

## Model Studies on the Photosensitized Isomerization of Bixin

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The photosensitized isomerization reaction of the natural *cis* carotenoid bixin (methyl hydrogen 9'-*cis*-6, 6'-diapocarotene-6, 6'-dioate) with rose bengal or methylene blue as the sensitizer in acetonitrile/methanol (1:1) solution was studied using UV-vis spectroscopy, high-performance liquid chromatography (HPLC), and time-resolved spectroscopic techniques, such as laser-flash photolysis and singlet oxygen phosphorescence detection. In both N<sub>2</sub>- and air-saturated solutions, the main product formed was all-*trans*-bixin. The observed isomerization rate constants,  $k_{obs}$ , decreased in the presence of air or with increase in the bixin concentration, suggesting the participation of the excited triplet state of bixin, <sup>3</sup>Bix\*, as precursor of the *cis*→*trans* process. On the other hand, bixin solutions in the absence of sensitizer and/or light did not degrade, indicating that the ground state of bixin is stable to thermal isomerization at room temperature. Time-resolved spectroscopic experiments confirmed the formation of the excited triplet state of bixin and its deactivation by ground state bixin and molecular oxygen quenching processes. The primary isomerization products only degraded in the presence of air and under prolonged illumination conditions, probably due to the formation of oxidation products by reaction with singlet molecular oxygen. An energy-transfer mechanism was used to explain the observed results for the bixin transformations, and the consequences for food color are discussed.

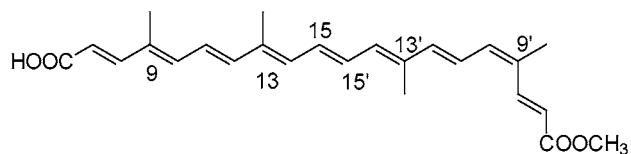
**KEYWORDS:** Bixin; photoisomerization; photosensitization; singlet oxygen and triplet states

### INTRODUCTION

Bixin (methyl hydrogen 9'-*cis*-6, 6'-diapocarotene-6, 6'-dioate, **Scheme 1**) is the main carotenoid existing in the seed coat of the annatto tree (*Bixa orellana* L.), which is found in tropical regions of South and Central America, Africa, and Asia (1, 2). Annatto extracts are widely used in the food industry as natural coloring agents in cheese, ice cream, yoghurt, sausages, margarine, snacks, and dressings, and compared to natural colors, are relatively inexpensive, thus increasing their commercial relevance (1, 2).

The characterization of the coloring components of the annatto extracts (3–5), annatto seeds (6–9), and their degradation products (10, 11) has received much attention. In particular, the stability of bixin was evaluated under the effect of several factors, such as light, oxygen concentration, temperature, and solvent (12, 13). Bixin showed great stability in the dark, in the presence or absence of oxygen, but under direct illumination was degraded (12). The thermo- and photostability of the 9'-*cis* double bond in methyl bixin was reported as being so great that the molecule underwent a second *trans*→*cis* isomerization

Scheme 1. Chemical Structure of the Natural *cis*-Carotenoid Bixin



giving considerable amounts of the 9, 9'-*di-cis* isomer, but in the presence of iodine and light, 9'-*cis*→*trans* isomerization occurred (14). Therefore, it seems that the fade of bixin could be strongly dependent on the experimental conditions (e.g., direct illumination, heating, photosensitization, etc).

Most studies on bixin stability focused on thermal degradation (10, 11), direct illumination (12), and the water activity solvent effect (13). In the thermal degradation of bixin, the main products formed were all-*trans*-bixin, a C17 carotenoid (*trans*-monomethyl ester of 4,8-dimethyl tetradecahexaenedioic acid) and volatile compounds, especially xylene (5, 10).

However, to our knowledge, there is little or no information about the photosensitized degradation of bixin either in model systems or in foods. Moreover, light can dramatically reduce food stability due to the promotion of autoxidation and photosensitized oxidation, the latter being more common, because many natural sensitizers are commonly found in foods.

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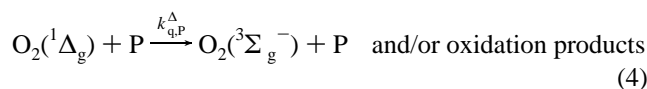
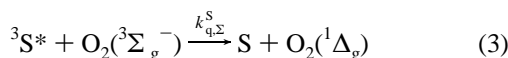
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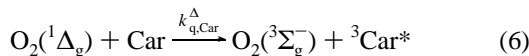
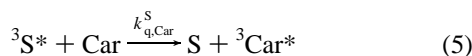
In photosensitized reactions, photons are absorbed by one type of molecule (known as a sensitizer, S), resulting in a long-lived energy-rich state(s) (typically triplet states) of the sensitizer, eq 1, which can undergo reactions producing chemical alteration of another molecule (substrate, P) in the system, eq 2 (15).



In its primary reactions, the excited sensitizer molecule can also react directly with some other molecule (frequently molecular oxygen  $O_2({}^3\Sigma_g^-)$ ) in the reaction mixture, giving products (usually singlet molecular oxygen,  $O_2({}^1\Delta_g)$ ) that in turn react with the substrate, eqs 3 and 4 (15, 16).



On the other hand, it is well-known that carotenoids are efficient quenchers of both excited triplet states and  $O_2({}^1\Delta_g)$ , principally by an energy-transfer mechanism producing the ground state of the precursor species and the carotenoid triplet state, as occurs in the photosynthetic organisms, eqs 5 and 6 (17).

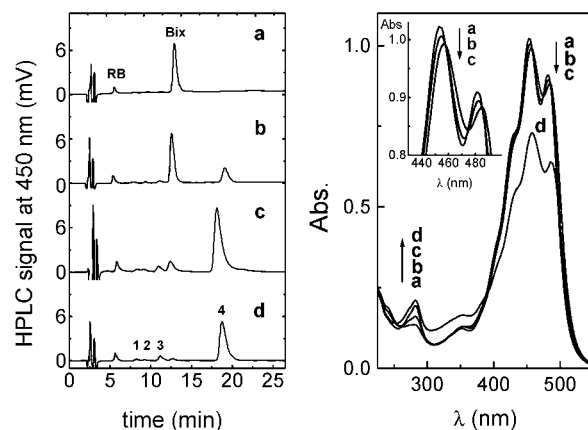


Once formed, the carotenoid triplet state returns harmlessly to the ground state with the liberation of heat. In general, the reactive quenching reaction between  $O_2({}^1\Delta_g)$  and the carotenoids yielding oxidation products is several orders of magnitude smaller than the physical quenching process, eq 6. The bleaching of all-*trans*-carotenoids by  $O_2({}^1\Delta_g)$ -mediated oxidation is commonly observed after prolonged photosensitization periods (18). However, for *cis* carotenoids, the photosensitized process produces the *trans* isomer (19, 20). Therefore, for bixin contained in foods and beverages, a similar photosensitized behavior can be expected, because in foods, several natural or artificial pigments (e.g., riboflavin, chlorophyll, and organic dyes) act as sensitizers (21).

The aim of this work was to present a kinetic study of the photosensitized transformation of bixin in an organic solvent mixture, using UV-vis spectroscopy, high-performance liquid chromatography (HPLC) and time-resolved spectroscopic techniques, such as laser-flash photolysis and  $O_2({}^1\Delta_g)$ -phosphorescence detection.

## EXPERIMENTAL PROCEDURES

**Materials.** Bixin (99% purity) was kindly donated by Dr. Werner Simon from Roche Vitamins (Basel, Switzerland). Rose bengal (RB) as its sodium salt and methylene blue (MB) were from Sigma Chemical Co. (St. Louis, MO) and used without further purification. 9, 10-Dimethylanthracene (DMA) from Aldrich (Milwaukee, WI) was recrystallized from ethanol before use. All the organic solvents used were HPLC grade from Mallinckrodt, and the distilled water was purified by the Milli-Q Plus system (Millipore).



**Figure 1.** HPLC chromatograms obtained with the  $C_{18}$ -Vydac 218TP54 column and monitored at 450 nm (left) and UV-vis spectra (right) of 8  $\mu$ M bixin in air-saturated ACN/MeOH (1:1) solutions at 20 °C in the presence of 10  $\mu$ M Rose bengal after different times of illumination of the sensitizer: (a) 0 min, (b) 2 min, (c) 10 min, (d) 150 min.

**Methods.** Stationary UV-vis spectra were obtained either with a Hewlett-Packard 8453 diode array spectrophotometer or a Beckman DU-640 spectrophotometer. HPLC analysis was carried out using a Waters HPLC system equipped with a photodiode array detector (Waters, model 996). The equipment also included an on-line degasser, a Rheodyne injection valve with a 20- $\mu$ L loop and an external oven. Data acquisition and processing were performed using the Millennium Waters software. The carotenoids were separated on a  $C_{18}$  Spherisorb ODS-2 column, 150  $\times$  4.6 mm i.d. (3- $\mu$ m particle size), with acetonitrile/2% v/v acetic acid/dichloromethane (63:35:2) as mobile phase at a flow rate of 1 mL/min, with the column temperature being set at 25 °C. The chromatograms were processed at max plot ( $\lambda_{max}$ ), and the spectra were recorded between 250 and 600 nm. The second HPLC system used was a Konik 500 A chromatograph coupled to a UV-vis detector Konik UV-vis 200, monitored at 450 nm. In this case, the column was a  $C_{18}$  Vydac 218TP54 (5- $\mu$ m, 4.6  $\times$  250-mm), and the mobile phase and flow conditions were identical to those described above. All samples and solvents were filtered before use, using 0.22  $\mu$ m and 0.45  $\mu$ m membranes, respectively.

The photosensitization experiments were performed in acetonitrile/methanol (1:1) solutions of bixin containing ca. 10  $\mu$ M RB or MB, illuminated with a 150 W filament lamp coupled to an orange cutoff filter to exclusively excite the band of the sensitizer above 520 nm. The reaction was monitored by HPLC at 5, 15, and 20 °C under  $N_2$  (99.99% purity) and in air-saturated solutions.

Laser-flash photolysis (LFP) experiments and the transient spectra were obtained with a homemade system previously described by Borsarelli et al. (22), using a Q-switched Nd:YAG laser (Spectron SL400) operating at the frequency-doubled output (532 nm, 20 ns halfwidth) as excitation source. The transient spectra were performed in both  $N_2$ - and air-saturated solutions using fresh solution after a few laser shots.

Singlet molecular oxygen  $O_2({}^1\Delta_g)$  sensitization in air-saturated solutions was measured using time-resolved detection of the phosphorescence of  $O_2({}^1\Delta_g)$  at 1270 nm, using the same laser as excitation source, as described previously (18).

In all cases, the experiments were performed in duplicate.

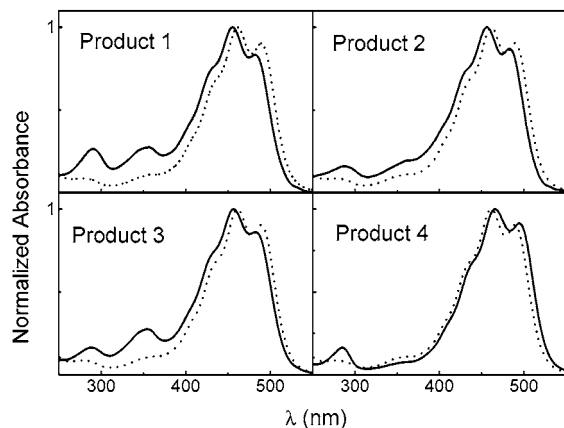
## RESULTS AND DISCUSSION

**Figure 1** shows the HPLC chromatograms and the UV-vis spectra of 8  $\mu$ M bixin in air-saturated solutions at 20 °C, observed after different times of illumination of the sensitizer RB. It can be observed that whereas the bixin peak was almost completely consumed after 10 min of photosensitization, slight changes in the UV-vis spectra were produced (**Figure 1**). In addition, the consumption of bixin was accompanied by the simultaneous formation of new peaks (labeled as products 1, 2,

**Table 1.** Retention Times  $t_R$ , Absorption Maximum Wavelengths  $\lambda_{\max}$ , and Band Ratios %III/II and %A<sub>B</sub>/A<sub>II</sub> Obtained from HPLC – Photodiode Array Analysis (PDA) during the Photosensitized Isomerization of Bixin in Aerated and Non-aerated ACN/MeOH (1:1) Solutions

peak label <sup>a</sup>	$t_R^b$ (min)	$t_R^c$ (min)	$\lambda_{\max}^d$ (nm)	% III/II	% A <sub>B</sub> /A <sub>II</sub>
1	8.0 ± 0.5	11.8 ± 0.2	290, 352, 420 (sh), 455, 482	10	12
2	9.3 ± 0.3	12.3 ± 0.2	285, 420 (sh), 457, 483	28	10
3	11.0 ± 0.9	13.1 ± 0.1	285, 354, 422 (sh), 459, 483	21	18
Bix	12.7 ± 1.0	13.3 ± 0.1	355, 425 (sh), 461, 489	39	5
4	19.2 ± 1.4	13.7 ± 0.2	285, 430 (sh), 466, 495	40	0

<sup>a</sup> Labeled according to the chromatogram from **Figure 1**. <sup>b</sup> C<sub>18</sub>–Vydac 218TP54 column (average value from 10 chromatographic runs). <sup>c</sup> C<sub>18</sub>–Spherisorb ODS-2 column (average value from 60 chromatographic runs). <sup>d</sup> (sh) = shoulder.

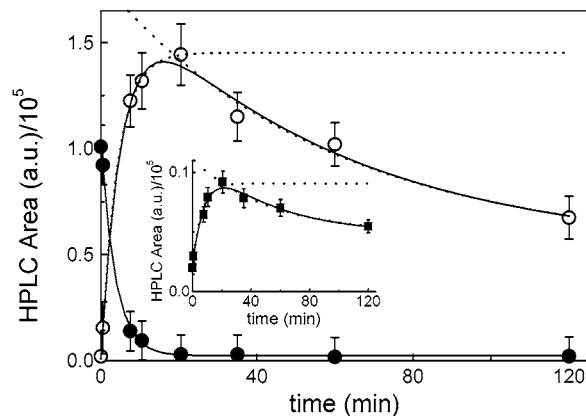


**Figure 2.** Normalized UV–vis spectra obtained from HPLC–PDA analysis of the photosensitized degradation products of bixin in ACN/MeOH (1:1) solutions. The dotted spectra correspond to the bixin spectrum.

3, and 4). The same HPLC pattern was observed in photosensitized experiments in N<sub>2</sub>-saturated solutions and with higher bixin concentrations. In all cases, peak 4 was the main product formed, with a greater retention time ( $t_R$ ) than bixin, whereas the minor products 1–3 presented shorter  $t_R$  values than bixin, **Table 1**.

In all experiments, the sensitizer RB was not degraded as confirmed by HPLC (**Figure 1**) and/or UV–vis spectra (data not shown). Moreover, no consumption of bixin was observed under dark conditions in the absence or presence of the sensitizer, indicating that bixin did not form products from the thermal degradation of the carotenoid and/or from direct reaction with the sensitizer.

**Figure 2** shows the normalized UV–vis absorption spectra of products 1–4 obtained from the photodiode array detection (PDA) of the HPLC peaks, and compared to that of bixin (dotted line spectrum). **Table 1** presents the %III/II ratio, calculated as the values of the height ratio between the longest and middle wavelength absorption bands (designated as III and II, respectively) by taking as the baseline the valley between both bands and of the relative intensity of the cis band (%A<sub>B</sub>/A<sub>II</sub>), calculated as the ratio between the height of the cis band (ca. 360 nm for bixin) and the band II. These are known to be useful parameters for the spectral characterization of carotenoids (23).



**Figure 3.** Kinetic curves obtained from the HPLC peak analysis of 8  $\mu$ M bixin in air-saturated ACN/MeOH (1:1) solutions in the presence of 10  $\mu$ M Rose bengal: (●) Bixin, (○) all-*trans*-bixin, and (■) isomer 3. The solid and dotted lines represent the exponential fitting of the experimental data (see text for details).

**Table 2.** Observed Rate Constant  $k_{\text{obs}}$  and Activation Parameters  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  for the Photosensitized Isomerization of Bixin in ACN/MeOH (1:1) Solutions

system	temp (deg C)	[Bix] ( $\mu$ M)	$k_{\text{obs}} \times 10^4$ (s <sup>-1</sup> )	$\Delta H^\ddagger$ (kcal/mol)	$\Delta S^\ddagger$ (cal/mol · K)
air saturated	5	76	2.3	5 ± 1	-59 ± 11
	15	76	3.0		
	20	76	3.7		
	20	8	42		
N <sub>2</sub> saturated	5	76	21	6 ± 2	-50 ± 30
	15	76	26		
	20	76	40		

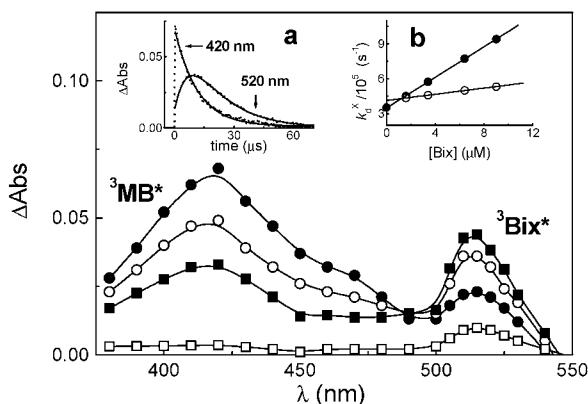
As compared with data from the literature, the observed spectral features strongly suggested that products 1–4 were geometrical isomers of bixin. For instance, the slight blue shift of the  $\lambda_{\max}$ , together with the increase in %A<sub>B</sub>/A<sub>II</sub> and decrease in %III/II for the minor products 1–3 indicated the formation of different *cis*-isomers of bixin (23). Although the UV–vis absorption spectra parameters reported in **Table 1** are very useful for structure characterization, they are not conclusive evidence for the assignment of the position of the *cis* double bonds in products 1–3. However, this is not the case for the major product 4, because the bathochromic shift, together with the total disappearance of the *cis*-band absorption and increased spectral fine structure, indicated the quantitative *cis*→*trans* conversion at the 9'-*cis* double bond of bixin, producing the all-*trans* form (14, 23). In addition, the  $\lambda_{\max}$  values of product 4 were similar to those reported for all-*trans*-norbixin in a similar solvent mixture (3) supporting, in this case, the assignment of product 4 to all-*trans*-bixin, with the present UV–vis data. By comparison of the HPLC peak areas, it can be concluded that bixin is almost completely transformed to all-*trans*-bixin (> 90%) and to a much less extension to the *cis*-isomers 1–3.

**Figure 3** shows the kinetic curves, obtained from the HPLC analysis, of bixin and products 3 and 4 (all-*trans*-bixin) as a function of the photosensitization time. In all cases, the disappearance of bixin followed an irreversible 1st-order kinetic law, with a similar observed rate constant  $k_{\text{obs}}$  to those for the formation of the product isomers 3 and 4 (**Table 2**). However, the formation of both bixin isomers was followed by slower 1st-order consumption only in air-saturated solutions. The solid lines in **Figure 3** represent the 1st-order fits for the decay of bixin, and the 1st-order growth and decay of the isomers 3 and

**Table 3.** Rate Constant Values for the Deactivation Processes Indicated in **Scheme 2**

process	rate constant
${}^3\text{S}^* \rightarrow \text{S}$	$k_{d,0}^{\text{S}} = (1.7 \times 10^4 \text{ s}^{-1})^a; (2.9 \times 10^4 \text{ s}^{-1})^b$
${}^3\text{S}^* + \text{Bix} \rightarrow \text{S} + {}^3\text{Bix}^*$	$k_{q,\text{Bix}}^{\text{S}} = (8 \times 10^9 \text{ M}^{-1}\text{s}^{-1})^a; (7 \times 10^9 \text{ M}^{-1}\text{s}^{-1})^b$
${}^3\text{S}^* + \Sigma \rightarrow \text{S} + \Delta$	$k_{q,\Sigma}^{\text{S}} = (1 \times 10^9 \text{ M}^{-1}\text{s}^{-1})^a; (2 \times 10^9 \text{ M}^{-1}\text{s}^{-1})^b$
$\Delta \rightarrow \Sigma$	$k_{d,0}^{\Delta} = 6.7 \times 10^4 \text{ s}^{-1}$
$\Delta + \text{Bix} \rightarrow \Sigma + {}^3\text{Bix}^*$	$k_{q,\text{Bix}}^{\Delta} = 1.3 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$
${}^3\text{Bix}^* \rightarrow \text{Bix}$	$k_{d,0}^{\text{Bix}} = 4.2 \times 10^4 \text{ s}^{-1}$
${}^3\text{Bix}^* + \text{Bix} \rightarrow 2\text{Bix}$	$k_{q,\text{Bix}}^{\text{Bix}} = 1.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$
${}^3\text{Bix}^* + \Sigma \rightarrow \text{Bix} + \Sigma$	$k_{q,\Sigma}^{\text{Bix}} = 7.0 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$
$\Delta + \text{Isomer products} \rightarrow \text{Oxidation products}$	$k_r^{\Delta} = 1.0 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$

<sup>a</sup> S = RB. <sup>b</sup> S = MB;  $\Sigma = \text{O}_2({}^3\Sigma_g^-)$ ;  $\Delta = \text{O}_2({}^1\Delta_g)$ .



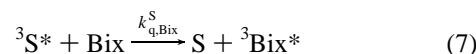
**Figure 4.** Transient absorption spectra of 10  $\mu\text{M}$  methylene blue (MB) in the presence of 9  $\mu\text{M}$  bixin in  $\text{N}_2$  saturated ACN/MeOH (1:1) solutions observed at different times after the 532 nm laser pulse: (●) 1  $\mu\text{s}$ , (○) 4  $\mu\text{s}$ , (■) 8  $\mu\text{s}$ , and (□) 40  $\mu\text{s}$ . Insets: (a) Decay traces at 420 and 520 nm, with the exponential and biexponential fittings (solid lines); (b) Dependence of the observed decay constant  $k_d^X$  for  ${}^3\text{MB}^*$  (●) and  ${}^3\text{Bix}^*$  (○), with the bixin concentration, eq 9.

4, fitted with a biexponential function derived from the sum of two consecutive 1st-order processes (dotted lines). **Table 2** also shows that the  $k_{\text{obs}}$  decreased with increase in bixin concentration and in the presence of air.

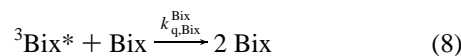
The temperature effect on  $k_{\text{obs}}$  was also evaluated according to the activated complex theory (24), allowing for the calculation of the activation parameters  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$ , **Table 2**. The activation parameters were very similar in both  $\text{N}_2$ - and air-saturated solutions, suggesting that the same degradation mechanism operated under both conditions.

To explore the participation of transient excited species in the photosensitized isomerization of bixin, laser-flash photolysis experiments were performed, using laser pulsed excitation at 532 nm. **Figure 4** shows the transient absorption spectra observed after laser excitation of the sensitizer MB in  $\text{N}_2$ -saturated solutions in the presence of 9  $\mu\text{M}$  bixin. The transient absorption due to the excited triplet state of the sensitizer,  ${}^3\text{MB}^*$ , with a maximum at 420 nm (25) decayed with the simultaneous build-up of an absorption band at ca. 520 nm, as confirmed by the kinetic curves in the inset **a** of **Figure 4**. After 40  $\mu\text{s}$  of the laser pulse, almost 95% of the  ${}^3\text{MB}^*$  state was depleted, and the residual transient spectrum was similar to those observed for the triplet state of carotenoids such as  $\beta$ -carotene or zeaxanthin (26, 27). The 1st-order kinetic analysis (solid lines in the inset **a**) of the decay at 420 nm and the growth at 520 nm, afforded similar rate constant values as a function of the bixin concentration, confirming that the decay of the sensitizer

(MB) triplet state  ${}^3\text{S}^*$  was kinetically associated with the formation of the  ${}^3\text{Bix}^*$ , via a bimolecular energy-transfer quenching pathway (17, 19), eq 7



where  $k_{q,\text{Bix}}^{\text{S}}$  is the energy-transfer quenching rate constant of  ${}^3\text{S}^*$  by bixin. In addition, the decay portion of the transient curve at 520 nm represents the actual decay of  ${}^3\text{Bix}^*$ , which is also dependent on the bixin concentration. Because no laser intensity effect was observed, the triplet-triplet annihilation process was ruled out. On the basis of this fact, the shortening of the decay time of  ${}^3\text{Bix}^*$  with increases in the carotenoid concentration was due to a self-quenching process (28) with a rate constant  $k_{q,\text{Bix}}^{\text{Bix}}$ , eq 8.



The dependence of the unimolecular decay rate constants  $k_d^X$  of the species X (with X =  ${}^3\text{S}^*$  or  ${}^3\text{Bix}^*$ ) showed a linear relationship with the concentration of the carotenoid, eq 9, as shown in inset **b** of **Figure 4**.

$$k_d^X = k_{d,0}^X + k_{q,\text{Bix}}^X [\text{Bix}] \quad (9)$$

where  $k_{d,0}^X$  represents the unimolecular decay rate constant of the species X in the absence of bixin. The quenching rate constants of  ${}^3\text{S}^*$  and  ${}^3\text{Bix}^*$  by bixin,  $k_{q,\text{Bix}}^{\text{S}}$  and  $k_{q,\text{Bix}}^{\text{Bix}}$ , respectively, are reported in **Table 3**. These values are equivalent to those of quenching processes almost controlled by diffusion. The natural lifetime of  ${}^3\text{Bix}^*$ ,  $(k_{d,0}^{\text{Bix}})^{-1} = 24 \mu\text{s}$  was calculated from the intercept value of the plot for the self-quenching process, with the value being in good agreement with recently reported data for several carotenoids with different numbers of conjugated double bonds (28).

On the other hand, in air-saturated solutions, the  ${}^3\text{S}^*$  was efficiently quenched by ground-state molecular oxygen  $\text{O}_2({}^3\Sigma_g^-)$ , eq 3, with  $k_{q,\Sigma}^{\text{S}} \approx 2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$  (**Table 3**). At 20  $^\circ\text{C}$ , the concentration of dissolved molecular oxygen in air-saturated methanol or acetonitrile was ca. 2 mM (25). Therefore, under these conditions,  ${}^3\text{S}^*$  was efficiently quenched by  $\text{O}_2({}^3\Sigma_g^-)$  producing  $\text{O}_2({}^1\Delta_g)$  and the ground state of the sensitizer, eq 3.

The formation of  $\text{O}_2({}^1\Delta_g)$  was followed by monitoring the phosphorescence decay of  $\text{O}_2({}^1\Delta_g)$  at 1270 nm (**Figure 5**). The tail part of the  $\text{O}_2({}^1\Delta_g)$  decays was fitted with an exponential function extrapolating to the same initial intensity value, due to the signal distortion by the residual luminescence of the

Scheme 2. Reaction Mechanism Proposed for the Photosensitized Isomerization of Bixin in Both Nonaerated and Aerated Solutions

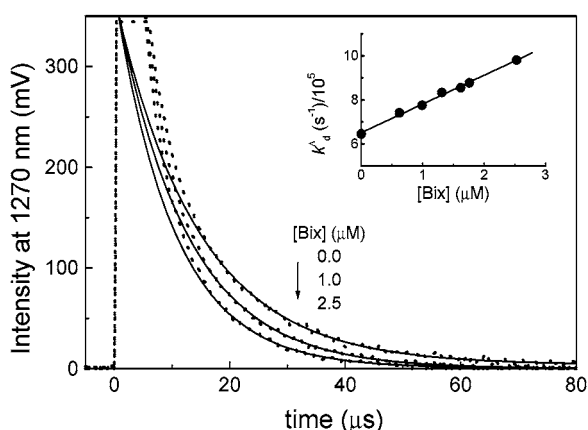
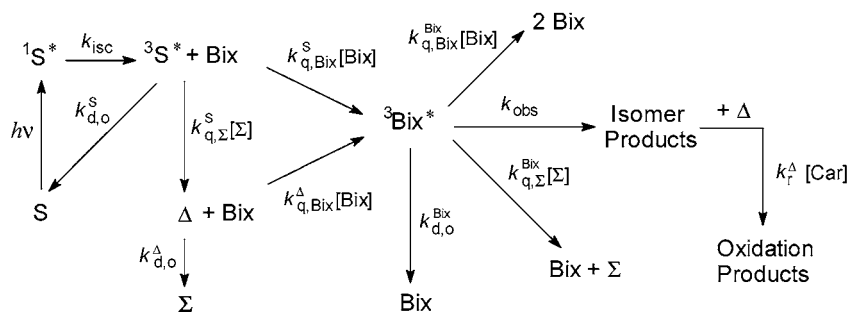
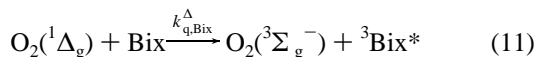


Figure 5. Effect of the bixin concentration on the phosphorescence decay of singlet molecular oxygen  $O_2(^1\Delta_g)$ . Inset: Dependence of the observed decay constant  $k_d^\Delta$  with the bixin concentration, eq 10.

sensitizer and the rise time of the signal response of the germanium detector ( $<5 \mu s$ ). In the absence of bixin,  $O_2(^1\Delta_g)$  decayed with a lifetime of  $15 \mu s$ , in good agreement with the lifetime expected for binary mixtures of similar polar organic solvents (16, 29). The addition of bixin reduced the observed decay rate constant of  $O_2(^1\Delta_g)$ ,  $k_d^\Delta$ , following eq 10.

$$k_d^\Delta = k_{d,o}^\Delta + k_{q,Bix}^\Delta [Bix] \quad (10)$$

A quenching rate constant of  $k_{q,Bix}^\Delta = 1.3 \times 10^{10} M^{-1}s^{-1}$  was obtained, Table 3, in good agreement with data obtained for bixin in other solvent systems (18, 30). It is well-known that the quenching process is more physical than chemical for carotenoids with  $\geq 9$  conjugated double bonds, because the chemical quenching rate constant  $k_r^\Delta$  is several orders of magnitude smaller than the physical one,  $k_q^\Delta$  (16, 18, 28, 29). Moreover, the HPLC analysis between 250 and 600 nm did not show the presence of extra peaks other than the reported products 1–4 during the consumption of bixin, confirming that the  $O_2(^1\Delta_g)$  quenching by bixin was totally physical, eq 11.



The formation of  ${}^3Bix^*$  during the reaction shown in eq 11 was also confirmed by the detection of the transient absorption spectra of  ${}^3Bix^*$  in air-saturated solutions (data not shown).

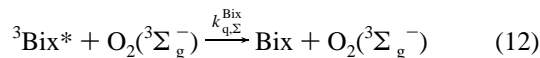
Therefore, all the above results support the participation of  ${}^3Bix^*$  in the isomerization reaction of bixin, according to the energy-transfer-based mechanism (19, 26, 31) proposed in Scheme 2. In this mechanism S,  ${}^1S^*$ , and  ${}^3S^*$  symbolize the ground, singlet, and triplet excited states of the sensitizer molecules (RB or MB), respectively;  $\Sigma$  and  $\Delta$  are  $O_2(^3\Sigma_g^-)$  and

$O_2(^1\Delta_g)$ ; and Bix and  ${}^3Bix^*$  are the ground and triplet excited states of bixin. According to this mechanism, the excited triplet state of  ${}^3Bix^*$  is produced under two different energy-transfer pathways: (i) bixin quenching of  ${}^3S^*$  in  $N_2$ -saturated solutions, and (ii) by sequential quenching of  $O_2(^1\Delta_g)$  by bixin, after deactivation of  ${}^3S^*$  by  $O_2(^3\Sigma_g^-)$  in air-saturated conditions.

The driving force for these processes is the energy cascade involving  ${}^3S^*$ ,  $O_2(^1\Delta_g)$  and  ${}^3Bix^*$ . The triplet energies  $E_T$  for the sensitizers RB and MB were 39 and 33 kcal/mol, respectively (25). In sequence, the  $O_2(^1\Delta_g)$  lays at 22.5 kcal/mol above the ground-state  $O_2(^3\Sigma_g^-)$  (15), but the precise energy level of  ${}^3Bix^*$  is still unknown. However, the  $E_T$  of  ${}^3Bix^*$  can be expected to be no higher than that for  $\beta$ -carotene, reported as 20 kcal/mol (32, 33), by considering the similar  $k_q^\Delta$  values observed for both carotenoids in the same solvent system (18). Thus, the  ${}^3Bix^*$  was the final long-lived excited state produced in the degradation energy cascade.

Therefore, the unimolecular cis $\rightarrow$ trans isomerization reaction of the 9'-double bond was occurring starting with  ${}^3Bix^*$ , explaining the 1st-order kinetic behavior of the consumption of bixin and the formation of the isomerization products. According to this mechanism, the same activation parameters in the absence and presence of air can be expected, as was experimentally found (Table 2).

On the other hand, the smaller values for  $k_{obs}$  observed by increasing the bixin or molecular oxygen concentrations, are explained by the competitive deactivation of the  ${}^3Bix^*$  by the bimolecular self-quenching of bixin, eq 8, or by an enhanced intersystem crossing quenching by  $O_2(^3\Sigma_g^-)$ , eq 12.

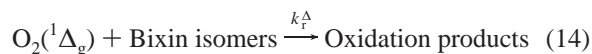


The rate constant quenching  $k_{q,\Sigma}^{Bix}$  was expected to be much smaller than the solvent diffusion limit (ca.  $2 \times 10^{10} M^{-1}s^{-1}$ ), because energy-transfer quenching to yield  $O_2(^1\Delta_g)$  is endergonic (see above) (34). According to the quenching rate constants reported in Table 3, the quenching efficiencies of  ${}^3S^*$  or  $O_2(^1\Delta_g)$  by  $76 \mu M$  bixin were almost in unity. Because both energy-transfer pathways yield  ${}^3Bix^*$  as common isomerization precursor, the amount of carotenoid triplet state formed per absorbed photon by the sensitizer was almost the same either in  $N_2$ - or air-saturated solutions. Thus, an estimation of the  $k_{q,\Sigma}^{Bix}$  can be obtained by using the  $k_{obs}$  values for the isomerization reaction in the absence and presence of air according to eq 13, because the quenching processes by  $O_2(^3\Sigma_g^-)$  competes with the unimolecular isomerization pathway.

$$k_{q,\Sigma}^{Bix} = \frac{(k_{obs}^{N_2}/k_{obs}^{Air}) - 1}{\tau_{d,Bix}[O_2]} \quad (13)$$

In eq 13,  $\tau_{d,Bix} (= 1/k_d^{Bix}) = 6.5 \mu s$  was obtained as the actual lifetime of  $^3Bix^*$ , considering the self-quenching of bixin at  $[Bix] = 76 \mu M$ , eq 9. Using the data reported in **Table 3**, the calculation in eq 13 yielded  $k_{q,\Sigma}^{Bix} = (7 \pm 2) \times 10^8 M^{-1}s^{-1}$ , this magnitude being in agreement with the previous assumption (34).

Finally, as shown in **Figure 3**, both all-*trans*-bixin and isomer **3** only degraded in aerated solutions, indicating a possibly slower reaction with  $O_2(^1\Delta_g)$ , eq 14.



This reaction is associated with the UV-vis spectral changes observed after prolonged photosensitization periods (e.g., spectrum d in **Figure 1**). A chemical quenching rate constant  $k_{qr}^\Delta = 1 \times 10^6 M^{-1}s^{-1}$  was calculated by comparing, under identical experimental conditions, the observed 1st-order rate constants ( $2.8 \times 10^{-4} s^{-1}$ ) for the degradation of the bixin isomers, with that observed for the reaction of the reference compound 9,10-dimethylanthracene (DMA) with  $O_2(^1\Delta_g)$ , in which the bimolecular reaction rate constant  $k_{r,DMA}^\Delta = 5 \times 10^7 M^{-1}s^{-1}$  (16). Current research activities are being focused on the isolation and identification of the  $O_2(^1\Delta_g)$ -mediated final oxidation products of the bixin isomers.

In summary, this study showed that in photosensitized processes, bixin isomerizes mainly to all-*trans*-bixin, independent of the presence of oxygen, via an energy-transfer based mechanism, giving the triplet state of bixin,  $^3Bix^*$ , as the isomerization precursor. Therefore, the isomerization rate is dependent on several factors that compete for the deactivation of  $^3Bix^*$ , such as self-quenching and  $O_2(^3\Sigma_g^-)$  quenching processes. In the presence of  $O_2(^1\Delta_g)$  the isomerization products are slowly degraded, resulting in small color changes in the solution.

These results are important, considering that despite the solvent used in the model-system, this study showed that carotenoids with *cis* conformation are able to retain their color during photosensitized oxidation reactions, because the main product formed is the correspondent *trans*-carotenoid, which shows a bathochromic shift of 2–5 nm. This is not the case for *trans*-carotenoids, such as all-*trans*- $\beta$ -carotene, which formed oxidation products with shorter conjugated bond chain (35), therefore presenting a softer color. Therefore, under the same photosensitized conditions, the color of the bixin system can be maintained, whereas that of  $\beta$ -carotene would fade.

## ABBREVIATIONS

RB, Rose Bengal; MB, Methylene Blue; DMA, 9,10-dimethylanthracene;  $O_2(^3\Sigma_g^-)$ , ground-state molecular oxygen;  $O_2(^1\Delta_g)$ , singlet molecular oxygen;  $^3Bix^*$ , bixin triplet excited state; HPLC, high performance liquid chromatography; PDA, photodiode array detection; LFP, laser-flash photolysis; TRPD, time-resolved phosphorescence detection.

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