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Antioxidant activity and mechanism of action of butein in linoleic acid

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Abstract

A kinetic analysis was performed to evaluate the antioxidant behaviour of butein in linoleic acid. The process was performed at high (kinetic regime) oxygen concentrations at 40 °C in the dark. Different kinetic parameters were determined, including the stabilization factor as a measure of effectiveness, the oxidation rate ratio as a measure of strength, the antioxidant activity that combines these two parameters, and the mean rate of inhibitor consumption. It was established that the effectiveness and strength of the equal percentage, and not molar concentration of butein was in part lower than butylated hydroxytoluene. Butein participated in one side reaction of chain propagation (with hydroperoxides). The radicals of butein at concentrations less than 0.10% did not participate in reactions of chain propagation but participated in more than one reaction of chain propagation (with linoleic acid) at concentrations more than 0.10%.

Keywords: Butein; Lipid oxidation; Linoleic acid; Kinetic analysis; Mechanism

1. Introduction

Lipid oxidation is a free radical chain process leading to the deterioration of lipids and lipid-containing materials. Antioxidant addition is one of the most effective means to retard oxidation. The action of antioxidant depends on its participation in a series of reactions involving radicals. Recently, the interest in natural antioxidants has been increased since the application of the most widely used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) has been questioned because of possible toxic and carcinogenic components formed during their degradation (Ito, Fukushima, & Tsuda, 1985; Maeura, Weisburger, & Williams, 1984). Phenolic compounds are the main class of natural antioxidants. One of these compounds is a chalcone compound known as butein (2′,4′,3,4–tetrahydroxy chalcone) (Fig. 1). Butein is one of the major active components of *Dalbergia odorifera T. Chen* (Fabaceae) (Wang, Weng, & Cheng, 2000). It is also found in *Acacia* heartwood, and its 4′-glucoside in the petals of *Coreopsis* spp. (Dziedzic & Hudson, 1983).

Butein has been reported to be an inhibitor of xanthine oxidase, and has inhibitory effects on lipid peroxidation in rat liver microsomes (Sogawa et al., 1994). In addition, it has been shown to be a specific protein tyrosine kinase inhibitor (Yang, Zhang, Cheng, & Mack, 1998) and also to induce apoptosis in human leukaemic HL-60 cells (Kim et al., 2001). There are no kinetic data available in the literature concerning the mechanism of antioxidant action of butein. The aim of this study was to investigate the antioxidant activity and the mechanism of action of butein in linoleic acid.

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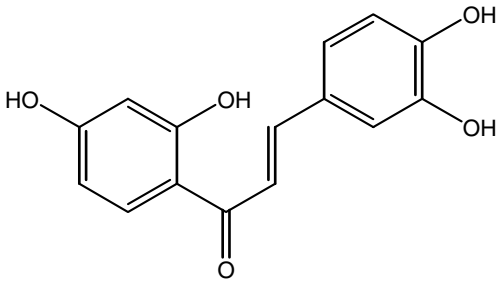

Fig. 1. Chemical structure of butein.

2. Materials and methods

2.1. Materials

Linoleic acid and antioxidants, butein and BHT, were purchased from Sigma Aldrich Company, England.

2.2. Sample preparation

Inhibition of the lipid substrate was achieved by adding aliquots of a solution of the antioxidant in purified acetone to a weighed lipid sample followed by the removal of the solvent under nitrogen. Samples containing 0.02% BHT by weight and 0.02%, 0.05%, 0.10% and 0.20% butein by weight were prepared (Yanishlieva, Marinova, Gordon, & Raneva, 1999).

2.3. Oxidation

Oxidation at 40 °C was performed in the dark using a 1-mm layer in a Petri dish with a diameter of 5 cm. Under these conditions, the process took place in a kinetic regime, i.e. at a sufficiently high oxygen concentration at which the diffusion rate does not influence the oxidation rate (Emanuel, Denisov, & Maizuss, 1965). The oxidation process was monitored by withdrawing samples (0.01–0.03 g) at certain time intervals and subjecting them to spectrophotometric determination of the peroxide value, PV (Shantha & Decker, 1994).

2.4. Kinetic parameters

The influence of antioxidants (or inhibitors, InH) on linoleic acid oxidation was estimated on the basis of the kinetic parameters characterizing the lipid oxidation during its initial stage: stabilization factor F, oxidation rate ratio ORR, activity A, and mean rate of inhibitor consumption W_{InH} (Table 1). The method of estimating the needed kinetic values for calculating these parameters is shown in Fig. 2 (Le Tutour & Guedon, 1992; Marinova, Yanishlieva, & Totseva, 1992; Yanishlieva & Marinova, 1992; Yanishlieva et al., 1999; Yanishlieva, Marinova, Raneva, Partali, & Sliwka, 2001).

2.5. Evaluation of mechanism of action

Mechanism of action of butein in linoleic acid was determined based on the participation of its molecules (InH) and the radicals formed from it (In$^{\bullet}$) in a series of reactions (Scheme 1) (Denisov & Khudyakov, 1987).

The effectiveness of inhibitor F represents the possibility of blocking the radical chain process by interaction with peroxide radicals (reaction 7), which is mainly responsible for the duration of the induction per-

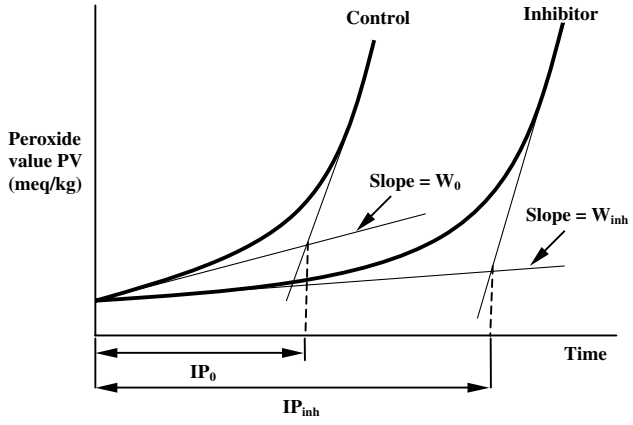

Fig. 2. A schematic kinetic curve of peroxide accumulation during oxidation of lipid systems, and the kinetic values which can be derived from it.

Table 1
Kinetic parameters characterizing the inhibited lipid oxidation during its initial stage

Parameter	Formula	Remarks
(1) Stabilization factor F	$F = IP_{inh}/IP_0$	A measure of effectiveness; IP_{inh}: induction period of inhibited oxidation; IP_0: induction period of non-inhibited oxidation
(2) Oxidation rate ratio ORR	$ORR = W_{inh}/W_0$	An inverse measure of strength so that if ORR > 1 there will be a pro-oxidant than antioxidant activity; W_{inh}: oxidation rate of inhibited oxidation W_0: oxidation rate of non-inhibited oxidation
(3) Activity A	$A = F/ORR$	A general parameter unifying the effectiveness of an antioxidant in termination of the autoxidation chain, and its ability to decrease the oxidation rate during the induction period
(4) Mean rate of inhibitor consumption W_{InH}	$W_{InH} = [InH]_0/IP_{inh}$	$[InH]_0$: initial concentration of the antioxidant (M); 1 meq/kg/h = 1.4×10^{-7} M/s

0. $2RH + O_2 \rightarrow 2R^{\bullet} + H_2O_2$

1. $R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$

2. $ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$

3. $ROOH \rightarrow RO^{\bullet} + {}^{\bullet}OH$

3′. $2ROOH \rightarrow ROO^{\bullet} + RO^{\bullet} + H_2O$

3″. $ROOH + RH \rightarrow RO^{\bullet} + R^{\bullet} + H_2O$

4. $R^{\bullet} + R^{\bullet} \rightarrow R\text{-}R$

5. $R^{\bullet} + ROO^{\bullet} \rightarrow ROOR$

6. $ROO^{\bullet} + ROO^{\bullet} \rightarrow$ products

7. $ROO^{\bullet} + InH \rightarrow ROOH + In^{\bullet}$

-7. $In^{\bullet} + ROOH \rightarrow InH + ROO^{\bullet}$

8. $In^{\bullet} + ROO^{\bullet} \rightarrow In\text{-}OOR$

9. $In^{\bullet} + In^{\bullet} \rightarrow$ products

10. $In^{\bullet} + RH \rightarrow InH + R^{\bullet}$

11. $InH + ROOH \rightarrow In^{\bullet-} + RO^{\bullet-} + H_2O$

12. $InH + O_2 \rightarrow In^{\bullet-} + HOO^{\bullet}$

13. $InOOR \rightarrow InO^{\bullet} + RO^{\bullet}$

14. $In^{\bullet} + O_2 \rightarrow InOO^{\bullet}$

Scheme 1. Non-inhibited (0–6) and inhibited (7–14) oxidation reactions. RH: oxidizing lipid substrate; ROO·: peroxide radical; InH: inhibitor.

iod, IP. The strength of inhibitor expresses the possibility for the inhibitor moieties to participate in other reactions, e.g. −7, 10, 11, 12, and 14, which leads to a change in oxidation rate during the induction period (Yanishlieva & Marinova, 1992).

Non-linear dependence of the parameter F with inhibitor concentration is attributed to the participation of the inhibitor molecules in reactions other than the main reaction of chain termination 7, namely reaction 11 and/or 12. In this case, there is a relationship between the mean rate of inhibitor consumption W_{InH} and the inhibitor concentration [InH], which is used for the theoretical interpretation:

$$W_{InH} = W_i/f + K_{eff}[InH]^n \quad (1)$$

where W_i is the mean rate of initiation during the induction period of the inhibited oxidation (M/s), f is the stoichiometric coefficient of inhibition determining how many radicals perish in an inhibitor molecule, and K_{eff} is the rate constant of inhibitor consumption in side reaction(s) of chain propagation. If W_{InH} is not dependent on its concentration, inhibitor will be consumed in a zero order rate side reaction ($n = 0$) which means that inhibitor molecule does not participate in side reaction(s). A linear dependence at $n = 1$ shows that inhibitor molecule is consumed in a first order rate side reaction which means that inhibitor molecule participates in only one side reaction of chain propagation of reaction 11 or reaction 12. Inhibitor molecule will participate in the two side reactions mentioned if there is a linear dependence at $n = 2$. Table 2 shows two kinetic parameters, W_i/f and K_{eff}, during oxidation of different lipid systems in the presence of different phenolic antioxidants (Emanuel et al., 1965; Le Tutour & Guedon, 1992; Marinova et al., 1992; Marinova, Yanishlieva, & Totseva, 2002; Marinova & Yanishlieva, 1992, 1994, 1996; Yanishlieva & Marinova, 1995; Yanishlieva et al., 1999, 2001; Yanishlieva, Kamal-Eldin, Marinova, & Toneva, 2002).

Kinetic results on inhibited oxidation have shown that the following relation is valid if the inhibitor radical In· does not participate in chain propagation:

$$W_{inh} \sim [InH]^{-1} \quad (2)$$

but if In· participates in one reaction of chain propagation the following correlation is valid (Denisov & Khudyakov, 1987);

$$W_{inh} \sim [InH]^{-0.5} \quad (3)$$

It has been shown that this reaction should be reaction 10 (Yanishlieva & Marinova, 1992). If neither of the above two relations is valid, it suggests that the antioxidant radicals participate in more than one reaction of chain propagation (Marinova et al., 1992). It is possible that the rate of inhibited oxidation in the presence of antioxidant, W_{inh}, does not depend on its concentration. This means that under experiment circumstances, antioxidant is so active that ROO· reacts faster with InH than with RH (Denisov & Khudyakov, 1987).

3. Results and discussion

Fig. 3 illustrates the kinetic curves of peroxide accumulation during oxidation of linoleic acid in the presence of 0.02% BHT, 0.02%, 0.05%, 0.10% and 0.20% butein at 40 °C in the dark. The kinetic curve of oxidation of non-inhibited lipid substrate is also presented.

The kinetic parameters obtained after processing the kinetic curves for 0.02% BHT and all studied concentrations of butein in linoleic acid are given in Table 3. It can be seen that the effectiveness and strength, and therefore, activity of 0.02% BHT is higher than 0.02% butein in linoleic acid oxidation. Of course, as shown in Table 3 the molarity of BHT is higher than butein at the same percentage concentration, and therefore, it is expected

Table 2
Kinetic parameters characterizing the mean rate of initiation, W_i/f, and the effect rate constant of inhibitor consumption, K_{eff}, during oxidation of different lipid systems in the presence of different phenolic antioxidants

Antioxidant	TGL[a]	TGOO[b]	TGSO[c]	TGSBO[d]	MEOO[e]	MESO[f]	MEL[g]	Temp. (°C)
$W_i/f \times 10^{10}$ (Mls)								
Thymol	3		7					22
Carvacrol	3.3		32					22
α-Tocopherol	0.1							22
α-Tocopherol			150	150				100
γ-Tocopherol			150	150				100
Trans-resveratrol	140		900					100
ρ-Coumaric acid		980			500			100
Ferulic acid		400	300		320	18,000		100
Caffeic acid		27	100	130	20	300	35	100
Sinapic acid			600			2000	150	100
Vanilic acid							7000	100
Syringic acid							390	100
3,4-Dihydroxybenzoic acid	200		1500			7000	170	100
$K_{eff} \times 10^{8}$ (s^{-1})								
Thymol	3.7							22
Carvacrol	3.7							22
α-Tocopherol	5.8							22
α-Tocopherol			1500	2500				100
γ-Tocopherol			1200	2000				100
Trans-resveratrol	230		3400					100
ρ-Coumaric acid		2140			3120			100
Ferulic acid		380	10,600		380	10,000		100
Caffeic acid		70	820	750	70	880	140	100
Sinapic acid			3200			2400	160	100
Vanilic acid							10,000	100
Syringic acid							1000	100
3,4-Dihydroxybenzoic acid	280		7300			4200	380	100

[a] Triacylglycerols of lard.
[b] Triacylglycerols of olive oil.
[c] Triacylglycerols of sunflower oil.
[d] Triacylglycerols of soybean oil.
[e] Methyl esters of olive oil.
[f] Methyl esters of sunflower oil.
[g] Methyl esters of lard.

that their equal molar concentrations at least at low levels (e.g. 0.02%) have approximately the same antioxidant activity.

Fig. 4 illustrates the changes of antioxidant activity A with increasing antioxidant concentration during oxidation of linoleic acid at 40 °C. As it can be seen, antioxidant activity increases with a milder slope above the concentrations about of 3.3×10^{-3} M (0.10%).

Fig. 5 illustrates the dependence of the stabilisation factor F on increasing butein concentration during oxidation of linoleic acid at 40 °C. It is clear that this dependence is not linear. The absence of F linearity is due to the participation of the inhibitor molecules in reactions other than the main reaction of chain termination 7, namely reaction 11 and/or 12.

The mean rate of butein consumption, W_{InH}, was plotted against concentration [InH] at $n = 1$ and 2. This plot was linear only at $n = 1$ (Fig. 6), which indicates that its participation in only one of the two side reactions of chain propagation (reaction 11 or 12). Previous investigations have shown that K_{eff} depends on the character of the lipid system and, in particular, on the different decomposition rate of linoleic and oleic acid hydroperoxides (Table 2). As seen from Scheme 1, reaction 11 is dependent on the hydroperoxide reactivity. Therefore, it has been concluded that the main side reaction in which the antioxidants in inhibited lipid oxidation participates is reaction 11 (Marinova & Yanishlieva, 1994, 1996; Yanishlieva & Popov, 1971, 1973; Yanishlieva & Marinova, 1995). The same should be true for butein in linoleic acid oxidation. From the slope of the line, the rate constant K_{eff} of this reaction was calculated to be 2.8×10^{-7} s^{-1}.

The parameter W_i/f takes into account the participation of the antioxidant in the initiation reactions. W_i/f was determined from Fig. 5 by extrapolation to zero concentration of butein (13.6×10^{-10} M/s). Table 2 shows the strong influence of the unsaturation type and degree of the lipid system on the W_i/f values of different phenolic antioxidants. The higher W_i/f, the more participation of antioxidant in chain initiation during oxidation.

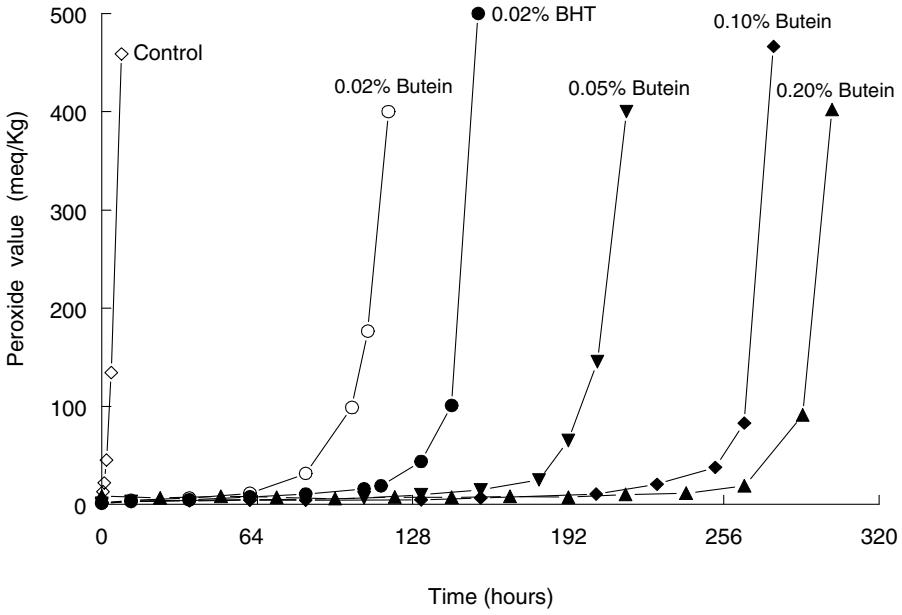

Fig. 3. Kinetic curves of peroxide accumulation (PV) during oxidation of linoleic acid at 40 °C in the absence and in the presence of 0.02% BHT, and 0.02%, 0.05%, 0.10% and 0.20% butein in the dark. All kinetic curves are the mean result of three independent experiments. The values obtained varied no more than 5%.

Table 3
Kinetic parameters characterizing the inhibited lipid oxidation of linoleic acid at 40 °C, $PV_0 = 0$ meq/kg, $IP_0 = 3.4$ h, $W_0 = 2.8 \times 10^{-6}$ M/s[a]

Antioxidant	Inhibitor concentration		F	ORR	A	W_{inh} ($\times 10^9$) (M/s)	W_{InH} ($\times 10^{10}$) (M/s)
	[InH] (%)	[InH] ($\times 10^3$) (M)					
Butein	0.02	0.66	33.80	0.00766	4410.76	21.5551	15.97677
	0.05	1.65	77.35	0.00181	42736.43	5.07724	17.45545
	0.10	3.31	116.47	0.00116	100406.03	3.26270	23.18447
	0.20	6.61	167.06	0.00100	167059.00	2.82086	32.32764
BHT	0.02	0.82	42.87	0.00706	6070.61	19.85998	15.56609

[a] All data are the mean result of three independent experiments. The values obtained varied no more than 5%.

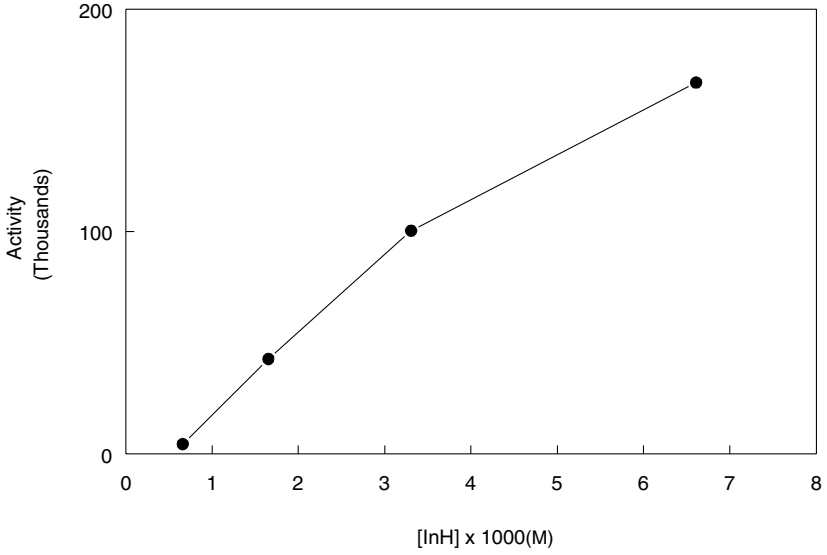

Fig. 4. Dependence of the antioxidative activity A of butein on its concentration [InH] during oxidation of linoleic acid at 40 °C in the dark.

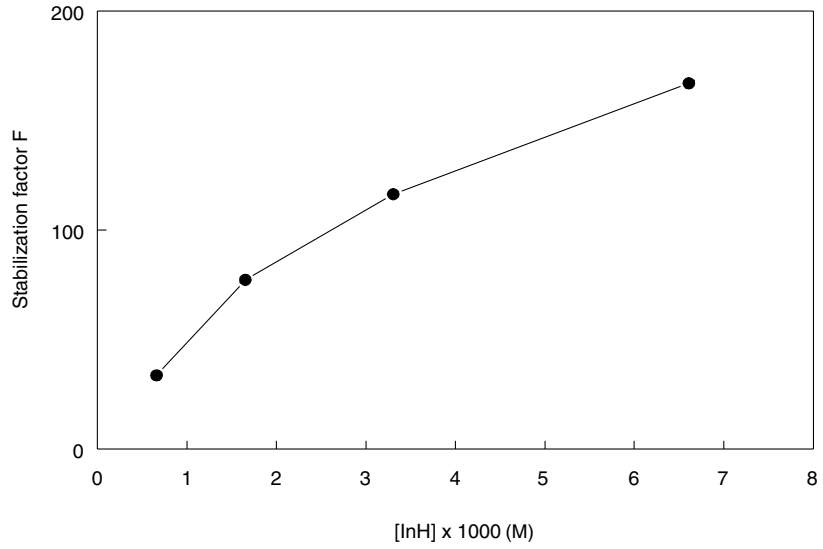

Fig. 5. Dependence of butein stabilization factor F on its concentration [InH] during oxidation of linoleic acid at 40 °C in the dark.

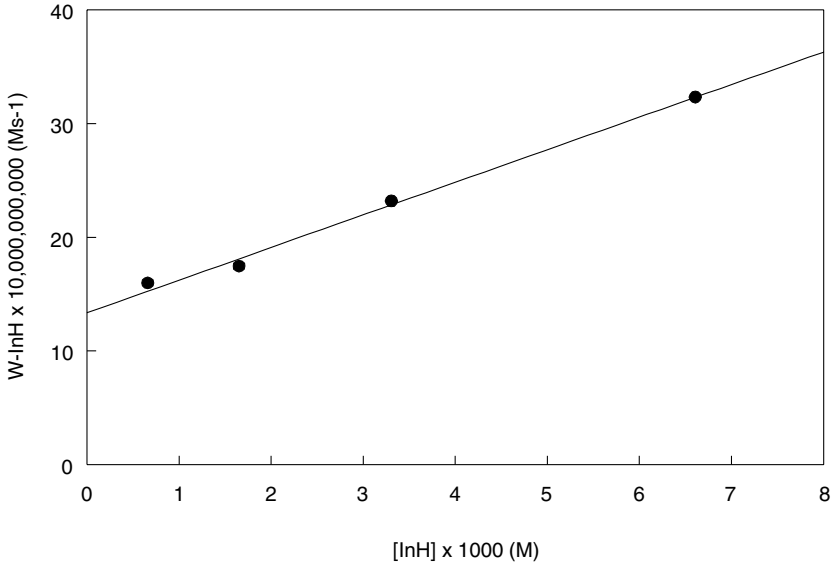

Fig. 6. Dependence of the mean rate of consumption W_{InH} of butein on its concentration [InH] during oxidation of linoleic acid at 40 °C in the dark.

Processing of the results obtained (Table 3) on the basis of Eqs. (2) and (3) for butein showed a good linear relationship at concentrations lower than 0.10%; meaning that butein at concentrations less than 0.10% does not participate in reactions of chain propagation, but participates in more than one reaction of chain propagation at concentrations more than 0.10%.

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