetate-methanol (98:2) as the eluent afforded compound **9** (109 mg, 66%), mp 170–172 °C (decomp.) as an amorphous orange solid.

(*RS*)-5-Benzoyl-3,3-(hydroxymethylmethyl)-8*a*-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazin-8(8a*H*)-one 10

When the controlled potential was fixed at +450 mV *vs.* SCE the above described procedure replacing Tris by 2-amino-2-methyl-1,3-propanediol (2.63 g, 25 mmol) afforded compound **10** (110 mg, 70%), as a mixture (65:35) of two diastereoisomers A and B that could not be separated.

(*RS*)-5-Benzoyl-3,3-bis(methyl)-8*a*-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazin-8(8a*H*)-one 11

When the controlled potential was fixed at +450 mV (*vs.* SCE) the above described procedure replacing Tris by 2-amino-2-methylpropanol (2.23 g, 25 mmol) afforded, after chromatography on silica gel with dichloromethane–methanol (98:2) as the eluent, compound **11** as an amorphous orange solid (45 mg, 30%), mp 140–142 °C (decomp.).

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