



# Pathway of anthracene modification under simulated solar radiation

Ali Mallakin, D. George Dixon, Bruce M. Greenberg \*

*Department of Biology, University of Waterloo, Waterloo, Ont., Canada N2L 3G1*

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## Abstract

Exposure of polycyclic aromatic hydrocarbons (PAHs) to sunlight results in rapid structural photomodification generally via oxidation reactions. These PAH modification products are in many cases more toxic than their parent compounds. In this study, anthracene (ANT), a rapidly photooxidized PAH, was irradiated with simulated solar radiation (SSR,  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in aqueous solution to examine the photomodification pathway. The photoproducts formed were identified by HPLC. The ANT product profile after 9 h in SSR was very complex, with more than 20 compounds detected. The photoproducts formed were anthraquinones, benzoic acids, benzaldehydes and phenols showing the process to be oxidative in nature. Some of the anthraquinones were themselves subject to photooxidation, and were thus intermediates in the product pathway. The kinetics of ANT photooxidation revealed a pseudo first-order reaction with a half-life of 2 h under the SSR source used. The kinetics of product formation allowed deduction of a probable photomodification pathway. This study indicates that PAH photooxidation products are likely to exist as complex, dynamically changing mixtures in PAH contaminated aquatic environments. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* Polycyclic aromatic hydrocarbons; Photomodification; Photooxidation; Sunlight; Toxicity

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of the most prevalent groups of aquatic environmental contaminants (Neff, 1979; Cook et al., 1983). Without an external stimulus, PAHs are extremely stable compounds (Neff, 1979; Krylov et al., 1997), making them a persistent environmental problem (Basu and Saxena, 1978). Due to their extensive  $\pi$ -orbital systems, PAHs absorb sunlight in the visible (400–700 nm) and the ultraviolet regions (290–400 nm) of the solar spectrum (Nikolaou et al., 1984; Huang et al., 1993). Photooxi-

dation of PAHs is a potentially important pathway for PAH modification in the environment (Huang et al., 1993; Katz et al., 1979). Upon absorbing sunlight, a PAH can be rapidly transformed to a variety of compounds, most of which are oxidation products. Compounding the problem, the photooxidation products of PAHs are in many cases as or more toxic than the parent compounds (Krylov et al., 1997; McConkey et al., 1997; Mallakin et al., 1999). Clearly, it is important that the modification pathways of PAHs are well understood. This will indicate which by-products are likely to accumulate in the environment allowing for more realistic analysis of PAH loads.

In this study, anthracene (ANT) was chosen for a detailed investigation of a PAH photomodification pathway. Upon exposure to natural or simulated solar radiation, ANT undergoes rapid photooxidation with

\* Corresponding author. Tel.: +1-519-888-4567.

*E-mail address:* greenber@sciborg.uwaterloo.ca (B.M. Greenberg).

high quantum efficiency (Katz et al., 1979; Krylov et al., 1997). This is because it absorbs strongly in the UV-B (290–320 nm) and UV-A (320–400 nm) spectral regions found in sunlight, and its central ring has low  $\pi$ -orbital stability making it susceptible to attack by  $O_2$  (Mezey et al., 1998). Further, the ANT photomodification pathway results in many toxic products (Mallakin et al., 1999; Huang et al., 1997). Indeed, we recently found that several specific photoproducts of ANT (anthraquinone and hydroxyanthraquinones) to be as toxic as ANT (Mallakin et al., 1999). In this study, we characterized the photooxidation products of ANT, to derive a complete environmentally relevant photomodification pathway based on kinetic data for ANT photooxidation and product formation. It was found that ANT was modified to 9,10-anthraquinone (ATQ) likely via an endoperoxide intermediate. ATQ is then hydroxylated forming hydroxyATQs (hATQs), and ATQ along with some of the hATQs are subject to ring fission to form a complex

mixture of various phenols, benzaldehydes and benzoic acids. This work represents the most complete analysis of a PAH photomodification pathway to date.

## 2. Experimental

### 2.1. Chemicals

The structures of anthracene and the identified photoproducts along with their abbreviations are given in Fig. 1. ANT was purchased in high purity (Sigma Chemical, St. Louis, MO) and used as received. The other compounds were purchased (Aldrich, Milwaukee, WI; or Sigma Chemical, St. Louis, MO) or synthesized as previously described (Mallakin et al., 1999). Solvents employed were HPLC-grade acetonitrile (Aldrich, Milwaukee, WI) and reagent-grade dimethyl sulphoxide (BDH, Toronto, Ontario). Water was purified by reverse

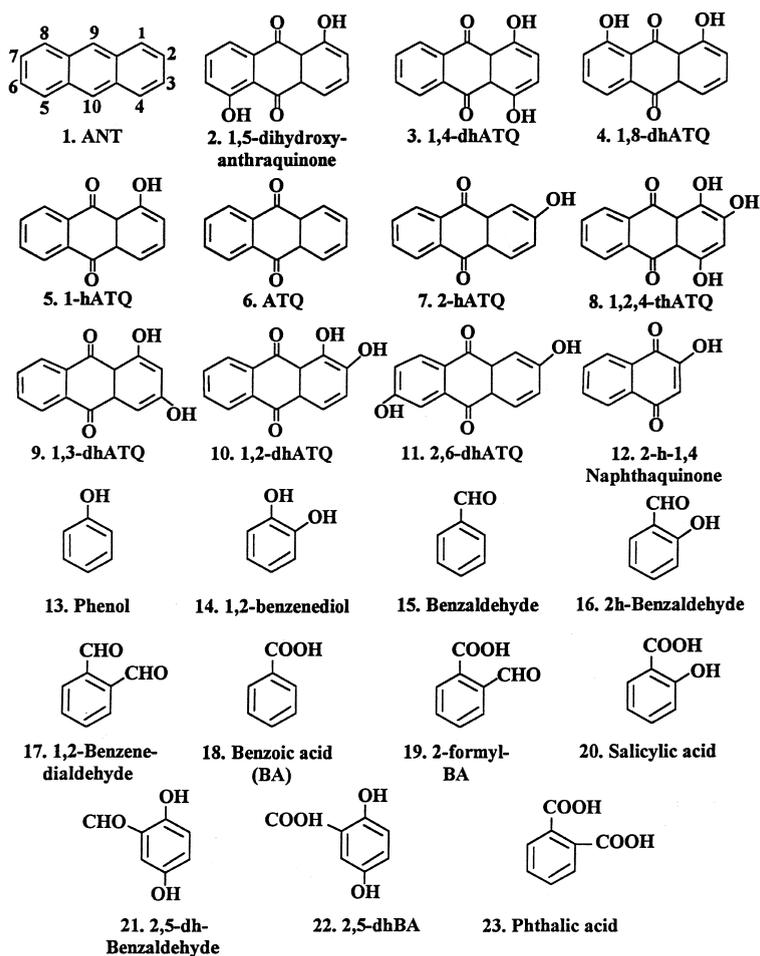


Fig. 1. Products formed from photomodification of ANT in SSR. Compounds are numbered according to the peaks in Fig. 2. Abbreviations, where appropriate, are given.

osmosis (RO) (Barnstead, Dubuque, IO). Reagent-grade phosphoric acid was used to adjust pH.

## 2.2. Photooxidation of ANT

Photooxidation of ANT was carried out in aqueous solution under simulated solar radiation (SSR). ANT was dissolved in DMSO to 5 mg ml<sup>-1</sup>, and 10 µl of this solution was transferred to 10 ml of RO water to give a final concentration of 5 µg ml<sup>-1</sup>. This results in an emulsified solution of ANT. Nonetheless, we have found that the kinetics of photooxidation are rapid and not multi-phasic under these conditions (Krylov et al., 1997; Huang et al., 1993), indicating all of the ANT is fully available for photoreaction. DMSO was used as a delivery solvent because it has been shown not to affect ANT toxicity or ANT photomodification (Huang et al., 1993; Mallakin et al., 1999). The aqueous solution containing ANT was exposed to 100 µmol m<sup>-2</sup> s<sup>-1</sup> of SSR for 10 h. Samples were taken for analysis every hour. The SSR source is described elsewhere (Huang et al., 1993; Mallakin et al., 1999).

## 2.3. Analysis of ANT photooxidation products by HPLC

A high performance liquid chromatograph (HPLC) with diode array detector (Shimadzu, Columbia, MD, USA) was used to examine the purity of intact ANT and detect the products of photooxidation (McConkey et al., 1997; Mallakin et al., 1999). Samples of 400 µl of the aqueous photooxidized solutions were loaded onto a HPLC column using an auto injector (Shimadzu, Columbia, MD). The columns used were a Supelco LC-18 column (25 cm × 4.6 mm ID, 5 µm particle size; Supelco, Mississauga, ON), a Phenomenex Envirosep-PP column (125 × 3.20 mm<sup>2</sup> ID, 120 µm particle size; Phenomenex, Torrance, CA), or a Supelcosil LC-PAH column (25.0 cm × 4.6 mm, 5 µm particle size; Supelco, Mississauga, ON). All gave similar separation profiles. The method for elution of ANT and the photoproducts from the column was an acetonitrile–water gradient: isocratic at 1% acetonitrile for 2 min, increased linearly to 90% acetonitrile over 32 min, and isocratic at 90% acetonitrile for 20 min (McConkey et al., 1997; Mallakin et al., 1999). The pH of the water was adjusted with phosphoric acid to pH = 2.8. The flow rate was 1 ml/min. A diode array detection system allowed simultaneous identification and quantification of ANT photoproducts. Data from the system was collected and evaluated using Shimadzu EZ-chrome software. Identification of photoproducts was achieved via comparison to the retention times and absorbance spectra of authentic material. Identification was considered positive only when both parameters matched.

## 3. Results and discussion

### 3.1. Photomodification of ANT and identification of the photooxidation products

ANT in aqueous solution was exposed to SSR for various lengths of time. The HPLC analysis of the product profile is shown for four time points (Fig. 2). In SSR, ANT was consumed with a concomitant appearance of photoproducts. Initially ATQ and hATQs were formed. After longer time exposures to SSR various benzoic acids, benzaldehydes and phenols derivatives

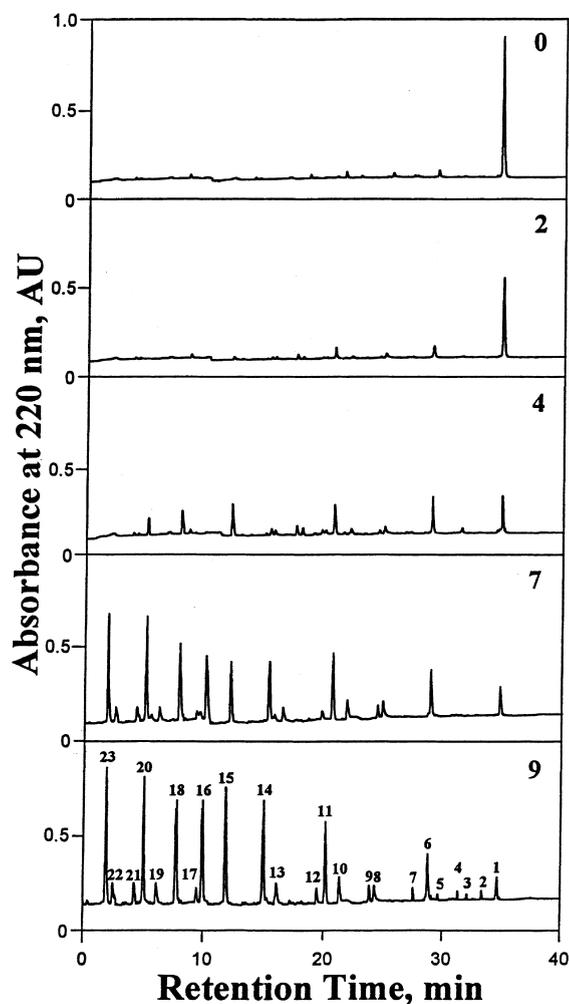


Fig. 2. High-performance liquid chromatography (HPLC) analysis of ANT photooxidation. Photooxidation was carried out in distilled water under 100 µmol m<sup>-2</sup> s<sup>-1</sup> of SSR. Samples were taken for analysis at 0, 2, 4, 7 and 9 h. A 400 µl aliquot of each sample was analyzed by HPLC equipped with a diode array detector. The chromatographs show the relative absorbance at 220 nm. Identified peaks (see Fig. 1) are based on comparison of retention times and spectra of authentic samples.

dominated the product spectrum. After 9 h of light exposure, more than 20 distinct compounds had formed and little ANT remained (Fig. 2). The parent compound and 22 photomodified products were identified by comparison to authentic material (Figs. 1 and 2).

### 3.2. Kinetics of ANT photooxidation

The photooxidation of ANT under SSR was investigated in more detail to assess the kinetics of photooxidation (Fig. 3). The photochemical reaction was carried out under  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  of SSR in distilled water and monitored by HPLC as above. The kinetics of consumption of ANT could be fit to an exponential decay curve (Fig. 3) according to the following equation:

$$[\text{ANT}]_t = [\text{ANT}]_0 e^{-k_p t}, \quad (1)$$

where  $[\text{ANT}]_t$  is the ANT concentration at time  $t$ ,  $[\text{ANT}]_0$  the ANT concentration at time 0 and  $k_p$  is the pseudo first-order rate constant.

The curve fit for exponential loss of ANT gave a  $k_p$  of  $0.314 \text{ h}^{-1}$  ( $r^2 = 0.96$  and  $P < 0.05$ ).

The first detectable product, ATQ, appeared after a 2 h lag time (Fig. 3). During this period approximately 50% of the ANT was consumed. This indicates there was an undetected intermediate in the ANT to ATQ photooxidation reaction. The ATQ concentration reached a maximum level after about 6 h in SSR, at which point it started to be depleted. As ATQ was depleted hATQs, benzoic acids, benzaldehydes and phenols started to form. Some of the hATQs (e.g. 2,6-dhATQ) were

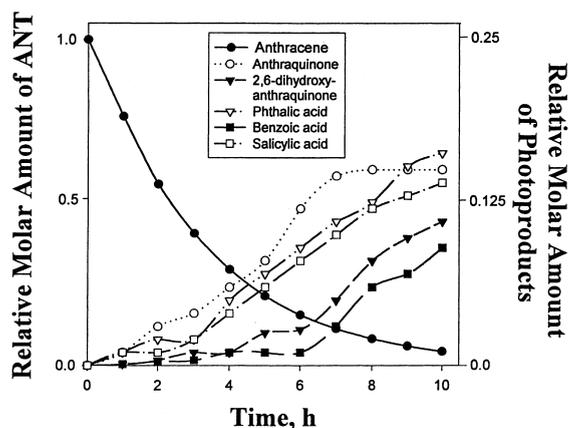


Fig. 3. Kinetics of ANT photooxidation due to exposure to  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  of SSR. HPLC analysis was as in Fig. 2. The amount of intact ANT remaining and production of ATQ, 2,6-dhATQ, phthalic acid, Salicylic acid and benzoic acid were quantified by HPLC and plotted against time. Loss of ANT was fit to an exponential decay function ( $r^2 = 0.96$ ;  $P < 0.05$ ).  $[\text{ANT}]_t$  at  $t_0$  was:  $28.05 \mu\text{mol}$ . The relative molar amount of the photoproducts are based on the data in Table 1.

formed after the single ring aromatics started to appear (e.g. phthalic acid) (Fig. 3). This indicates that ATQ can be cleaved directly to single ring compounds. As well, some of the hATQs are photolabile (Table 1), and are therefore also sources of single ring aromatics. Interestingly, other hATQs are photostable (e.g. 2-hATQ) and some of these were found to be toxic (Mallakin et al., 1999). Thus, these products are specifically expected to accumulate in the environment and would represent a potential hazard. Indeed, in recent work some ANT photoproducts were detected at significant levels in PAH contaminated sediments (Greenberg et al., unpublished observations).

### 3.3. Pathway of ANT photomodification

Based on the kinetics for photomodification of ANT and product formation, a photomodification pathway can be proposed (Fig. 4). ANT is converted to ATQ via an intermediate. The most likely intermediate in the ANT to ATQ conversion is the 9,10-endoperoxide of ANT. This molecule would subsequently be oxidized to ATQ with the concomitant generation of hydrogen peroxide (Fig. 4). ATQ is then hydroxylated or its central ring is cleaved (Fig. 4). We assume ATQ can form one-ring aromatics directly as they form with a time frame similar to or faster than hATQ formation (see Figs. 2 and 3). Three of the hATQs (1,2-dhATQ, 1,4-dhATQ and 1,2,4-thATQ) also are subject to ring fission forming benzoic acids, benzaldehydes and phenols. The single ring aromatics and some of the hATQs products are photochemically stable and can therefore be classified as end-products.

Table 1

Representatives ANT photooxidation products quantified by calibration to external standards. Photomodification was performed under SSR for 9 h. Molar yields ratio and standard error of triplicate samples are presented

Compound	Yield <sup>a</sup>	$t_{1/2}$ , h in SSR
ANT	$0.006 \pm 0.008$	2
ATQ	$0.146 \pm 0.018$	1.8
1-hATQ	$0.018 \pm 0.002$	EP <sup>b</sup>
2-hATQ	$0.062 \pm 0.003$	EP
1,2-dhATQ	$0.027 \pm 0.002$	3.0
1,4-dhATQ	$0.077 \pm 0.006$	6.0
1,5-dhATQ	$0.010 \pm 0.001$	EP
1,8-dhATQ	$0.080 \pm 0.018$	EP
2,6-dhATQ	$0.105 \pm 0.015$	EP
Phthalic acid	$0.148 \pm 0.006$	EP
Salicylic acid	$0.140 \pm 0.025$	EP
Benzoic acid	$0.090 \pm 0.020$	EP

<sup>a</sup>  $\mu\text{mol}$  of compound at ( $t = 9$ ) h in SSR.

<sup>b</sup> End products.

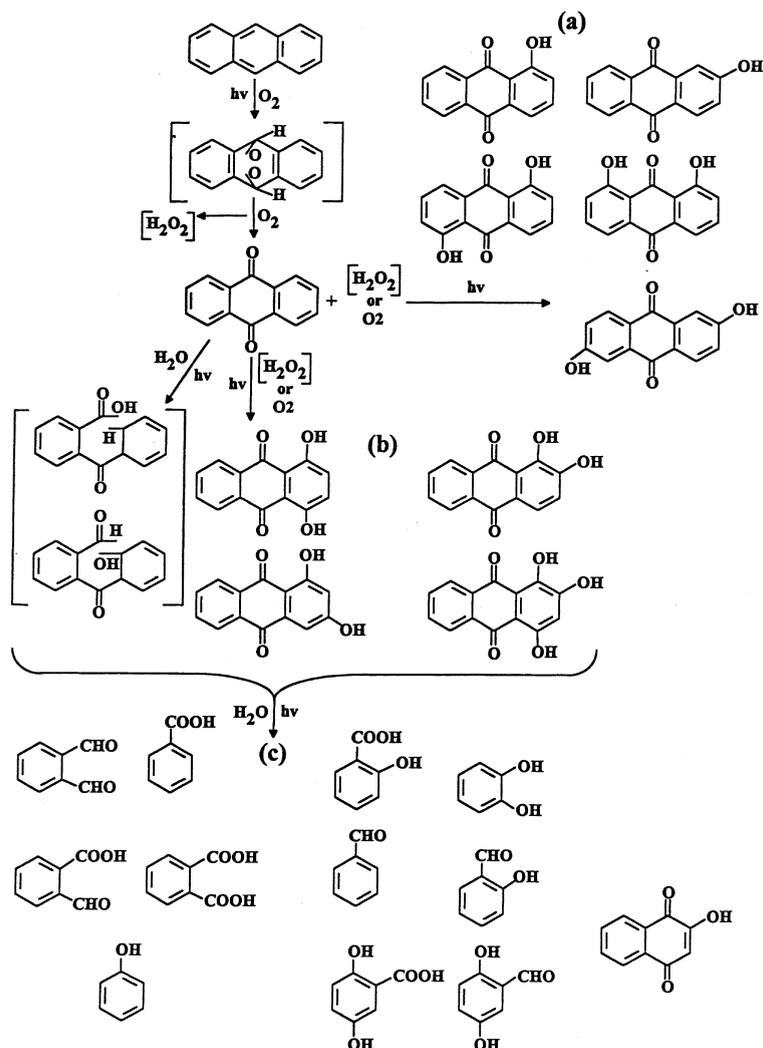


Fig. 4. Proposed pathway of anthracene photooxidation in simulated solar radiation. The generalized mechanism is based on the identified products and kinetics of their appearance. The brackets indicate unidentified intermediates. (a) Photostable hATQs. (b) Photolabile hATQs. (c) One ring aromatic end products.

#### 4. Conclusions

In this study, we have shown that ANT was oxidized to ATQ, hATQs, benzoic acid, benzaldehyde and phenols in the presence of SSR. Photooxidation of PAHs and product formation have been described in previous studies (Katz et al., 1979; McConkey et al., 1997; Mallakin et al., 1999; Ren et al., 1997). This study is consistent with previous work which demonstrated that PAHs can be photomodified via oxidation reactions (Katz et al., 1979; Huang et al., 1997; McConkey et al., 1997; Ren et al., 1997). However, this research greatly expanded on the number of identified products and presents a full photooxidation pathway for the first time. Many of the photoproducts identified are intermediates

and subject to further photooxidation. The action of light on PAHs can also result in the formation of active oxygen species including hydrogen peroxide, singlet oxygen, superoxide ion and organo-peroxy radicals (Larson and Berenbaum, 1988). In particular, based on the proposed photomodification pathway of ANT, hydrogen peroxide is a likely by-product. Interestingly, hydrogen peroxide has been shown to be at higher levels in aquatic environments exposed to sunlight (Scully et al., 1995), and our work shows one way it can be formed.

In solution, there are two possible routes of photomodification of PAHs; type II and type I photosensitization reactions (Foote, 1979). In type II photosensitization, after absorption of a photon, ground-state

ANT goes to the excited triplet-state ( $^3\text{ANT}$ ) via inter-system crossing from the excited singlet-state  $^1\text{ANT}$ . The experimental solution was open to the air, so the main quencher of  $^3\text{ANT}$  would be ground triplet-state oxygen  $^3\text{O}_2$ . This is because the concentration of  $^3\text{ANT}$  would be low in the aqueous medium relative to  $^3\text{O}_2$  concentrations (0.25 mM) (Krylov et al., 1997; Robinson and Cooper, 1970). This means the rate of reaction of  $^3\text{ANT}$  with  $^3\text{O}_2$  should be diffusion limited with an average interval of less than  $10^{-6}$  s between collisions (Krylov et al., 1997). Because the ANT excited triplet state is relatively long lived ( $10^{-6}$ – $10^{-3}$  s), the majority of the excited  $^3\text{ANT}$  should be quenched by oxygen, generating singlet-state oxygen  $^1\text{O}_2$  (Lakowics, 1983). These singlet  $^1\text{O}_2$  molecules could then react with ANT forming the endo-peroxide. In type I photosensitization reactions, as in type II reactions, after absorbance of a photon, ANT goes to  $^3\text{ANT}$  via intersystem crossing from the excited singlet state. The  $^3\text{ANT}$  does not react with  $^3\text{O}_2$ , but instead with another PAH or solvent to generate a PAH free radical or solvent free radical. These PAH radicals would also be reactive with  $\text{O}_2$ , forming oxidation products. Assuming the 9,10-endoperoxide of ANT is the initial oxidation reaction, a type II photosensitization mechanism is more likely.

Our studies show that the initial  $^1\text{O}_2$  attack is exclusively at the 9,10 position in the central ring of ANT. The electron density of this ANT central ring is very low (Mezey et al., 1998), explaining why the initial reaction is so specific to this position. This low electron density of the central ANT ring is also a key factor in the very rapid rate of photooxidation.

The initial ANT concentration (5  $\mu\text{g}/\text{ml}$ ) in water used in this study is well above its solubility limit. This was necessary so we would have sufficient material for analysis. Interestingly, the ANT did not precipitate, but rather formed an emulsified solution. Further, the kinetics of photooxidation were pseudo first-order, mono-phasic and rapid. From this we conclude that PAHs do not have to be fully solubilized to be subject to photomodification. This has environmental implications, as PAHs in aquatic environments are often bound to suspended particulate matter. Our data suggest that under these conditions photomodification can occur.

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