

Correlation between the OH bond dissociation energies of ellipticine hydroxylated derivatives and their cytotoxic properties

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Received 24 May 1989

The O–H bond dissociation energies (BDEs) of ellipticine-derived hydroxylated compounds have been evaluated by a kinetic method and are shown to correlate with the cytotoxic activity of these derivatives.

Ellipticine compound; Bond dissociation energy; Hydroxyl group; Cytotoxicity

1. INTRODUCTION

The cytotoxic activity of intercalating hydroxylated compounds derived from ellipticine has been shown to correlate with their antioxidant properties when the latter are measured during the induced oxidation of methyl linolenate [1]. These antioxidant properties are probably linked to the mobility of the phenolic hydrogen atom, however, no bond dissociation energy (BDE) has yet been measured on these phenols. A kinetic method which is based on activation energy measurements for the following reaction:



where $[\phi\text{CO}_2^-]_2$ denotes benzoyl peroxide (POB) and ArOH the studied phenol, has been recently proposed and discussed [2].

This method is used here to compare the OH BDEs of several hydroxylated compounds derived from ellipticine and to attempt correlation of the phenolic OH BDEs of ellipticine compounds with their cytotoxic activity.

The ellipticine compounds which have been studied are described in table 1 and fig.1.

2. MATERIALS AND METHODS

Ellipticine and its derivatives were synthesized according to conventional procedures [3–7]. Heptanol and other reactants were puriss or purum Fluka products.

Reactions were monitored by analyses of POB with respect to time. All experiments were performed in a heptanol/ethanol (80:20, v/v) mixture, a solvent of intermediate polarity in which all of the reactants are well solubilized. Reactions were studied over the range 294–348 K.

Benzoyl peroxide and benzoic acid were analyzed by high-pressure liquid chromatography on a Waters chromatograph using a Waters RP-18 (5 μM) column and an ultraviolet detector set at 240 nm. The eluent for benzoyl peroxide and benzoic

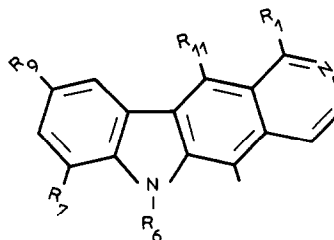


Fig.1. Structure of ellipticine derivatives (see table 1).

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Table 1
Structure of ellipticine derivatives (see fig.1)

Names	R ₁	R ₆	R ₇	R ₉	R ₁₁	Abbreviation
9-Hydroxyellipticine	H	H	H	OH	CH ₃	9-OH-E
7-Hydroxyellipticine	H	H	OH	H	CH ₃	7-OH-E
9-Hydroxyolivacine	CH ₃	H	HO	H	H	9-OH-O
Demethyl-9-hydroxyellipticine	H	H	H	OH	H	desMe-9-OH-E
6-N-Methyl-9-hydroxyellipticine	H	CH ₃	H	OH	CH ₃	6-Me-9-OH-E
Ellipticine	H	H	H	H	CH ₃	E
9-Methoxyellipticine	H	H	H	OCH ₃	CH ₃	9-OMe-E
6-N-Methyl-9-methoxyellipticine	H	CH ₃	H	OCH ₃	CH ₃	6-Me-9-OMe-E

acid was a methanol/water mixture (76:24 and 40:60, v/v, respectively) with 0.2% acetic acid.

The cytotoxic properties of the ellipticine derivatives were measured by inhibition of the growth rate of L1210 cultured cells in vitro as described by Paoletti et al. [8].

3. RESULTS

Fig.2 shows an example of POB consumption and of benzoic acid formation during the time for a reaction between POB and 9-OH-E, at 45°C. The initial rates of POB consumption and benzoic acid formation are first order in 9-OH-E (see fig.3a) and in POB (see fig.3b). Consequently, the rate law for POB consumption is given by:

$$- (d[\text{POB}]/dt)_0 = cte[9\text{-OH-E}]_0[\text{POB}]_0$$

We verified that this rate law was valid for all of the hydroxylated compounds studied.

Since POB is also known to react with pyridine or pyrrole, we investigated the reaction of POB with non-hydroxylated derivatives of ellipticine (E, 9-OMe-E, 6-Me-9-OMe-E). POB actually disappears in the presence of these compounds. The rate law for POB consumption is the same as that found for the corresponding hydroxylated derivatives, however, the reaction rates are much less significant.

A detailed kinetic study of POB reaction with hydroxylated and non-hydroxylated compounds (the corresponding methoxy derivatives) was performed in order to evaluate the activation energy of reaction 1, alone. Fig.4 illustrates an example of the Arrhenius plots which were obtained for 9-OH-E and 9-OMe-E; the correct rate constant of process 1 is simply given by the difference $k(9\text{-OH-E}) - k(9\text{-OMe-E})$.

Typical Arrhenius plots for k_1 are given in fig.5. The rate constants k_1 , pre-exponential factors A_1 and activation energies E_1 obtained for reaction of POB with the hydroxylated compounds of ellipticine and with phenol are listed in table 2.

In a previous paper [2], we showed that, for a given solvent, the activation energy E_1 and phenolic OH BDEs are connected by the relationship:

$$D_{\text{O-H}} = cte + E_1$$

By employing a selected reference value of $D_{\text{O-H}} = 88.2 \text{ kcal} \cdot \text{mol}^{-1}$ [9] and our measured activation energy $E_1 = 20.5 \text{ kcal} \cdot \text{mol}^{-1}$ for phenol, we were able to evaluate $D_{\text{O-H}}$ for all the compounds tested (see table 2). We also report data concerning α -

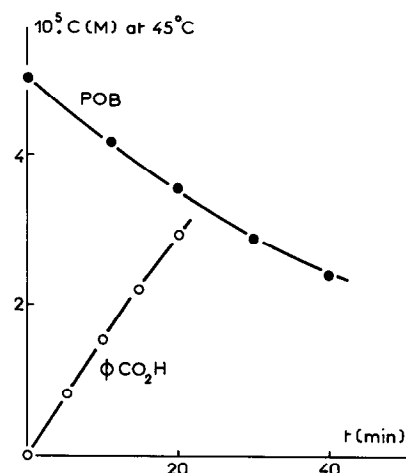


Fig.2. Reaction of POB with 9-OH-E at 45°C in a mixture of heptanol and ethanol (8:2, v/v). POB consumption (●) and $\phi\text{CO}_2\text{H}$ formation (○) as a function of time.

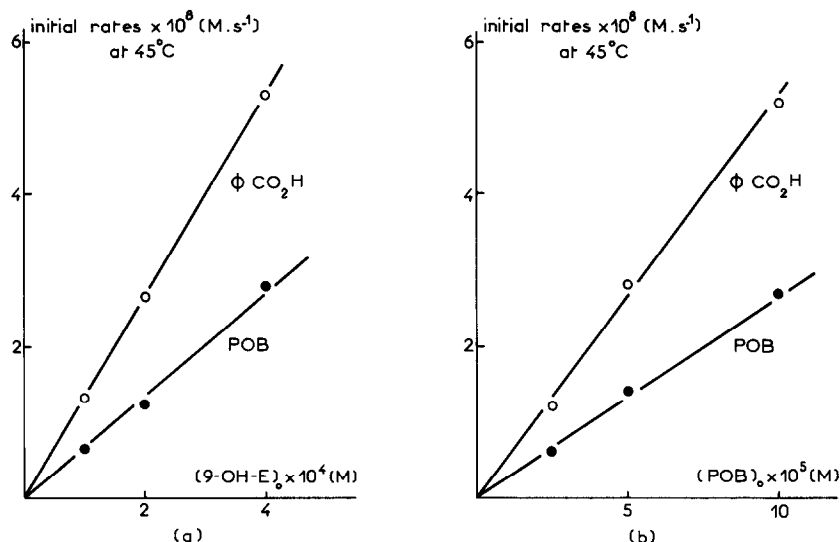


Fig.3. Reaction of POB with 9-OH-E at 45°C in a heptanol/ethanol (8:2, v/v) mixture. (●) POB consumption, (○) ϕ CO₂H formation. (a) [POB]₀ = 5 × 10⁻⁵ M, [9-OH-E]₀ variable; (b) [9-OH-E]₀ = 2 × 10⁻⁴ M, [POB]₀ variable.

tocopherol (vitamin E) which is considered here as a standard.

We attempted to correlate these data with the cytotoxic activity of the drugs. In fig.6, we have plotted log k_1 vs log (1/ID₅₀) at 37°C. Let us recall that ID₅₀ corresponds to the dose which reduces by 50% the L1210 cell growth rate as compared to controls after 48 h. No correlation is observed (fig.6). In contrast, plots of D_{O-H} vs log (1/ID₅₀) yield a good correlation, as indicated by fig.7.

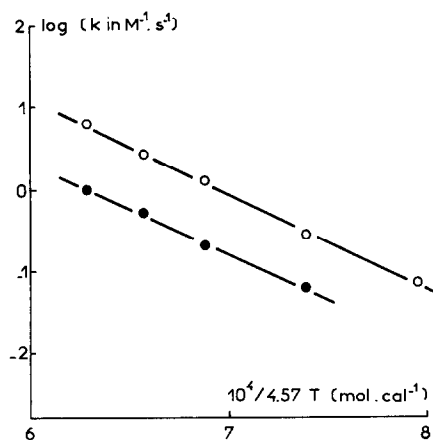


Fig.4. Arrhenius plots: log (k) vs $1/T$ for 9-OH-E (○) and 9-OMe-E (●).

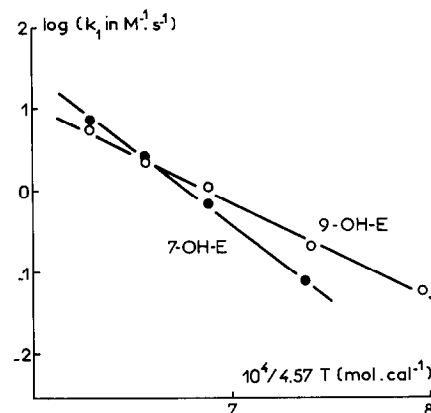
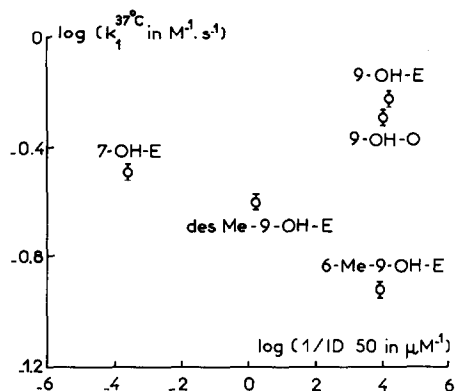
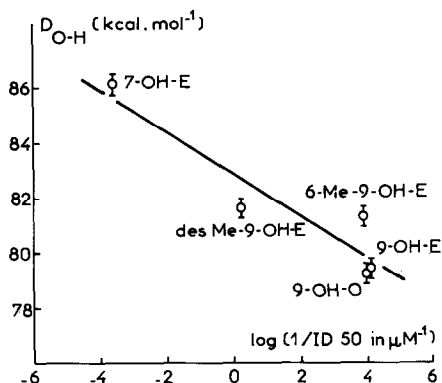


Fig.5. Arrhenius plots: log (k_1) vs $1/T$ for 9-OH-E (○) and 7-OH-E (●).

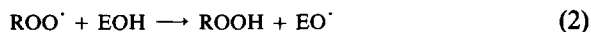
Table 2

EOH or phenolic derivatives	(k_1) _{37°C} (M ⁻¹ .s ⁻¹)	A_1 (M ⁻¹ .s ⁻¹)	E_1 (kcal.mol ⁻¹)	D_{O-H} (kcal.mol ⁻¹)
α -Tocopherol	0.072	10 ^{6.26}	10.5	78.2
9-OH-O	0.513	10 ^{7.96}	11.7	79.4
9-OH-E	0.602	10 ^{8.17}	11.9	79.6
6-Me-9-OH-E	0.120	10 ^{8.81}	13.8	81.5
DesMe-9-OH-E	0.250	10 ^{9.34}	14.1	81.8
7-OH-E	0.320	10 ^{12.62}	18.6	86.3
Phenol	6.5 × 10 ⁻⁶	10 ^{9.27}	20.5	88.2

Fig.6. $\log(k_1)$ vs $\log(1/ID_{50})$.Fig.7. D_{O-H} vs $\log(1/ID_{50})$.

4. DISCUSSION AND CONCLUSIONS

It has been shown that only the hydroxylated derivatives of ellipticine exhibit both antioxidant properties and cytotoxic activities [1]. The antioxidant properties were measured either by the rate constant of process 2:



where $ROO^{\cdot}$ is a peroxy-linolenoyl free radical and EOH the ellipticine derivative, or by the induction period of linolenate oxidation in the presence of these ellipticine compounds [1]. A fair correlation was established between cytotoxic activity and antioxidant properties [1].

The present results show that the cytotoxic activity of the studied ellipticine derivatives is correlated with the H atom mobility of their phenolic

OH whereas no such correlation is observed with rate constants k_1 .

Antioxidant properties are often evaluated from complex kinetic parameters (k_2 , inhibition periods). The OH BDEs measured in the present work probably give the best measure of these characteristics. Minor structural modifications result in a wide range of values: from $79.4 \text{ kcal} \cdot \text{mol}^{-1}$, slightly more than the value for α -tocopherol to $86.3 \text{ kcal} \cdot \text{mol}^{-1}$, a little under that of unsubstituted phenol. The most striking result is the significance of the relative positions of the indolic and hydroxyl groups; if the OH group is in the *para* position of the indolic group (9-OH-E, 9-OH-O) rather than *ortho* (7-OH-E), the O-H BDE is about $7 \text{ kcal} \cdot \text{mol}^{-1}$ lower. Suppression of the methyl group at the C_{11} position also results in an increase in the O-H BDE. On the other hand, a methyl group in the C_1 or C_{11} position is irrelevant, since we obtain nearly the same OH BDE for 9-OH-E and 9-OH-O.

The pre-exponential factors A_1 of the rate constants, k_1 , regularly increase with activation energies E_1 , from 9-OH-O to 7-OH-E. This results in a compensating effect. Hence, the k_1 values which are given in the second row of table 2 follow the trend of neither A_1 nor E_1 . This accounts for the fact that k_1 does not correlate with the cytotoxic properties whereas E_1 does. Let us recall that k_1 is the rate constant of process 1 and that its global value should normally depend on the properties of both reactants, POB and EOH. By contrast, the activation energy of process 1 is bound to D_{O-H} , a specific property of the hydroxy derivative of ellipticine.

A suitable exploration for the mobility of the phenolic hydrogen atom being connected to the cytotoxicity of the drug is difficult to find. The latter property may either produce or consume free radicals depending on the medium polarity. It is also difficult to assess which of these properties is important *in vivo*. Nevertheless, when discussing the mechanism of action of the mentioned drugs for the situation *in vivo*, it would be difficult to ignore the existence of the present correlation.

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