Biosynthesis of the *Erythrina* Alkaloids. The Incorporation of Tyrosine-2-C^{14} into the Erythroidines[1]

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Abstract: Radioactive α- and β-erythroidine were isolated from *Erythrina berteroana* plants which had been fed DL-tyrosine-2-C^{14}. Systematic degradation of these alkaloids indicated that essentially all the activity was located at C-8 and C-10, and was equally divided between these positions. These results are consistent with the participation of a symmetrical C_6–C_2–N–C_2–C_6 compound as an intermediate in the biosynthesis of the *Erythrina* alkaloids. The administration of DL-phenylalanine-2-C^{14} to *E. berteroana* plants did not lead to the formation of radioactive alkaloids, indicating that phenylalanine is not converted to tyrosine in this species.

Although there have been numerous speculations[3-9] on the biosynthesis of the *Erythrina* alkaloids, no tracer work *in vivo* has been reported to justify any of these hypotheses. Recently Scott[10] and Mondon,[11] and their co-workers, have been successful in obtaining the tetracyclic ring system present in the aromatic *Erythrina* alkaloids by the *in vitro* oxidation of the symmetrical amine **4** (R = CH_3). A tentative hypothesis for the formation of the *Erythrina* alkaloids is illustrated in Figure 1. It is suggested that dopamine (**2**) and 3,4-dihydroxyphenylacetaldehyde (**3**), both of which can be derived from tyrosine by unexceptional metabolic steps, condense to yield the Schiff base **5**, which on reduction affords the amine **4** (R = H). Oxidative coupling[9] of the phenolic rings yields the diphenoquinone **6**. The spiro ring system in **7** then results by condensation of the amino group on the quinone as indicated. The dienone **7** is then converted to the aromatic *Erythrina* alkaloids such as erysopine (**9**) by unexceptional reductions and dehydrations. Recently Barton and co-workers[12] have described compounds which are probable intermediates in this transformation. Woodward fission[13] of the catechol ring of erysopine yields compound **8**, which on decarboxylation and hydration of an olefinic double bond affords **10**. α-Erythroidine (**11**) is formed by lactone formation, and isomerization of the double bond in the lactone ring yields β-erythroidine (**12**).

We have now tested this hypothesis by feeding DL-tyrosine-2-C^{14} (**1**) to seven-month-old *Erythrina berteroana* plants by means of cotton wicks inserted in the stems. Extraction of the aerial parts of the plants 3 weeks later yielded a mixture of α- and β-erythroidines, both of which were radioactive (0.025% incorporation). Since these alkaloids are difficult to separate, the α-erythroidine in this mixture was converted to β-erythroidine by treatment with sodium hydroxide.[14] If the tyrosine-2-C^{14} were incorporated into the erythroidines in accordance with the scheme illustrated in Figure 1, radioactivity would be expected at C-8 and C-10. Degradations carried out on radioactive β-erythroidine are illustrated in Figure 2. In the degradation by route A, apo-β-erythroidine (**13**) was obtained by heating β-erythroidine with hydrobromic acid.[15] A Hofmann degradation[16] on the methiodide of **13** yielded des-N-methylapo-β-erythroidine (**14**), which was hydrogenated affording dihydro-des-N-methylapo-β-erythroidine (**15**). A Kuhn–Roth oxidation of this compound yielded a mixture of acetic and propionic acids which were separated on silicic acid.[17] The acids were assayed as their α-naphthylamides. The acetic acid was subjected to a Schmidt reaction yielding carbon dioxide and methylamine assayed as N-methylbenzamide (representing the activity at C-10 in β-erythroidine). Attempted further degradation of compound **15** to obtain information regarding the activity at C-8 was unsuccessful. We therefore subjected the radioactive β-erythroidine to a second degradation by route B, based on the recent work of Boekelheide and Wenzinger.[18] Reduction of β-erythroidine with hydrogen in the presence of platinum afforded tetrahydro-β-erythroidine (**16**), which on treatment with lithium aluminum hydride yielded tetrahydro-β-erythroidinol (**17**). Cyclization with phosphoric acid yielded anhydrotetrahydro-β-erythroidinol (**18**). Methylation and a subsequent Hofmann degradation yielded des-N-methylanhydrotetrahydro-β-erythroidinol (**19**). Hydrogenation yielded compound **23**, which was methylated and subjected to a Hofmann degradation which proceeds *via* a 1,4 elimination yielding des-N,N-dimethylanhydrohexahydro-β-erythroidinol (**22**, a mixture of *cis* and *trans* isomers). This compound was converted to its methiodide, the pyran double bond reduced, and the product subjected to a third Hofmann degradation afford-

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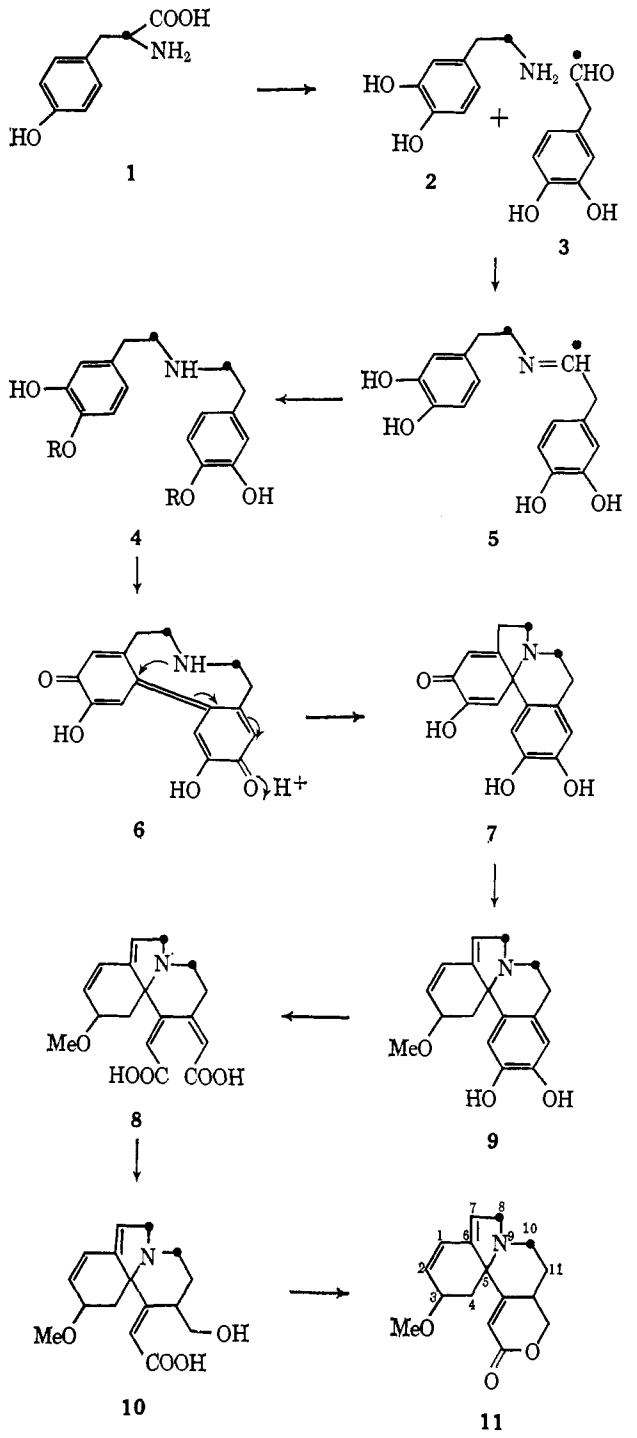

Figure 1. Hypothesis for the biosynthesis of the *Erythrina* alkaloids (carbon-14 indicated with ●).

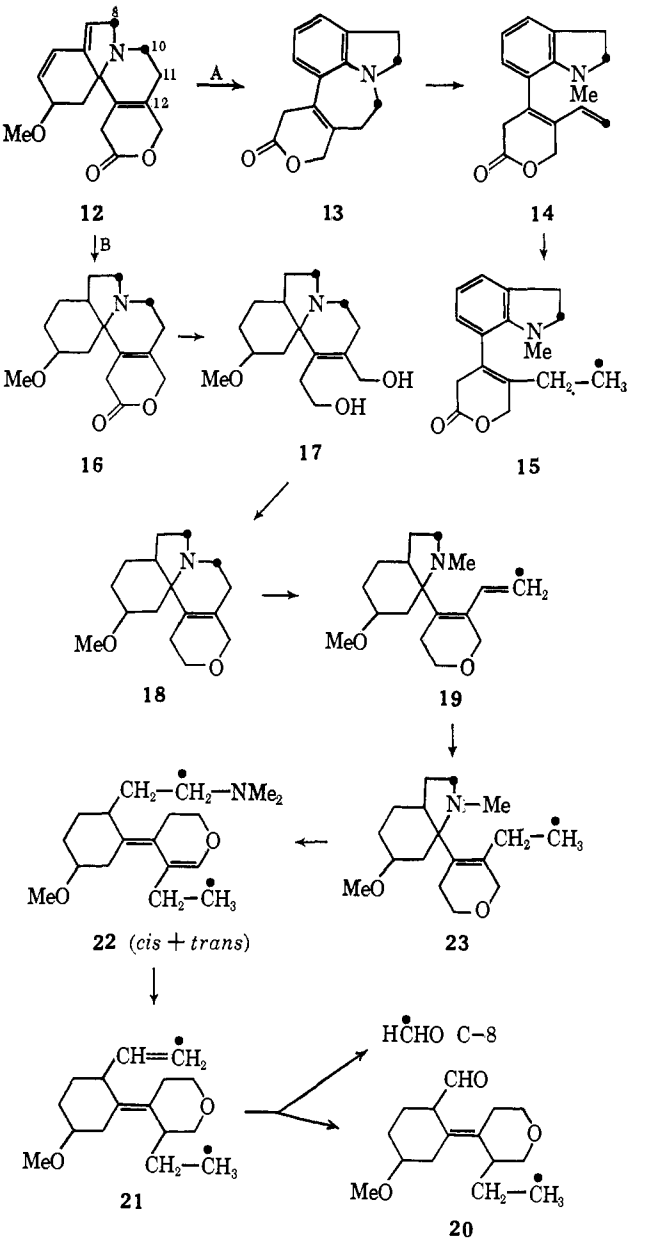

Figure 2. Degradation of β-erythroidine (carbon-14 indicated with ●).

ing desazaanhydrooctahydro-β-erythroidinol (**21**). In contrast to previous work[18] we could not detect any of the ethylidene isomer, and subsequent ozonolysis yielded only formaldehyde (derived from C-8 of β-erythroidine) and no acetaldehyde. The aldehyde **20**, also obtained from the ozonolysis, was subjected to a Kuhn–Roth oxidation yielding a mixture of acetic and propionic acids. Further degradation of the acetic acid by a Schmidt reaction yielded confirmatory data on the activity at C-10. The activities of β-erythroidine and its degradation products are recorded in Table I. It is seen that essentially all the activity of the alkaloid was located at C-8 and C-10 and was equally divided between these positions. These results thus provide strong support for the previously discussed biosynthetic scheme and are consistent with the formation of a symmetrical compound such as **4** as an intermediate in the formation of the *Erythrina* alkaloids. However the results can be interpreted without involving a symmetrical intermediate. The two halves of the erythroidine molecule could be derived from different metabolites of tyrosine which acquired the same specific activity during the 3-week period the plants were allowed to grow in the presence of radioactive tyrosine. It is satisfying to discover that "Woodward fission" is a valid biochemical concept in the biosynthesis of this class of alkaloids, whereas it is apparently irrelevant for the indole[19] and Ipecacuanha[20] alkaloids.

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Table I. Activities of β-Erythroidine and Its Degradation Products

	Specific activity, dpm/mmole × 10^{-3}	Relative activity	Carbon atom
Degradation by route A			
β-Erythroidine hydrochloride	5.0		
Apo-β-erythroidine (**13**)	5.2	100	All
Dihydro-des-N-methylapo-β-erythroidine (**15**)	5.1		
1-Propionamidonaphthalene[a]	2.4	47	10–12
1-Acetamidonaphthalene[a]	2.3	45	10,11
N-Methylbenzamide[b]	2.8	55	10
Barium carbonate[b]	<0.01	0	11
Degradation by route B			
β-Erythroidine hydrochloride	3.2	100	All
Tetrahydro-β-erythroidine (**16**)	3.2	100	All
Anhydrotetrahydro-β-erythroidinol (**18**)	3.4	106	All
Des-N-methylanhydrotetrahydro-β-erythroidinol (**19**)	3.3	103	All
Des-N,N-dimethylanhydrohexahydro-β-erythroidinol (**22**)	3.7	116	All
Desazaanhydrooctahydro-β-erythroidinol (**21**)	3.1	97	All
Formaldehyde dimedone[c]	1.4	44	8
1-Propionamidonaphthalene[d]	1.8	56	10–12
1-Acetamidonaphthalene[d]	1.5	47	10,11
N-Methylbenzamide[e]	1.6	50	10
Barium carbonate[e]	<0.01	0	11

[a] Derivatives of the acetic and propionic acids obtained by a Kuhn–Roth oxidation of compound **15**. [b] Obtained by a Schmidt reaction on the previously mentioned acetic acid. [c] Obtained by ozonolysis of compound **21**. [d] Derivatives of the acetic and propionic acids obtained by a Kuhn–Roth oxidation of the aldehyde **20**. [e] Obtained by a Schmidt reaction on the previously mentioned acetic acid.

In preliminary work DL-phenylalanine-2-C^{14} was fed to *E. berteroana* plants; however the α- and β-erythroidines isolated 2 weeks later had negligible activity. Thus apparently this species, like *Colchicum*,[21] *Amaryllidaceae*,[22] and *Salvia*,[23] lacks the enzymes to convert phenylalanine to tyrosine.

Experimental Section

Melting points are uncorrected. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation spectrometer, Model 724, using as solvents either toluene or dioxane–water with the usual scintillators.[24]

Administration of DL-Tyrosine-2-C^{14} to *Erythrina* plants and Isolation of the Alkaloids. DL-Tyrosine-2-C^{14} [25] (13.25 mg, 0.1 mcurie) dissolved in water (5 ml) was fed to five 7-month-old *Erythrina berteroana* plants (about 60 cm tall) growing in soil in a greenhouse (July 1965), by means of cotton wicks inserted in the stems. After 20 days the aerial parts of the plants (fresh wt, 711 g) were mascerated in a Waring blender with a mixture of methanol (2 l.) and **15** *N* ammonia (50 ml). The mixture was then filtered through cloth and the filtrate evaporated *in vacuo*. The greenish yellow residue was dissolved in a mixture of water (50 ml) and concentrated hydrochloric acid (5 ml). The solution was filtered and extracted with chloroform. The aqueous solution was made basic with sodium bicarbonate and extracted with chloroform. Evaporation of this dried extract yielded the crude alkaloids (activity 1.45 × 10^5 dpm). This mixture was dissolved in benzene and chromatographed on Woelm alumina (activity II). Elution with benzene afforded a mixture of α- and β-erythroidines. Thin layer chromatography of this mixture on silica gel G (E. Merck, AG), eluting with 5% methanol in chloroform indicated that α- and β-erythroidines (R_f values 0.60 and 0.53, respectively) were present in the ratio of 1:4 approximately. Radioactivity was detected at positions coincident with these alkaloids. The alkaloid mixture was dissolved in methanol (2 ml) and hydrogen chloride gas was passed in. On the addition of ether colorless needles (121 mg) of a mixture of α- and β-erythroidine hydrochlorides separated. This active mixture was diluted with an inactive mixture of α- and β-erythroidine hydrochlorides (650 mg) which was obtained by extraction of *Erythrina berteroana* seeds.[26] The diluted mixture of alkaloids was refluxed for 2 hr in 10 ml of 12% aqueous sodium hydroxide. The cooled solution was neutralized with hydrochloric acid and extracted with chloroform. Evaporation of the dried extract yielded β-erythroidine which was converted to its hydrochloride and crystallized to constant activity (2.26 × 10^4 dpm/mmole) from a mixture of methanol and ether. Further dilution of this material was carried out prior to subsequent degradations. Activity of the diluted alkaloid is recorded in Table I.

Degradation of β-Erythroidine by Route A. β-Erythroidine hydrochloride having a specific activity of 5.0 × 10^3 dpm/mmole was used in the following degradation. Apo-β-erythroidine (680 mg), mp 137° (lit.[15] mp 144°), was obtained from β-erythroidine hydrochloride (1.2 g) by the procedure of Koniuszy and Folkers.[15] Apo-β-erythroidine (670 mg) was converted to its methiodide,[16] and a Hofmann degradation carried out using 40% potassium hydroxide at 80°.[16] Hydrogenation of the resultant des-N-methylapo-β-erythroidine (255 mg) in the presence of Adams catalyst yielded dihydro-des-N-methylapo-β-erythroidine (202 mg), mp 129–130° (lit.[16] mp 130–130.5°).

Kuhn–Roth Oxidation of Dihydro-des-N-methylapo-β-erythroidine. The dihydro compound (150 mg) was added to a solution of chromium trioxide (5 g) in 2 *N* sulfuric acid (10 ml) and the mixture distilled. Distillation was continued until about 65 ml of distillate had been collected, water being added to the oxidation mixture to maintain the volume in the distillation flask at 10–15 ml. The distillate was titrated to a phenolphthalein end point with 0.1 *N* sodium hydroxide and then evaporated to dryness. Paper chromatography[27] of the residue indicated the presence of sodium acetate and propionate. Separation on silicic acid[17] yielded sodium propionate (9.6 mg) and sodium acetate (20.5 mg). These compounds were assayed by conversion to their α-naphthylamides.[28] A Schmidt reaction was carried out on the sodium acetate with sodium azide and concentrated sulfuric acid as previously described.[23]

Degradation of β-Erythroidine by Route B. β-Erythroidine hydrochloride (3.2 × 10^3 dpm/mmole, 1.826 g) was hydrogenated using the procedure of Boekelheide, *et al.*,[6] yielding tetrahydro-β-erythroidine (1.7 g) as a pale yellow oil. This compound was converted to desazaanhydrooctahydro-β-erythroidinol (**21**) (505 mg) using the method of Boekelheide and Wenzinger.[18]

Ozonolysis of Desazaanhydrooctahydro-β-erythroidinol. The vinyl compound **21** (150 mg) dissolved in ethyl acetate (15 ml) was cooled to 0° and treated with excess ozone. The solvent was removed *in vacuo* and the residual oil stirred with water (20 ml) and powdered zinc (0.3 g) for 5 min on a steam bath. The mixture was then filtered, and the filtrate and residue were washed with chloroform. Evaporation of the dried chloroform extract yielded 3-ethyltetrahydro-4-(2-formyl-5-methoxycyclohexylidene)pyran (**20**) as a colorless oil (109 mg). This aldehyde had an absorption in the infrared at 1720 cm^{-1} and a parent mass peak at 252 in the mass spectrum corresponding to $C_{15}H_{24}O_3$. The aqueous solution from which this aldehyde had been extracted was distilled into an aqueous solution of dimedone affording formaldehyde–dimedone (41 mg) which was purified by sublimation (120°, 0.001 mm) and melted at 192–193°.

Kuhn–Roth Oxidation of the Aldehyde 20. The aldehyde **20** (100 mg) was oxidized with a mixture of chromium trioxide (5 g) and 1 *N* sulfuric acid (15 ml) using the procedure previously described,

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and yielded a mixture of propionic acid (9.5 mg) and acetic acid (11.4 mg).

Administration of DL-Phenylalanine-2-C^{14} to *Erythrina* plants. DL-Phenylalanine-2-C^{14} [29] (6.6 mg, 0.1 mcurie) dissolved in water (5 ml) was fed to five 6-month-old *E. berteroana* plants by means of cotton wicks inserted in the stems. After 14 days the plants (500 g) were worked up as previously described. The resultant crude mixture of α- and β-erythroidines (320 mg) had negligible radioactivity.

(29) Purchased from Calbiochem, Los Angeles, Calif.

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Communications to the Editor

Reactions of Alkoxy Radicals. II. The Absolute Rate Constant for the Combination of *t*-Butoxy Radicals[1]

Sir:

The reaction of *t*-butoxy radicals with organic substances in the liquid phase has received considerable attention in recent years (see, for example, ref 2 and subsequent papers in this series). However, although the relative rates of hydrogen atom abstraction from many compounds have been measured, no absolute rate constants have been directly determined. Walling and co-workers[2] have developed a method for the production of *t*-butoxy radicals by the photolysis of *t*-butyl hypochlorite. We have employed this source of *t*-butoxy radicals to determine the absolute rate of combination of *t*-butoxy radicals at room temperature and to determine the absolute rate of hydrogen atom abstraction from toluene and diphenylmethane by these radicals.

Photolysis of the hypochlorite (BOCl) in the presence of an organic substance RH and in the absence of oxygen leads to a chain reaction which can be represented by the following reaction scheme.[2]

$$\mathrm{BOCl} \xrightarrow{h\nu} \mathrm{BO}\cdot + \mathrm{Cl}\cdot \quad (1)$$

$$\mathrm{BO}\cdot + \mathrm{RH} \longrightarrow \mathrm{BOH} + \mathrm{R}\cdot \quad (2)$$

$$\mathrm{R}\cdot + \mathrm{BOCl} \longrightarrow \mathrm{RCl} + \mathrm{BO}\cdot \quad (3)$$

There are three possible bimolecular chain termination processes, each of which gives a different kinetic expression for the over-all rate of reaction when it dominates termination

Chain Termination		$-\mathrm{d[RH]/d}t$	
$\mathrm{R}\cdot + \mathrm{R}\cdot \longrightarrow$	inactive products	$k_3[\mathrm{BOCl}]^{3/2}(KI/2k_4)^{1/2}$	(4)
$\mathrm{R}\cdot + \mathrm{BO}\cdot \longrightarrow$		$(k_2k_3[\mathrm{RH}])^{1/2}[\mathrm{BOCl}](KI/2k_5)^{1/2}$	(5)
$\mathrm{BO}\cdot + \mathrm{BO}\cdot \longrightarrow$		$k_2[\mathrm{RH}][\mathrm{BOCl}]^{1/2}(KI/2k_6)^{1/2}$	(6)

where I represents the intensity of the light, and $KI[\mathrm{BOCl}]$ is the rate of chain initiation (R_i).

In our experiments, degassed solutions of hypochlorite and toluene in several solvents were photolyzed with light of wavelength 468–550 mμ. The reaction rate was measured by following the temperature rise registered by a thermocouple[3] situated at the center of a 25-ml cylindrical cell. As long as the conditions remained adiabatic (10–20 sec after the start of photolysis) the temperature rise gave a direct measure of the rate. Absolute rate constants were determined by the rotating sector technique. The rates of chain initiation in each mixture of reactants were measured by the induction period method[4] using both phenolic inhibitors[5] and oxygen which also retards the reaction.

An induction period was always observed in the reaction. This was attributed to traces of oxygen that the normal freeze–thaw cycle did not remove from the reactants. The oxygen probably converts R· radicals to peroxy radicals, thus interfering with step 3 of the propagation cycle. For kinetic purposes, all rate measurements (including measurements of the length of the second induction period produced when phenols or oxygen were deliberately added) were made after the rate reached a constant maximum value. The light was switched on for a series of short periods (~30 sec, with time allowed for thermal equilibration between these periods) until the initial rate reached a steady value.

The kinetics of the consumption of toluene in carbon tetrachloride could be represented by

$$(-\mathrm{d[RH]/d}t) \propto [\mathrm{RH}]^{0.92}[\mathrm{BOCl}]^{0.65}I^{0.55}$$

over a range of toluene concentrations from 0.185 to 9.20 *M* and hypochlorite concentrations from 0.165 to 1.65 *M*, and over a 500-fold change in light intensity. These kinetics imply that chain termination is bimolecular and occurs mainly by reaction 6. Walling[6] has studied the steady-state kinetics of this same reaction using thermal initiation, which always gives more reproducible rates than photochemical initiation. He concludes that reaction 6 is the major chain termination process only at [RH]/[BOCl] ratios of 3 or less. At higher ratios reaction 5 and, subsequently, reaction 4 become more important. Our own rate measurements, which are admittedly less accurate, were mostly made at [RH]/[BOCl] ratios in the range 0.1–3.0. Our conclusion

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