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KINETICS STUDY OF NORBIXIN'S FIRST STAGE THERMAL DECOMPOSITION, USING DYNAMIC METHOD

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Cis-norbixin isomer obtained by hydrolysis of *cis*-bixin and isolated by solvent extraction from annatto seeds. The thermal decomposition data of the *cis*-norbixin samples were analyzed by thermogravimetric analysis at different heating rates in the 25–900°C temperature range. DSC curves showed that thermal decomposition reactions for *cis*-norbixin occurred in the solid phase. The kinetic parameters, such as activation energy and pre-exponential factor were determined using integral and approximate methods: Coats–Redfern, Madhusudanan, Horowitz–Metzger and Van Krevelen. F1 mechanism describes well the first stage of the thermal decomposition.

Keywords: *kinetic, norbixin, thermal degradation*

Introduction

The seeds of *Bixa orellana* L., a native shrub from Tropical America are rich source of orange-red pigments that have been largely used for food coloring in the industry. These pigments are commercially known as annatto (E160b) and their major coloring component is bixin ($C_{25}H_{30}O_4$), an unusual carotenoid having a free carboxyl and an esterified carboxyl as end groups. Approximately 80% of the pigments present in annatto seeds correspond to bixin [1] and more recently several other minor carotenoids have been isolated and identified [2–4]. The hydrolytic removal of the methyl ester group from bixin by saponification results norbixin.

($C_{24}H_{28}O_4$), a water-soluble carotenoid, also found in annatto preparations but in lower amounts than bixin. Notwithstanding the large use of annatto pigments as food colorant, practically no information exists about their toxicological properties in human and animal tissues. Toxicity was mostly determined in animals using commercial annatto preparations containing undetermined amounts of bixin and norbixin [5, 6].

Effect of carotenoid has been studied when norbixin was added to drinking-water. It was given to masculine Swiss rats with a greasy diet. The diet without norbixin decreases the level of high-density lipoprotein (HDL) – cholesterol and increases the level of very low-density lipoprotein (VLDL) – cholesterol/low-density lipoprotein (LDL) – cholesterol. When the high fat diet was ingested with norbixin, HDL–cholesterol and VLDL/LDL–cholesterol remained near at control levels while plasma triglycerides were not altered [7].

Toxicological data about annatto pigments are limited. It can be due that food additives derived from natural sources have been given the warranty certification, i.e. they can be safely used without in-depth chemical and toxicological analysis [8]. However, current scientific works state that annatto extract does not exert any genotoxicity or carcinogenicity through assays of toxicity in high concentrations [9–14].

Several studies pointed that under certain conditions carotenoids can act as chain breaking antioxidants by scavenging and deactivating free radicals both in vitro and in vivo. Antioxidant activity of carotenoids in organic solution is related to oxygen concentration, the chemical structure of carotenoids and the presence of other antioxidants [15]. Carotenoids have been shown to maintain the stability of oils against photo oxidation [16]. There are few reports on the antioxidant properties of annato carotenoids, and recently antioxidant activity was reported for norbixin [17]. The kinetic of the thermal decomposition of *cis*-bixin isomer isolated by solvent extraction from the annatto seeds was investigated [18].

The theoretical basis of the kinetic calculations using dynamic thermogravimetry is summarized in the following Eq. (1):

$$g(\alpha)=\frac{A}{\theta}\int_0^T \exp\left(\frac{E_a}{RT}\right)\mathrm{d}T \qquad (1)$$

where A is the frequency factor, T is temperature, R is the universal gas constant, θ is the heating rate, E_a is the activation energy and a represents the decomposed fraction. Equation (1) represents the reaction mecha-

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nism at a fixed heating rate. However, the right term of Eq. (1) cannot be solved analytically. Thus, several numerical methods to calculate this integral have been proposed giving rise to the different methods for the determination of the kinetic parameters from the TG curves [19–22]. In this study Coats–Redfern [23] and Madhusudanan [24] integral methods and the Horowitz–Metzger [25] and Van Krevelen [26] approximation methods have been used.

Experimental

The annatto seeds from 'Embrapa II' species were heated in chloroform in a Soxhlet extraction apparatus. *Cis*-bixin was got in agreement with Silva *et al.* [18]. *Cis*-norbixin was prepared through alkaline hydrolysis of *cis*-bixin (at 40–50°C), then it was acidified with hydrochloric acid and filtered under vacuum. The obtained precipitate was recrystallized from acetone. Its structure was identified by FTIR, ^{1}H and ^{13}C NMR spectroscopy techniques and mass spectrometry.

Decomposition temperatures were determined using Microquimica/MQAPF-301 with a controlled heating rate and Shimadzu differential scanning calorimeter, model DSC-50, at 10°C min^{-1} heating rate and nitrogen purging. The infrared absorption spectra have been recorded in KBr pellets, using a Bomem/MB-102 spectrophotometer operating in the 4000–400 cm^{-1} range. The mass spectrum was obtained using LC-MS positive electrospray using a Quattro LC–Micromass (Waters). TLC was done using silica gel Kieselgel 60 from Merck and spots were visualized by UV radiation. ^{1}H and ^{13}C NMR spectra were obtained using a Varian 200 MHz spectrometer.

The dynamic TG/DTG curves were recorded using Shimadzu TGA-50 Thermobalance in a nitrogen atmosphere (flow rate: 50 mL min^{-1}), alumina crucibles, at heating rates of 5, 10 and 20°C min^{-1}. The initial sample masses were 2.5–3.0 mg and the TG curves were recorded in the 25–900°C temperature range. The DSC curves were obtained using Shimadzu DSC-50 equipment in nitrogen flow (50 mL min^{-1} flow rate) in aluminium crucibles, with an initial sample masses of 2.5–3.0 mg, at a heating rate of 10°C min^{-1} in the 25 to 500°C temperature range.

Results and discussion

Cis-norbixin was obtained as an amorphous powder with a decomposition temperature higher than 280°C. This compound is soluble only in tetrahydrofuran and acetic acid, being insoluble in other organic solvents and water.

The known corant *cis*-norbixin was identified by spectral data, mainly by ^{1}H and ^{13}C NMR spectra compared to the literature values [27, 28].

The IR spectrum (KBr) indicated absorptions at 3456 cm^{-1} ($\nu_{\max(O–H)}$) and conjugated carbonyl function in 1677 cm^{-1} [27]. The LC-MS positive electrospray of *cis*-norbixin showed molecular peak at m/z=381 daltons [MH$^+$]. The ^{1}H and ^{13}C (APT) NMR data of *cis*-norbixin and the [MH$^+$] allowed us to deduce the molecular formula, $C_{24}H_{28}O_4$ for *cis*-norbixin.

Comparative analysis of APT and HMQC spectra was used to identify the number of signals attributed to six quaternary [$(C)_6$, all sp^2, including two carbonyl group of α–β unsaturated carboxylic acid δ_C (167.9)], fourteen methine sp^2 $(CH)_{14}$, and four methyl $(CH)_3$. The ^{1}H (1D and 2D ^{1}H–^{1}H–COSY) and ^{1}H–^{13}C–COSY–$^nJ_{CH}$ (n=1, HMQC; n=2 and 3, HMBC) spectra are in agreement with these deductions.

The ^{1}H NMR spectra (1D and 2D ^{1}H–^{1}H–COSY) of *cis*-norbixin displayed signals of two AB systems α–β unsaturated with carboxylic acid (J=15 Hz) attributed to H-19 (δ_H 5.8); H-3 (δ_H 7.3) and H-2 (δ_H 5.6). The remaining signals observed in the ^{1}H to methine hydrogens between δ_H 6.4 and 6.8, beyond of the intense signals in δ_H 2.0 and 1.9 were used to establish the structure of *cis*-norbixin, which was also confirmed by long-range ^{1}H–^{13}C correlations obtained from HMBC experiment that reveals connection between quaternary carbons C-1 and C-20 (δ_C 167.9) with H-3 (δ_H 7.3) and H-18 (δ_H 7.8).

The thermoanalytical curves of the colorant were recorded in a temperature range of 25–900°C, using heating rates of 5, 10 and 20°C min^{-1} (Fig. 2). The selection of the kinetic parameters for the first mass loss step was done using thermochemistry and materials laboratory (LTM) software of UFPB. This program employs the $g(\alpha)$ function considering the maximum values of linear correlation coefficient and minor standard deviation. The thermogravimetric profiles

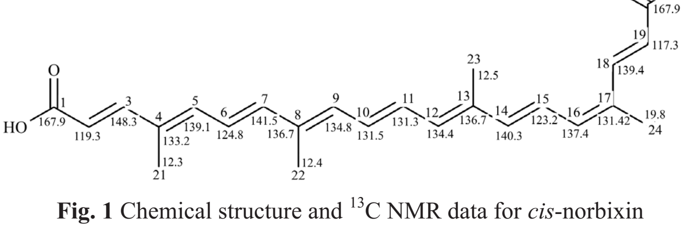

Fig. 1 Chemical structure and ^{13}C NMR data for *cis*-norbixin

Table 1 Kinetic parameters of the *cis*-norbixin

Heating rates/°C min^{-1}	Kinetic parameters	Models			
		Coats–Redfern	Madhusudanan	Horowitz–Metzger	Van Krevelen
5	n	1.92	2.02	2.13	2.07
	E/kJ mol^{-1}	154.12	159.95	178.07	169.66
	A/s^{-1}	4.47E-12	2.05E-13	9.07E-14	5.38E-20
	r	0.9969	0.9970	0.9970	1
10	n	1.71	1.79	1.98	1.84
	E/kJ mol^{-1}	131.35	135.41	157.88	144.85
	A/s^{-1}	1.75E-10	5.15E-10	6.52E-12	1.26E-18
	r	0.9982	0.9983	0.9982	1
20	n	1.67	1.71	2.01	1.90
	E/kJ mol^{-1}	99.44	101.15	116.49	109.98
	A/s^{-1}	6.33E-06	1.04E-07	7.16E-08	2.29E-13
	r	0.9995	0.9995	0.9994	0.9980

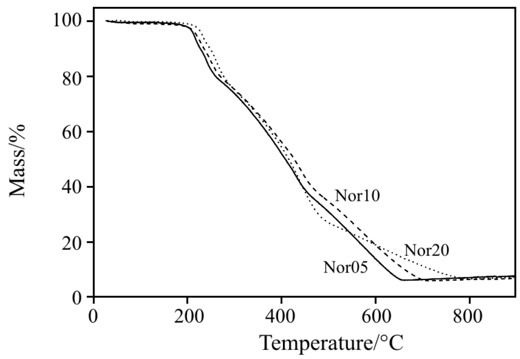

Fig. 2 TG curves of *cis*-norbixin at different heating rates

were similar to each other presenting three stages of thermal decomposition. The kinetic study was done for the first stage. The selection of the probable mechanism that describes the thermal decomposition reactions was certain for the function $g(\alpha)$ using Coats–Redfern method for α=0.1–0.9. F1 mechanism described best the experimental data for all heating rates. Kinetic parameters were calculated from the thermogravimetric curves using Coats–Redfern (CR), Madhusudanan (MD), Horowitz–Metzger (HM) and Van Krevelen (VK) (Table 1) equations.

Conclusions

Cis-norbixin dye was obtained by alkaline hydrolysis from natural dye *cis*-bixin. The structure of *cis*-norbixin was determined by melting point comparison, infrared spectroscopy and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy including 2D techniques. The dye was obtained as an amorphous powder, which decomposes above 280°C, soluble in tetrahydrofuran and acetic acid, showing low solubility in other organic solvents and insoluble in water.

The kinetic parameters obtained by the integral and approach methods exhibited good correlation. However, the values obtained by the integral methods were lower than obtained by approach methods, which is due to the different mathematical treatments of each method. The model that best described the reaction of thermal decomposition was F1, first order reaction.

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