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Photodegradation kinetics of some indolecarboxylic acids and their regulation effects on plant growth (groundnut and haricot bean)☆

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Abstract

Indolecarboxylic acids belong to the auxins group. They constitute a class of phytohormones, which play an important role in plant growth. This paper deals with the kinetics of six indolecarboxylic acids and their regulation effects on the growth of groundnut (*Arachis hypogea*) and haricot bean (*Phaseolus vulgaris*). The results obtained enabled us to establish a relationship between photodegradation rate constants and their regulation properties on the growth of these two plants. The paper shows that the compounds yielding the highest photodegradation rates were efficient growth regulators. In this regard, a few explanations are provided.

Keywords: Indolecarboxylic acids; Groundnut; Haricot bean; Plant growth; Regulation effect; Kinetics; Photodegradation; Absorption; Rate constant; Half-time; Caulogenesis; Rhizogenesis

1. Introduction

In line with our previous studies on indole compounds [1–5] and in particular on indole acids [6–8], there is need to study the kinetics of indolecarboxylic acid photodegradation before establishing correlations and evaluating their regulation effect on plant growth.

It is also widespread knowledge that indolecarboxylic acids belong to the auxins family. They constitute a class of phytohormones, which play an important role in plant growth.

Many papers have been published concerning indolecarboxylic acids as growth regulators. Van Sachs [9] postulated in 1880 that hormones regulated plant growth. Many years later, in

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1934, Koyl and Exterben [10] isolated indole-3-acetic acid (AIA) as a growth regulator. Their study has also shown, among others, that its physiologic effects may exert such influence on micro-organisms as cell elongation, rhizogenesis, and ribonucleic acid and protein synthesis. This was substantiated in 1985 by Rockem and Goldberg [11], then by Liang et al. [12]. They postulated that plant growth regulation is one of the most important factors affecting cell growth and tissue culture, although regulators do not always play the same role. Thus, indole-3-butyric acid (IBA), known to play a more efficient role in the possible formation of roots, has been identified as an endogenous auxin of peas, tobacco and maize [13]. A study by Kita [14] has also indicated that tissue culture of panax ginseng roots requires the presence of 2,4-dichlorophenoxy acetic acid (2,4-D). Likewise, certain authors [15] have shown that the synthesis of saponins in ginseng cals is facilitated by 2,4-D and is inhibited by AIA. The effect of auxins depends on both their concentration and the environment. For example, Kim et al. have shown that by using 2,4-D (5 mg l^{-1}) or kinentin (2 mg l^{-1}) in saponin for the culture of panax ginseng tissue, the quantity of saponin becomes very important [16].

Studies by Quattrochio [17] have shown that highly concentrated AIA causes the formation of hair in potatoe. Likewise, Nakamura et al. [18] have pointed out that maximum growth of tobacco was achieved with a lower concentration of AIA. Posthumus et al. [19] have also shown that an AIA concentration higher than 5.7 mM inhibits DNA synthesis in tobacco stems. However, a concentration of 11.4 mM of IBA increases DNA content in these stems. Studies by Houle and Babeux [20] have shown that female cuttings of *Juniperus communis*, var depressa, take root faster than male ones when IBA concentration is low. However, inoculation of highly concentrated IBA inhibits rooting in female individuals. In addition to concentration, ambient conditions may also alter the properties of auxin. Accordingly, Muller et al. [21] point out that AIA is altered during alkaline hydrolysis in a lighted setting and inoculation of AIA in maize turns it into IBA in low light conditions, while AIA turns entirely into IBA under intense light.

While IAA and IBA have been used as growth regulators in peas, tobacco, maize and potato, none of these two compounds has, to our knowledge, been tested in groundnut (*Arachis hypogea*) and haricot bean (*Phaseolus vulgaris*).

The objective of this work is to study the kinetics of photodegradation based on the electronic absorption spectra versus irradiation time, along with the effects of indolecarboxylic acid effects on rhizogenesis (root development) and caulogenesis (elongation of stems) of groundnut (*A. hypogea*) and haricot-bean (*P. vulgaris*). Lastly, this study enabled us to explore a possible correlation between photodegradation rate and the regulating power of these indolecarboxylic acids.

2. Experimental

2.1. Chemicals

AIA, indole-2-carboxylic acid, indole-5-carboxylic acid, 3-formylindole and 5-methoxyindole-2-carboxylic acid were purchased from Aldrich-Europe. All these compounds were used without further purification. Their purity was checked by determining their melting point, which was consistent with the values reported in the literature on this topic.

The solvents used for the study of kinetics were absolute ethanol (spectrophotometric grade, Gold Label, Aldrich Chemical Co) and distilled water (pH 7). As for the regulation effects on plant growth, drinking water (pH 7.8) from the laboratory outlet was used.

Our previous work [6–8] has shown that all indolecarboxylic acids are sensitive to visible UV. All stock solutions were consequently protected by aluminium foil and kept in a refrigerator. Kinetics and application of compounds took place at room temperature (24 °C). Growth regulators are altered by solar radiation on both the soil and the plant.

2.2. Instrumentation

Photodegradation of these indolecarboxylic acids was examined using a Beckman model 3600 UV–vis spectrophotometer and a photochemical reactor including an Oriel light source model 6137, equipped with a 200 W-lamp and an Oriel power supply box model 8500, a magnetic agitator, a quartz cell (1 cm optical path), placed 60 cm from the lamp. We also used Petri dishes for studying the effects of indole acids on the growth the two plants selected.

2.3. Photodegradation kinetics

We have followed the kinetics by studying the absorbance variation of wavelength band, which usually has the highest molar absorption coefficient values with respect to the time of irradiation. We have noticed the decrease in the absorbance of this band with the irradiation time. Generally, we have not noticed the shift of this band during the irradiation.

2.4. Rhizogenesis and caulogenesis

We used Petri dishes with cotton laid inside to maintain moisture. Groundnut and haricot bean seeds were purchased at a local market were placed over the cotton. There were three seeds of groundnut or haricot bean in each box. A specific concentration of the regulator (indole acid) was applied to all boxes in order to determine its effect on plant growth. At the same time, water was applied on two other boxes, which served as control boxes. We determined the mean number of roots in each box. As for caulogenesis (stem growth), we determined the average height.

Measurement took place the fourth day after planting and covered a period of 33–40 days, depending on the product used. Application of compounds was performed every other day.

2.5. Determination of rate constant (k) and half-life ($t_{1/2}$)

Consider a compound B absorbing radiation and which becomes altered and yields photoproducts according to the equation:

$$B + h\nu \rightarrow \text{Photoproduct(s)}$$

We can monitor photodegradation kinetics by examining absorbance variation based on irradiation time. We assumed that the photoproducts absorbance did not occur in the same areas. Most photoreactions were first order reactions, second order reactions being seldom observed.

- For first order reactions, taking into account the Beer–Lambert Law, we can write:

$$\ln\frac{A_0}{A} = kt \qquad (1)$$

where A_0 represents the indole acid absorbance at time $t = 0$ (before irradiation), A acid absorbance at time $t \neq 0$, and k the rate constant with $t_{1/2} = \frac{0.693}{k}$

- For second order reactions, we have:

$$\frac{1}{A} - \frac{1}{A_0} = \frac{[B]_0}{A_0}\, kt \qquad (2)$$

A_0, A, k will then have the same meaning as in Eq. (1), and $[B]_0$ represents the concentration of indole acid at time $t = 0$ with $t_{1/2} = \frac{1}{k[B]_0}$.

3. Results and discussion

3.1. Electronic absorption spectra

We determined the electronic absorption spectra of irradiated and non-irradiated indolecarboxylic acids in water and in ethanol. Spectral properties are presented in the Table 1. Most non-irradiated indole acids present a very intense broad band, about 260–320 nm, and correspond to the π–π* (^1L_a and ^1L_b) transitions [6,22–26]. Sometimes, several vibronic components are observed (Figs. 1 and 2).

In general, no significant shift of the absorption band limits of indolecarboxylic acids occurs when solvent polarity is changed. Furthermore, we did not notice any shift of the band during irradiation.

Table 1
The characteristic parameters of the photodegradation kinetic of the studied compounds

Compound	Solvent	ε_{max} (M^{-1} cm^{-1})[a]	Slope[b]	k (min^{-1})[c]	$t_{1/2}$ (min)[d]	R[e]	Order[f]	λ_{max} (nm)[g]	$[B]_0$ (mol l^{-1})[h]
Indol-3 acetic acid	Water	9600	0.68	0.68	1	0.98	1	243	10^{-4}
	Ethanol	9400	0.03	0.03	23.1	0.99	1	244	10^{-4}
Indole-5 carboxylic acid	Water	22 000	0.086	1892	21.2	0.99	2	236	2.5×10^{-5}
	Ethanol	39 333	0.119	0.119	5.82	0.98	1	237	3×10^{-5}
3-Formylindole	Water	7333	0.234	0.234	2.96	0.97	1	300	1.5×10^{-4}
	Ethanol	11 000	0.038	0.038	18.3	0.99	1	294	1.5×10^{-4}
Indole-2 carboxylic acid	Water	7666	0.039	0.039	17.8	0.98	1	288	6×10^{-5}
	Ethanol	14 500	0.049	0.049	14.2	0.99	1	290	4×10^{-5}
5-Methoxy-indole-2 carboxylic acid	Water	7000	0.136	0.136	5.1	0.98	1	290	5×10^{-5}
	Ethanol	12 200	0.049	0.049	14.2	0.99	1	290	5×10^{-5}

[a] Molecular extinction coefficient at the maximum absorption wavelength.
[b] Slope of the straight line.
[c] Rate constant.
[d] Half-life of reaction.
[e] Correlation coefficient.
[f] Order of the kinetics.
[g] The maximum absorption wavelength measurement.
[h] Initial concentration of the compound.

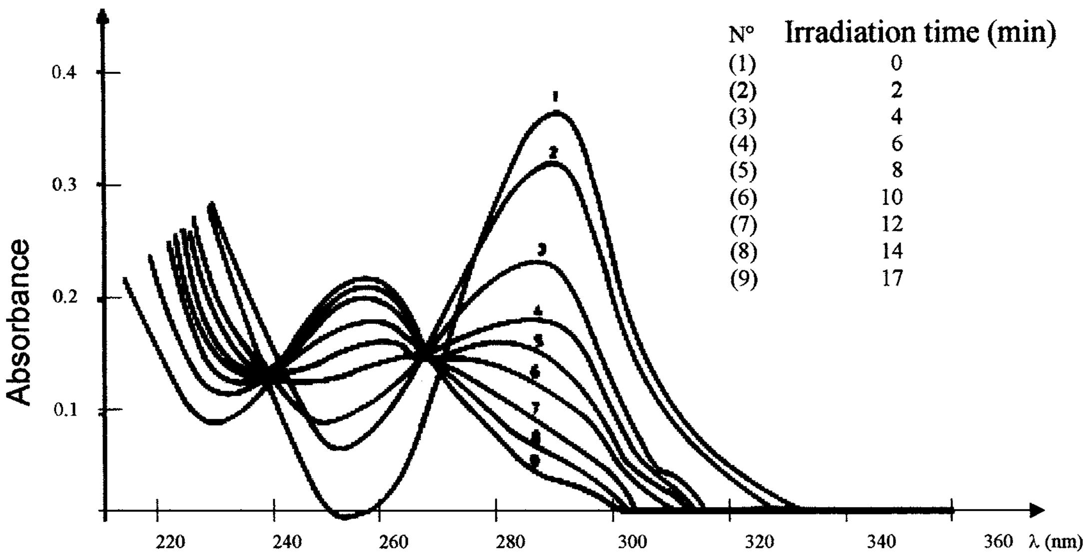

Fig. 1. Electronic absorption spectra of irradiated and non-irradiated 5-methoxy indole-2-carboxylic acid in water.

However, we noted a decrease in absorbance of electronic absorption band versus irradiation time. Sometimes, a new band appears and its absorbance increases along with irradiation time. This is more significant with water than with ethanol (Figs. 1 and 2).

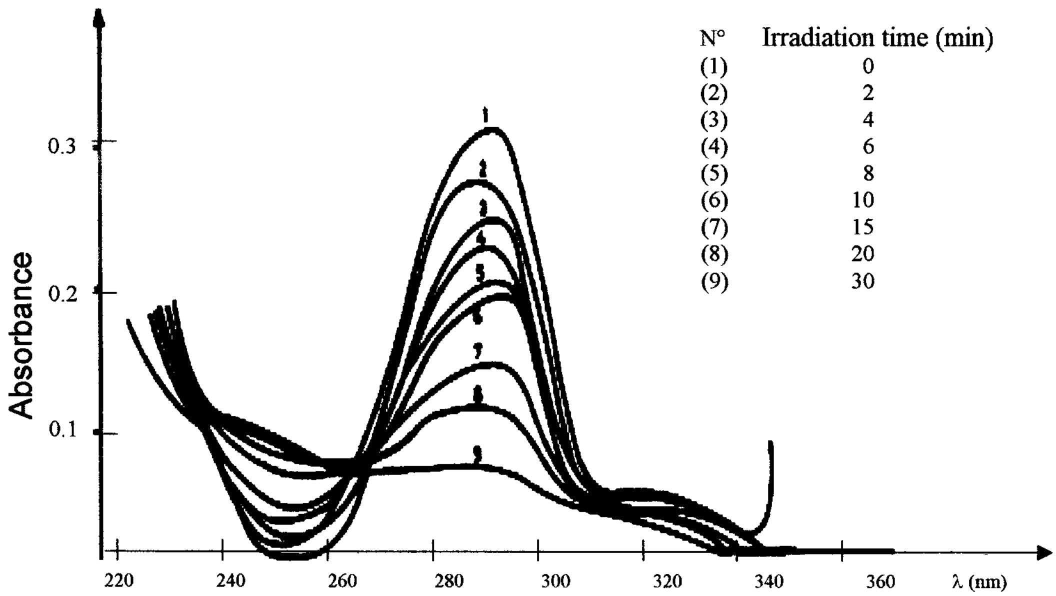

Fig. 2. Electronic absorption spectra of irradiated and non-irradiated 5-methoxy indole-2-carboxylic acid in ethanol.

3.2. Photodegradation kinetics of indolecarboxylic acids

In order to examine the photodegradation kinetics of indolecarboxylic acids, we monitored the evolution of absorbance of the broad band versus time. We then plotted the curve:

$$\ln\frac{A_0}{A}=f(t)$$

where A_0 and A are, respectively, the absorbance of indole acid at $t=0$ (before irradiation) and at $t\neq 0$ (during excitation). We obtained significant linear correlations, in all our tests (Fig. 2a), except for the experience with indole-5-carboxylic acid in water. These correlations thus show that the photodegradation rate of indole carboxylic acid is a first order reaction. Examining the slopes, we determined k rate constants with precision (correlation coefficients ranging from 0.97 to 0.99, see Table 1).

Concerning indole-5-carboxylic acid in water, we plotted the curve:

$$\frac{1}{A}-\frac{1}{A_0}=f(t)$$

where A_0 and A have the same meaning as in Eq. (2). We also obtained a significant linear correlation. The photodegradation kinetics of this indole acid is, therefore, of second order reaction (Fig. 3b).

Results: rate constant (k), half-life ($t_{1/2}$), absorption wavelength (λ_A), initial concentration of indole acids $[B]_0$, correlation coefficient (R) are presented in the Table 1.From the Table 1, we noted that half-life periods were shorter in water than in ethanol. Accordingly, these indole acids, under radiation, decompose faster in water than in ethanol, except for indole-5- and indole-2-carboxylic, where we note the opposite.

Based on the photodegradation kinetics of indolecarboxylic acid, we can conclude that these compounds are very unstable under solar radiation. Consequently, these radiations may play an important role in plant growth.

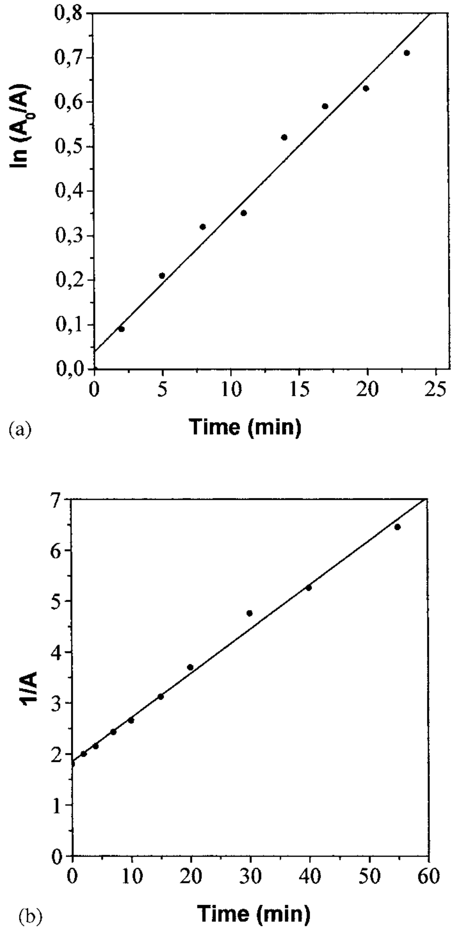

Fig. 3. (a) The photodegradation kinetics of AIA 10^{-4} M. (b) The photodegradation kinetics of indole-5-carboxylic acid (2.5×10^{-5} M).

3.3. Regulation effects of indole acids on the rhizogenesis and caulogenesis of groundnut and haricot bean

We studied the effect of indole acids on the rhizogenesis (root formation) and caulogenesis (elongation of stems) of these plants. We used concentrations ranging from 10^{-3} to 10^{-11} M of indole acids. Plotting growth parameters versus time (number of days), we obtained curves of sigmoid appearance, characterised by the three following phases: a phase of slow growth which

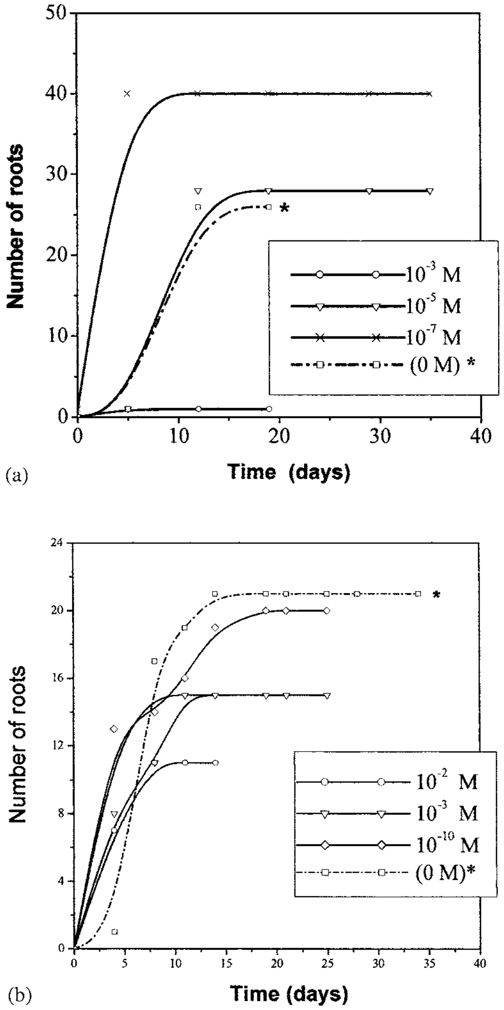

Fig. 4. (a) Action of 3-formylindole on the rhizogenesis of haricot bean. (*) Blank curve. (b) Action of indole-5-carboxylic acid on the rhizogenesis of haricot bean. (*) Blank curve.

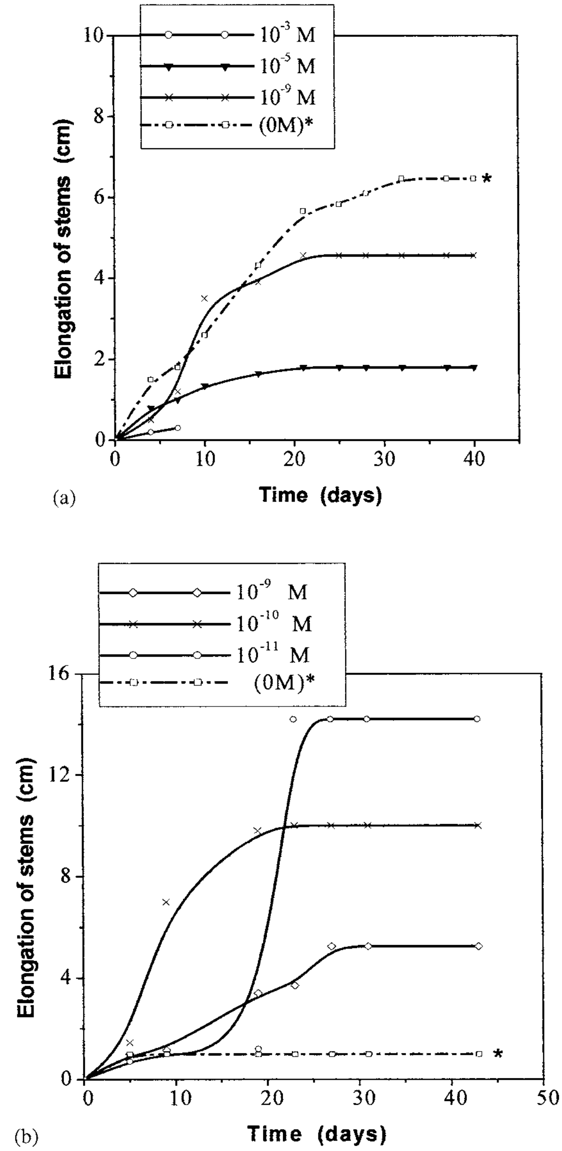

Fig. 5. (a) Action of indole-2-carboxylic acid on the caulogenesis of groundnut. (*) Blank curve. (b) Action of 5-methoxyindole-2-carboxylic acid on the caulogenesis of groundnut. (*) Blank curve.

corresponds to the time for the germ to adapt to the setting, a second phase of exponential growth, which is followed by a third stationary phase (Fig. 4a and b, Fig. 5a and b).

In general, such experimental curves show that the growth rate (tangent to one point of the sigmoid curve) are relatively slow at the beginning, which accelerates and reaches a maximum at inflection point, and finally decreases until growth seems to be roughly constant.

We also noted that growth parameters are more sensitive to low concentrations of growth regulators, while higher concentrations ($>10^{-7}$ M) inhibit, more often than not, plant growth. Indeed, accumulation of growth hormones has a toxic effect on plants. For this reason, most efficient concentrations range from 10^{-7} to 10^{-11} M.

Trials on the groundnut revealed that AIA is more sensitive to stem elongation than root development. AIA presents the better results with a 10^{-11} M concentration. However, 5-methoxyindole-2-carboxylic acid yielded the best results when we consider the whole set of concentrations used (Fig. 5b). Except for, indole-5- and indole-2-carboxylic acids, all the compounds used appear to be efficient growth regulators, at least for low concentrations.

As for haricot bean, 5-methoxyindole-2-carboxylic acid and 3-formylindole presented the fastest elongation of stems and root development. All of the concentrations used yield better results than the control box. In this case also, all the indole acids used are efficient regulators, except for indole-5- and indole-2-carboxylic acids (Fig. 4b, Fig. 5a). These inhibit growth.

3.4. Relationship between regulation effect and photodegradation half-life

The study of the effect of indole acids on groundnut and haricot bean rhizogenesis and caulogenesis has shown that the compounds used are generally efficient regulators. We noted, however, that only compounds with the shortest half-life reactions yielded the best results. Indole-5- and indole-2-carboxylic acids had an inhibiting effect. For indole-2-carboxylic acid, we noted the low value of rate constant photodegradation and, consequently, greater half-life values, with first order reactions. For indole-5-carboxylic acid, rate constants were high, with second order reactions; the concentrations applied were low and, consequently, half-life values were greater too.

This points to the existence of a relationship between regulation effect and photodegradation half-life. The shorter the half-life period, the more significant the regulation effect. This may be explained by the fact that the compounds are altered by light and their concentration decreases, thus inducing better cell growth. In contrast, when degradation is slow, compounds tend to accumulate in the plant and result in higher concentration. They become toxic.

4. Conclusion

The main conclusion we can draw from this study is the rapid degradation of indole carboxylic acids under radiation. Photodegradation of these acids is of first order kinetics, except for indole-5-carboxylic in water, for which kinetics is of second order. We also noted that most compounds studied were efficient growth regulators, with the exception of indole acids, whose photodegradation half-life lasts longer (indole-2- and indole-5-carboxylic acids).

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