

Thermal Degradation Kinetics of Bixin in an Aqueous Model System

ALESSANDRO DE O. RIOS,[†] CLAUDIO D. BORSARELLI,^{*,‡} AND
 ADRIANA Z. MERCADANTE^{*,†}

Departamento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, UNICAMP, Cx. Postal 6121, 13083-970, Campinas, SP, Brazil, and Instituto de Ciencias Químicas, Universidad Nacional de Santiago del Estero, Av. Belgrano (S) 1912, 4200 Santiago del Estero, Argentina

The kinetics of the thermal degradation of the natural *cis* carotenoid bixin in a water/ethanol (8:2) solution was studied as a function of temperature (70–125 °C), using high-performance liquid chromatography. The curves for the decay of bixin and formation of products (e.g., di-*cis* and all-*trans* isomers and a C17 degradation compound) did not adjust well to a first-order rate law, but very good fits were obtained using a biexponential model. This mathematical modeling gave the rate constant values for the formation of the primary products from bixin, and the energy barrier for each step was obtained. The di-*cis* isomers were formed immediately (15 kcal/mol) together with the decay of bixin, followed by a slow consumption, indicating their role as reaction intermediates. In fact, the di-*cis* isomers could easily revert to bixin ($E_a \approx 3$ kcal/mol) or yield the primary C17 degradation product, with an energy barrier of 6.5 kcal/mol. In turn, 24 kcal/mol was necessary for the Bix → all-*trans* step, explaining its slower formation.

KEYWORDS: Bixin; annatto; *Bixa orellana*; heating; kinetics; degradation mechanism

INTRODUCTION

Bixin (methyl hydrogen 9'-*cis*-6,6'-diapocarotene-6,6'-dioate, C₂₅H₃₀O₄; **Figure 1**) is the main carotenoid found in the seeds of the tropical annatto tree (*Bixa orellana* L.) (1, 2). Bixin is one of the few naturally occurring *cis* carotenoids, whose formation is thought to occur by oxidative degradation of the parent C₄₀-carotenoid (3, 4). Recently, using molecular biology, the formation of bixin was confirmed to occur from lycopene, with bixin dialdehyde and norbixin as intermediates, but no mention of the geometry of the 9' double bond was made (5). Small amounts of norbixin (**Figure 1**) (9-*cis*-6,6'-diapocarotene-6,6'-dioic acid, C₂₄H₂₈O₄) are also found in the annatto seeds, and this becomes the major carotenoid of the aqueous annatto extracts.

Commercial annatto preparations have been added to numerous foods to impart yellow to red colors. They include oil soluble colors and annatto powder, which contain bixin as the main pigment, water dispersible colors with norbixin as the main colorant, and emulsions, which may contain a combination of both bixin and norbixin or only norbixin (6). The main application of annatto is in the food industry for coloring sausages, margarine, snacks, and cheese.

High-temperature processes are used either to extract pigments from natural sources or in food treatments. In the case

of carotenoids, which are susceptible to thermal degradation, this type of treatment leads to a reduction of the active carotenoids or to their transformation into degradation products with different colors and properties.

One of the processes for the production of annatto extracts uses edible oils and applies high temperatures, which can reach 125 °C under vacuum (1). Under these conditions, the major carotenoids also include all-*trans*-bixin and a yellow thermal degradation product (1, 6–8) (**Figure 1**). The structure of the yellow degradation product was proposed by Mckeown (7, 8) and confirmed by Scotter (9) as being 4,8-dimethyl-tetradeca-hexaene-dioic acid monomethyl ester (C17) (**Figure 1**). Heating of bixin solutions also formed volatile compounds, especially xylene and toluene (7–10).

Scotter et al. (10) evaluated the thermal stability of bixin at the boiling point of different alcohol solvents. A di-*cis*-isomer was rapidly formed while both all-*trans*-bixin and C17 were formed at a much lower rate. These authors have postulated a mechanism for the complete formation of the thermal degradation products, reporting an activation energy (E_a) of 8.5 kcal/mol. However, Berset and Marty (11) reported an E_a of 30 kcal/mol for the thermal degradation of the annatto pigments (mainly bixin) in vaseline as solvent, also assuming a first-order behavior for the pigment consumption. Regardless of the use of different solvents and considering the same degradation mechanism, there is a large difference in the reported E_a , indicating some controversy in the kinetic analysis or misinterpretation of the experimental data.

* To whom correspondence should be addressed. (C.D.B.) Fax: 54-385-4509585. E-mail: cborsa@unse.edu.ar. (A.Z.M.) Fax: 55-19-37882153. E-mail: azm@fea.unicamp.br.

[†] UNICAMP.

[‡] Universidad Nacional de Santiago del Estero.

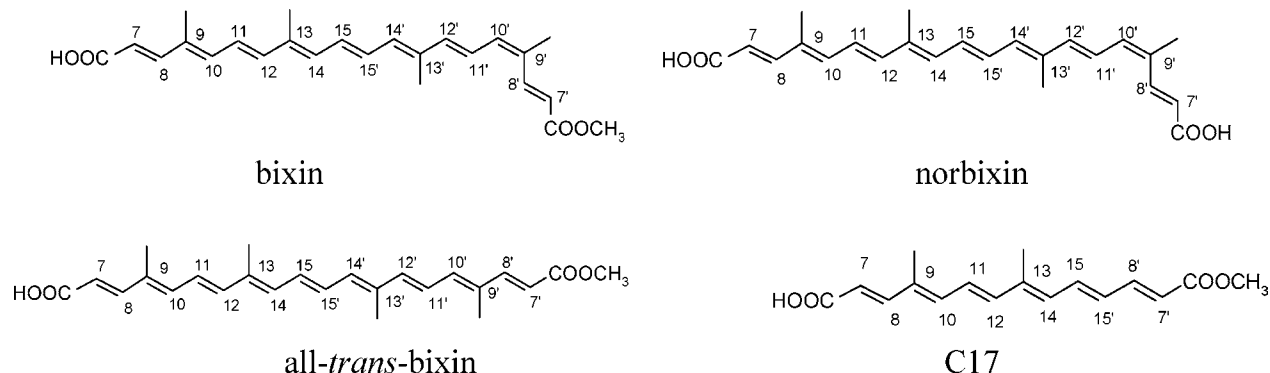


Figure 1. Structures of the main apocarotenoids from annatto and some of their degradation products.

It is interesting to note that bixin was stable at room temperature but was easily transformed to all-*trans*-bixin in the presence of a sensitizer molecule and light (12). In this case, an energy transfer-based mechanism involving the excited triplet state of bixin, $^3\text{Bix}^*$, as the precursor of the isomerization step was reported, and the activation energy for this step was about 6 kcal/mol (12). At first sight, these findings indicate that a greater energy barrier can be expected for the thermal *cis* \rightarrow *trans* isomerization of bixin.

In this report, a detailed kinetic study of the thermal degradation of bixin in aqueous 20% ethanol solutions (a solvent system closer to those found in foods), using high-performance liquid chromatography (HPLC) for monitoring the reaction, is presented. The rate constants and activation parameters for the different primary degradation steps are reported and discussed as a function of previous degradation mechanisms.

MATERIALS AND METHODS

Materials. Bixin crystals, with 99% purity as determined by HPLC, were kindly supplied by Dr. Simon Werner from DSM (Basel, Switzerland). All of the organic solvents used were HPLC grade from Mallinckrodt, and the distilled water was purified by a Milli-Q Plus system (Millipore).

Methods. Bixin (76 μM) was dissolved in air-saturated aqueous 20% ethanol solutions and heated in tightly sealed glass tubes at 70, 77, 84, 98, and 125 $^{\circ}\text{C}$ in the dark. Heating at 125 $^{\circ}\text{C}$ was conducted in a sand bath, whereas the other temperatures were achieved in a water bath. At different heating times, the tubes, in duplicate, were taken from the bath, immediately cooled in an ice bath, and analyzed by HPLC.

All systems were monitored using a Waters HPLC system (Waters Corporation, Milford, MA) equipped with a photodiode array detector (PDA) (Waters, model 996). The equipment also included an on-line degasser, a Rheodyne injection valve with a 20 μL loop, and an external oven. Millennium Waters software performed the data acquisition and processing. Separation was carried out using a C_{18} Spherisorb ODS-2 column, 150 mm \times 4.6 mm i.d. (3 μm particle size), with acetonitrile/2% acetic acid (65:35) (13) or acetonitrile/2% acetic acid/dichloromethane (63:35:2) (12) as the mobile phases at a flow rate of 1 mL/min and the column temperature set at 29 $^{\circ}\text{C}$. The absorption spectra were obtained between 250 and 600 nm, and the chromatograms were processed at the maximum absorption wavelengths (λ_{max}) for quantification purposes. Because there are no standards available for the di-*cis* isomers, quantification was carried out by external calibration with a bixin standard.

UV-visible (UV-vis) spectrum features were expressed as a fine structure (%III/II), calculated as the values of the height ratio between the longest wavelength absorption band (designated III) and the middle length absorption band (II, usually λ_{max}) by taking as the baseline or zero value the valley between both bands and of the relative intensity of the *cis* band (% $A_{\text{B}}/A_{\text{II}}$), calculated as the ratio between the height of the *cis* peak (ca. 360 nm for bixin) and that of band II (14).

In addition to these experiments, heating experiments monitored by the UV-vis spectra using a diode array spectrophotometer (Hewlett-

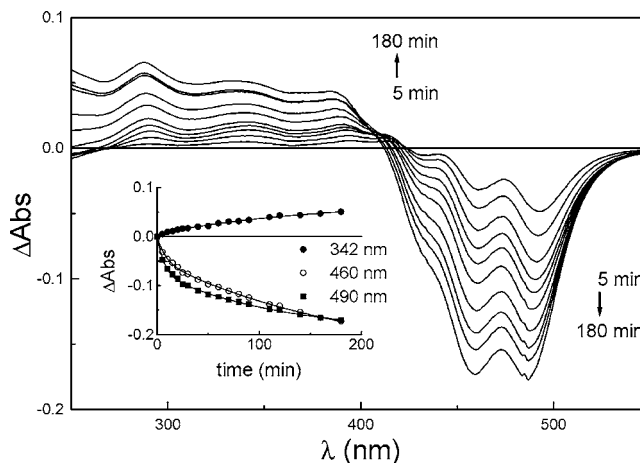


Figure 2. UV-vis spectral changes, ΔAbs , produced on heating 76 μM bixin at 92 $^{\circ}\text{C}$ in 20% aqueous ethanol solutions. The inset shows the kinetic profile at several wavelengths. The solid lines represent the fitting of the experimental data from the sum of two exponential functions.

Packard 8453) equipped with a thermostated cell holder were also conducted at 63, 81, and 92 $^{\circ}\text{C}$. The experiments were performed in duplicate.

RESULTS AND DISCUSSION

Figure 2 shows the UV-vis spectral changes produced in a bixin solution contained in a sealed quartz cell on heating at 92 $^{\circ}\text{C}$. The consumption of the visible band of bixin (400–500 nm) was accompanied by an increase in the absorbance below 400 nm, without the presence of clear isosbestic points. In addition, as can be seen in the inset of **Figure 2**, the kinetic curves showed nonsimple exponential behavior, whose fitting parameters were strongly dependent on the monitoring wavelength, precluding a simple kinetic analysis from the UV-vis absorption spectra. The same result was observed at all temperatures, indicating the formation of several bixin degradation products (including bixin isomers) at different rate constants.

The formation of several degradation products was confirmed by the HPLC chromatogram shown in **Figure 3**, where the main degradation products were labeled as peaks 1–6. Only product 6 presented a greater retention time (t_{R}) than bixin (**Table 1**). The same products were produced at lower heating temperatures. At all temperatures, the order of peak appearance with heating time was 4, 5 > 1–3 > 6 \gg 2.

To compare the spectral features, **Figure 4** shows the normalized UV-vis absorption spectra of the degradation products 1–6 and bixin (bold line) as obtained by PDA. **Table 1** presents the maximum absorption wavelengths, the spectral

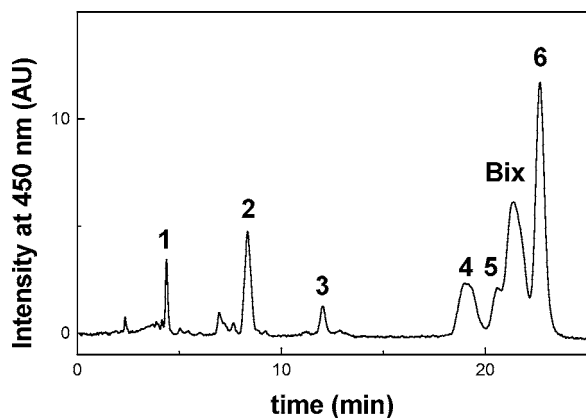


Figure 3. Chromatogram obtained after heating a bixin solution at 125 °C for 120 min. Chromatographic conditions: acetonitrile/2% acetic acid (65:35) at 1 mL/min. Peak characterization is shown in **Table 1**.

fine structure (%III/II), and the intensity of the *cis* peak (% A_B/A_{II}) values for all of the reported peaks.

The retention times of products 4–6 were close to that of bixin, indicating that these products could be geometrical isomers of bixin. The spectrum of product 6 showed a bathochromic shift together with the complete disappearance of the *cis* band absorption at 355 nm, e.g., % A_B/A_{II} = 0. These changes correspond to the *cis* → *trans* isomerization process at the 9'-C, producing all-*trans* bixin (12, 14). In contrast, the spectral features of compounds 4 and 5, e.g., hypsochromic shifts, and the variation of their %III/II and % A_B/A_{II} ratios strongly suggest that these products are different di-*cis* isomers of bixin (14). 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 4 and 5.

Product 2 showed a smaller retention time than those of bixin and products 4 and 5. However, its visible band was slightly shifted to the red and a new band ca. 290 nm was observed. These features are coincident with those reported for all-*trans*-norbixin in a similar solvent mixture (13). On the other hand, products 1 and 3 showed λ_{\max} at 400 nm and smaller retention times. The strong blue shift of the main absorption band of these compounds indicated a shorter double conjugated system as compared to that of bixin. In addition, their earlier elution on a reversed phase column indicated that these products had a lower molecular weight than bixin. The spectral features of product 1 were identical to those previously reported for the all-*trans*-C17 product (7, 9). Therefore, product 1 was tentatively identified as such a compound. Product 3 also showed a strong absorption band at 275 nm and a lack of fine structure of the visible band at 400 nm. In this case, it is very difficult to identify this product with the present information.

Figure 5 shows the kinetic curves for bixin and their degradation products during heating at 98 °C, obtained from the HPLC analysis of the peak areas. Similar kinetic curves were observed for the di-*cis* isomers 4 and 5, and their average values were plotted. These products were immediately formed together with the decay of bixin, followed by a slower consumption indicating their possible role as reaction intermediates. The relative concentration changes of C17 appear tiny because the C17 area was much lower as compared to the di-*cis* isomers areas. In turn, the formation of C17 and all-*trans*-bixin showed a small induction period and only at 125 °C were slowly degraded (data not shown).

At all temperatures, the decay curves did not adjust well to a first-order rate law (exponential fitting), as indicated by the

dashed lines in **Figure 5**. In fact, very good fits (solid lines) of the kinetic data were obtained using the following biexponential eq 1

$$A_t - A_\infty = \Lambda_1 \exp(-\alpha t) + \Lambda_2 \exp(-\beta t) \quad (1)$$

where A_t and A_∞ are the transitory and final HPLC areas, Λ_1 and Λ_2 are the preexponential factors, and α and β are, respectively, the observed fast and slow first-order rate constants. In all cases, similar α and β values were obtained from the biexponential fitting of the kinetic data. This biexponential behavior confirms the presence of a reversible step in the bixin degradation mechanism that accounts for the role of the di-*cis* isomers as reaction intermediates, according to the following coupled reaction systems (15, 16) (**Figure 6**).

According to this mechanism, the di-*cis*-bixin isomers (peaks 4 and 5) can be considered as reaction intermediates that produce C17 irreversibly (peak 1) or return reversibly to bixin. The isomerization of bixin to all-*trans*-bixin (peak 6) can be considered almost irreversible, taking into account the remarkable thermal stability observed for all-*trans*-bixin (9) and all-*trans*-methylbixin (17). The differential equations for the system are

$$\frac{d[\text{Bix}]}{dt} = k_2[\text{di-cis}] - (k_1 + k_4)[\text{Bix}] = k_2[\text{di-cis}] - X[\text{Bix}] \quad (2)$$

$$\frac{d[\text{di-cis}]}{dt} = k_1[\text{Bix}] - (k_2 + k_3)[\text{di-cis}] = k_1[\text{Bix}] - Y[\text{di-cis}] \quad (3)$$

$$\frac{d[\text{C17}]}{dt} = k_3[\text{di-cis}] \quad (4)$$

$$\frac{d[\text{all-trans}]}{dt} = k_4[\text{Bix}] \quad (5)$$

Assuming that the boundary conditions are such that, at $t = 0$, $[\text{Bix}] = [\text{Bix}]_0$ and $[\text{di-cis}]_0 = [\text{C17}]_0 = [\text{all-trans}]_0 = 0$, the mathematical solutions for bixin and the di-*cis* isomers equations are (15, 16) as follows:

$$\frac{[\text{Bix}]}{[\text{Bix}]_0} = \gamma_1 \exp(-\alpha t) + \gamma_2 \exp(-\beta t) \quad (6)$$

$$\frac{[\text{di-cis}]}{[\text{Bix}]_0} = \gamma_3 \{\exp(-\beta t) - \exp(-\alpha t)\} \quad (7)$$

where $\gamma_1 = (X - \beta)/(\alpha - \beta)$, $\gamma_2 = (X - \alpha)/(\alpha - \beta)$, $\gamma_3 = k_1/(\alpha - \beta)$, $\alpha = 1/2\{(X + Y) + [(X - Y)^2 + 4k_1k_2]^{1/2}\}$, and $\beta = 1/2\{(X + Y) - [(X - Y)^2 + 4k_1k_2]^{1/2}\}$. Combining eqs 4 with 6 and 5 with 7, the solving of the respective first-order differential equations yielded the same biexponential behavior for C17 and all-*trans*-bixin, according to the fitting used in eq 1. Thus, the constituent rate constants k_1 , k_2 , k_3 , and k_4 were calculated using the preexponential factors γ_1 , γ_2 , and γ_3 together with the observed first-order rate constants α and β . **Table 2** shows the results obtained by fitting the kinetic curves of bixin and of the di-*cis* isomers with eqs 6 and 7 as a function of temperature.

These results agree with those reported by Scotter et al. (10), who observed nonsimple exponential decay for bixin degradation in a homologous series of *n*-alcohols heated under refluxing conditions at the boiling point of each alcohol. In addition, they

Table 1. Retention Times (t_R), Absorption Maximum Wavelengths (λ_{max}), Spectral Fine Structure (%III/II), and Intensity of the *cis* Peak (% A_B/A_{II}) Obtained Using HPLC–PDA of Bixin and Its Heat Degradation Products^a

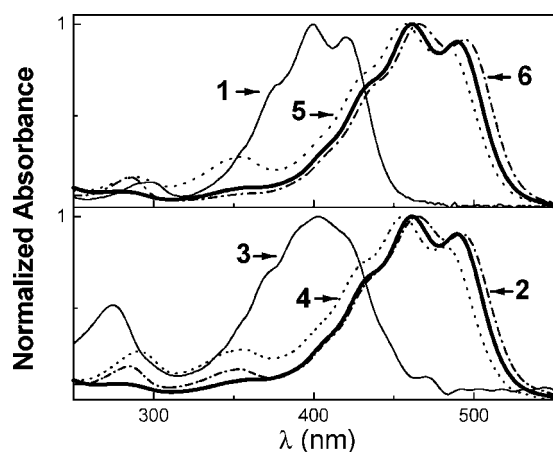
peak no. ^b	carotenoid	t_R^c (min)	λ_{max}^d (nm)	%III/II	% A_B/A_{II}
1	all- <i>trans</i> -C-17 ^e	4.4 ± 0.1	296, (380), 400, 420	39	10
2	all- <i>trans</i> -norbixin ^e	8.2 ± 0.1	285, 353, 428, 465, 489	20	15
3	not identified	12.6 ± 0.2	275, (370), 403, (420)	0	0
4	di- <i>cis</i> -bixin	18.7 ± 0.2	290, 352, (420), 455, 482	10	15
5	di- <i>cis</i> -bixin	20.7 ± 0.1	287, 356, (425), 460, 482	11	23
6	all- <i>trans</i> -bixin	23.0 ± 0.3	285, (430), 466, 495	40	0
	bixin	21.7 ± 0.2	355, (425), 461, 489	39	5

^a Brackets indicate a shoulder instead of a peak. ^b Numbered according to the chromatogram from Figure 3. ^c Average of 40 runs. ^d Solvent: acetonitrile/2% acetic acid (65:35). ^e Tentatively identified.

Table 2. Preexponential Factors A and B and the Observed Rate Constants α and β Obtained from the Biexponential Fitting of the Kinetic Data for the Thermal Degradation of Bixin^a

temp (°C)	$\gamma_1 (\times 100)$	$\gamma_2 (\times 100)$	$\gamma_3 (\times 100)$	$\alpha \times 10^2$ (min ⁻¹)	$\beta \times 10^3$ (min ⁻¹)	$k_1 \times 10^3$ (min ⁻¹)	$k_2 \times 10^3$ (min ⁻¹)	$k_3 \times 10^3$ (min ⁻¹)	$k_4 \times 10^3$ (min ⁻¹)
70	7 ± 2	93 ± 3	7 ± 2	6 ± 2	1.3 ± 0.1	4 ± 1	48 ± 15	12 ± 4	0.1 ± 0.2
98	15 ± 3	85 ± 5	20 ± 3	10 ± 2	4.4 ± 0.4	19 ± 7	61 ± 12	24 ± 5	1.4 ± 0.5
125	35 ± 5	65 ± 5	44 ± 5	19 ± 3	19.1 ± 1.2	76 ± 15	80 ± 10	45 ± 8	15 ± 8

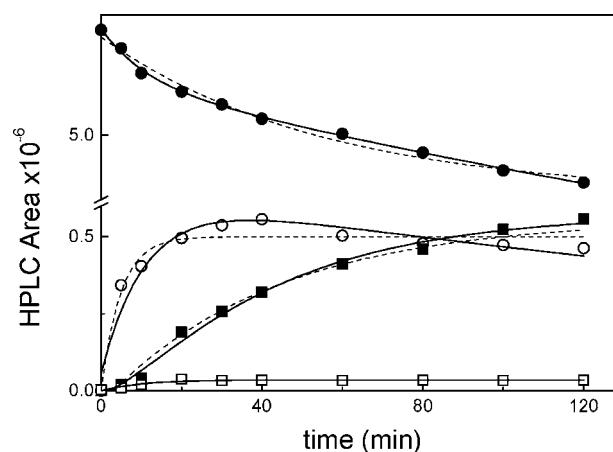
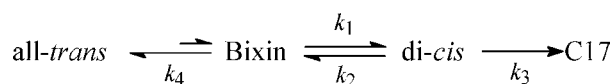
^a First-order rate constants k_i for the elementary steps according to the proposed degradation mechanism (Figure 6).

**Figure 4.** Normalized UV–vis spectra obtained using HPLC–PDA in acetonitrile/2% acetic acid (65:35) of bixin (bold line) and its main thermal degradation products.

also observed the faster formation of di-*cis* bixin isomers and C17, whereas all-*trans*-bixin was formed at a much lower rate.

Figure 7 shows the Arrhenius plots for the calculation of the activation parameters of the individual steps, which are reported in Table 3. By inspection of the E_a values, some conclusions can be extracted. For instance, a moderate energy barrier of ca. 15 kcal/mol accompanied the Bix → di-*cis* isomerization, while the Bix → all-*trans* step needed ca. 24 kcal/mol, explaining its slower formation. In addition, these results show the greater thermostability of the 9'-*cis* double bond of bixin, and thus, the molecule preferentially underwent a second *trans* → *cis* isomerization to yield di-*cis* isomers. Similar qualitative results were reported for the heating of methyl-bixin (17). In turn, the di-*cis* isomers can return easily to bixin ($E_a \approx 3$ kcal/mol) or react to yield the primary C17 degradation product with an energy barrier of 6.5 kcal/mol. As mentioned in the Introduction, the mechanism postulated for the thermal degradation of bixin includes a concerted electrocyclic process from the 9',13'-di-*cis*-bixin isomer, followed by the elimination of *m*-xylene and the formation of the main product C17 (7–9).

The process consists of three sequential steps: an eight-electron conrotatory and a six-electron disrotatory electrocyclic

**Figure 5.** Monoexponential (dashed lines) and biexponential fitting (solid lines) of the kinetic HPLC data for the thermal degradation of bixin in a 20% ethanolic solution at 98 °C: Bixin (●); di-*cis*-bixin (average of peaks 4 + 5) (○); all-*trans*-bixin (peak 6) (■); and C17 (peak 1) (□).**Figure 6.** Coupled reaction scheme proposed for the degradation of bixin and the formation of its primary products.

reaction followed by the opening of a four-membered ring (9). It is clear that a multi-*cis* configuration is a prerequisite for the chain folding and elimination steps, which are facilitated by the 9'-*cis* configuration of bixin, but do not exist in the all-*trans*-bixin isomer, explaining its relatively greater thermal stability.

The mechanism proposed does not explain the formation of compound 2 (tentatively all-*trans*-norbixin). However, this product was only detected on heating at 125 °C for more than 60 min, and the most probable pathway for its formation was the hydrolysis reaction of all-*trans*-bixin. Unfortunately, both the product and the kinetic analyses after prolonged heating periods are very complex, due to the formation of several secondary degradation products (data not shown).

Scotter et al. (10) reported an $E_a = 8.5$ kcal/mol for the loss of bixin performing a simple first-order analysis of the initial

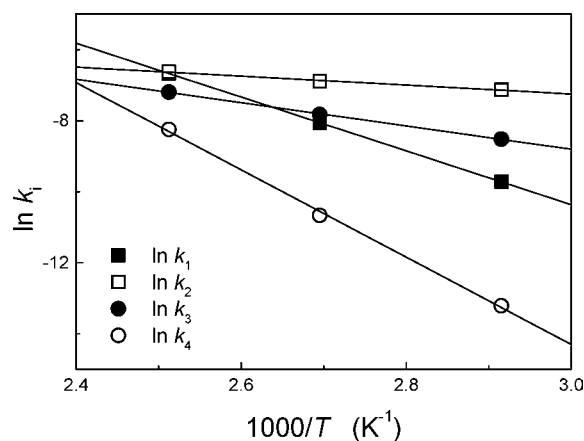


Figure 7. Arrhenius plot for the temperature dependence of the rate constants k_1 , k_2 , k_3 , and k_4 associated with the first-order processes represented in the mechanism scheme for the thermal degradation of bixin.

Table 3. Preexponential Factor A and Energy E_a Calculated from the Arrhenius Analysis of the First-Order Rate Constants for the Individual Steps of the Thermal Degradation Model of Bixin in a Water/Ethanol (8:2) Solution

	Bix \rightarrow di- <i>cis</i>	di- <i>cis</i> \rightarrow Bix	di- <i>cis</i> \rightarrow C17	Bix \rightarrow all- <i>trans</i>
Ln A	12.3 \pm 0.7	-3.5 \pm 0.4	1.0 \pm 0.3	22.6 \pm 1.5
E_a (kcal/mol)	15.0 \pm 0.5	2.5 \pm 0.3	6.5 \pm 0.3	24.4 \pm 1.0

phase of the reaction with the temperature. Nevertheless, Berset and Marty (11) reported an E_a ca. 30 kcal/mol for the thermal degradation of the annatto pigments in vaseline using the simple first-order model of the complete decay. In fact, an Arrhenius plot using the initial decay component for the bixin degradation (α values from Table 2) yielded an $E_a = 8.0$ kcal/mol, which is coincident with the data from Scotter et al. (10). Thus, it is clear that the simple first-order rate law does not account for the actual degradation mechanism of bixin; therefore, the above E_a values could be misinterpreted.

The E_a value of 24 kcal/mol obtained in our study for the *cis* \rightarrow *trans* isomerization is the same as that reported for the *trans* \rightarrow *cis* isomerization of β -carotene given by El Oualja et al. (18). Considering the sum of the E_a of the individual isomerization steps of bixin ($E_{a1} + E_{a2} + E_{a4}$) reported in our study, a value of 36.9 kcal/mol for the total bixin isomerization was obtained. This value is much higher than those previously reported for thermal isomerization equilibrium of C-40 carotenoids in safflower seed oil (19), e.g., β -carotene (26.2 kcal/mol) and 9-*cis*- β -carotene (25.1 kcal/mol), and for astaxanthin (20) in dichloromethane (25.3 kcal/mol). This fact confirms the greater bixin stability observed during its isolation and manipulation (9, 12, 17).

LITERATURE CITED

- (1) Preston, H. D.; Rickard, M. D. Extraction and chemistry of annatto. *Food Chem.* **1980**, *5*, 47–56.
- (2) Mercadante, A. Z.; Pfander, H. Carotenoids from annatto: A review. *Recent Res. Dev. Agric. Food Chem.* **1998**, *2*, 79–91.
- (3) Mercadante, A. Z.; Steck, A.; Pfander, H. Isolation and structure elucidation of minor carotenoids from annatto (*Bixa orellana* L.) seeds. *Phytochemistry* **1997**, *46*, 1379–1383.

- (4) Britton, G. Overview of carotenoid biosynthesis. In *Carotenoids: Biosynthesis and Metabolism*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser: Basel, 1998; Vol. 3, pp 13–147.
- (5) Bouvier, F.; Dogbo, O.; Câmara, B. Biosynthesis of the food and cosmetic plant pigment bixin (annatto). *Science* **2003**, *27*, 2089–2081.
- (6) Mercadante, A. Z. Composition of carotenoids from annatto. In *Chemistry and Physiology of Selected Food Colorants*; ACS Symposium Series 775; Ames, J. M., Hofmann, T. F., Eds.; American Chemical Society: Washington, DC, 2001; pp 92–101.
- (7) Mckeown, G. G. Composition of oil-soluble annatto food colors. 2. Thermal degradation of bixin. *J. Assoc. Off. Agric. Chem.* **1963**, *46*, 790–796.
- (8) Mckeown, G. G. Composition of oil-soluble annatto food colors. 3. Structure of the yellow pigment formed by the thermal degradation of bixin. *J. Assoc. Off. Agric. Chem.* **1965**, *48*, 835–857.
- (9) Scotter, M. J. Characterization of the coloured thermal degradation products of bixin from annatto and a revised mechanism for their formation. *Food Chem.* **1995**, *53*, 177–185.
- (10) Scotter, M. J.; Castle, L.; Appleton, G. P. Kinetics and yields for the formation of coloured and aromatic thermal degradation products of annatto in foods. *Food Chem.* **2001**, *74*, 365–375.
- (11) Berset, C.; Marty, C. Utilisation potentielle de l'Annatto en cuisson-extrusion. *Lebensm. Wiss. Technol.* **1986**, *19*, 126–131.
- (12) Montenegro, M. A.; Rios, A. de O.; Mercadante, A. Z.; Nazareno, M. A.; Borsarelli, C. D. Model studies on the photosensitized isomerization of bixin. *J. Agric. Food Chem.* **2004**, *52*, 367–373.
- (13) Scotter, M. J.; Wilson, L. A.; Appleton, G. P.; Castle, L. Analysis of annatto (*Bixa orellana*) food coloring formulations. 1. Determination of coloring components and colored thermal degradation products by high-performance liquid chromatography with photodiode array detection. *J. Agric. Food Chem.* **1998**, *46*, 1031–1038.
- (14) Britton, G. UV/visible spectroscopy. In *Carotenoids: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser: Basel, 1995; Vol. 1B, pp 13–62.
- (15) Espenson, J. H. *Chemical Kinetics and Reaction Mechanisms*, 2nd ed.; McGraw-Hill: New York, 1995.
- (16) Capellos, C.; Bielski, B. H. J. *Kinetic Systems. Mathematical Description of Chemical Kinetics in Solution*; Wiley-Interscience: New York, 1972.
- (17) Zechmeister, L.; Escue, R. B. A stereochemical study of methylbixin. *J. Am. Chem. Soc.* **1944**, *66*, 322–330.
- (18) El Oualja, H.; Perrin, D.; Martin, R. Kinetic study of the thermal oxidation of all-*trans*- β -carotene and evidence for its antioxidant properties. *New J. Chem.* **1995**, *19*, 863–872.
- (19) Henry, L. K.; Catignani, G. L.; Schwartz, S. J. Oxidative degradation kinetics of lycopene, lutein, and 9-*cis* and all-*trans* β -carotene. *J. Assoc. Oil Chem. Soc.* **1998**, *75*, 823–829.
- (20) Yuan, J.-P.; Chen, F. Kinetics for the reversible isomerization reaction of *trans*-astaxanthin. *Food Chem.* **2001**, *73*, 131–137.

Received for review November 4, 2004. Revised manuscript received January 11, 2005. Accepted January 12, 2005. We thank the Funding Agencies from Argentina (CONICET and Fundación Antorchas) and from Brazil (FAPESP, CAPES, and CNPq) for their financial support.

JF0481655