

Thermal analysis of prednicarbate and characterization of thermal decomposition product

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Abstract In the present work, the thermal behavior of prednicarbate was studied using DSC and TG/DTG. The solid product remaining at the first decomposition step of the drug was isolated by TG, in air and N₂ atmospheres and was characterized using LC-MS/MS, NMR, and IR spectroscopy. It was found that the product at the first thermal decomposition step of prednicarbate corresponds to the elimination of the carbonate group bonding to C₁₇, and a consequent formation of double bond between C₁₇ and C₁₆. Structure elucidation of this degradation product by spectral data has been discussed in detail.

Keywords Thermal analysis · Prednicarbate · NMR · LC-MS/MS · IR

Introduction

Prednicarbate (Fig. 1) is a nonhalogenated potent corticosteroid, double-ester derivative of prednisolone. This drug has a low effect in the suppression of interleucin (IL)-1 α and IL-6 in fibroblasts, resulting in low potential in causing skin atrophy [1–3]. The application of this substance in a semi-solid pharmaceutical form is frequently used in the treatment of atopic dermatitis, disease characterized by making the skin dry, inflamed and with intense itching, and its prevalence and gravity, in general, decrease with the aging process. The topical corticosteroids are used frequently for the dermatitis atopic treatment due to its wide activity in immune and inflammatory systems [4–6].

Thermal analysis, mainly differential scanning calorimetry (DSC), thermogravimetry (TG) and derivative thermogravimetry (DTG) techniques, have been used for more than 30 years by pharmacists, applied for the characterization of materials and preformulation study. These techniques measure the physical properties of substances and/or its reaction products in relation to the temperature while the substance is subjected to a controlled temperature program [7–11]. Thermal analysis makes possible to obtain results quickly and requires relatively simple experimental conditions [12]. In several situations the results of thermal analysis need to be associated to those obtained by other physicochemical and/or analytical techniques [9, 13, 14].

The products obtained in the thermal decomposition stages of the studied substance can be identified and have its chemical structure elucidated by the association between the results of thermal analysis to those obtained by other techniques such as mass spectrometry (MS), nuclear resonance magnetic (NMR), and infrared (IR) spectroscopy [15–17].

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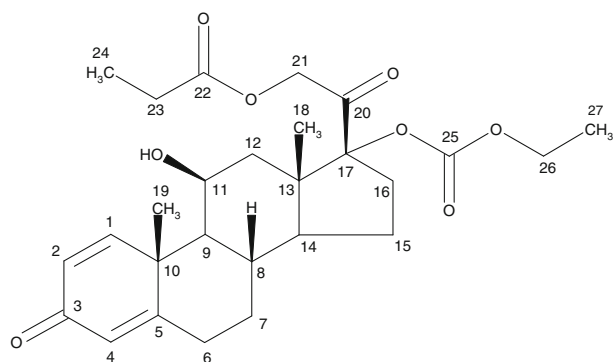


Fig. 1 Chemical structure of prednicarbate

The aim of the present study was to accomplish the characterization of prednicarbate through TG and DSC techniques, and to elucidate the chemical structure of the solid product formed at the first thermal decomposition step of this corticosteroid using high performance liquid chromatography (HPLC) coupled to triple quadrupole mass spectrometry (LC-MS/MS), NMR, and IR.

Materials and methods

Materials

The prednicarbate was obtained from the Hawon Biochemical Science Co., Ltd. This compound (Fig. 1) has a molecular mass and formula of $488.58 \text{ g mol}^{-1}$ and $\text{C}_{27}\text{H}_{36}\text{O}_8$, respectively. For developing MS analysis, acetonitrile (lot No. E02C71) and methanol (lot No. C48E28) HPLC grade obtained from JTBaker were used. Ammonium acetate (lot No. 1306658) was purchased from Fluka and purified water from Mili-Q, Millipore.

Methods

Thermal analysis

TG/DTG curves for the thermal characterization of material were obtained using a thermobalance model TGA 50 (Shimadzu) in temperature range of 25–900 °C, using Pt crucibles with approximately 4 mg of sample, under dynamic N_2 and air atmospheres (50 mL min^{-1}) and at a heating rate (β) of $10 \text{ }^\circ\text{C min}^{-1}$.

In other to obtain the solid product remaining at first step of thermal decomposition of the prednicarbate, a thermobalance model TGA 51 (Shimadzu) carrying Pt crucibles with approximately 80 mg of sample was used, under dynamic N_2 and air atmospheres (both 50 mL min^{-1}), using two different heating rates, β_1 of $20 \text{ }^\circ\text{C min}^{-1}$ up to 254 °C

and β_2 of $2 \text{ }^\circ\text{C min}^{-1}$ up to 264 °C, keeping this isothermal temperature for 30 min.

DSC curves were obtained in a DSC-50 cell (Shimadzu) using Al crucibles with approximately 2 mg of sample, under dynamic N_2 atmosphere (100 mL min^{-1}) and β of $10 \text{ }^\circ\text{C min}^{-1}$ between 25 and 600 °C. The DSC cell was calibrated with In^0 (m.p. = 156.6 °C; $\Delta H_{\text{fus}} = 28.7 \text{ J g}^{-1}$) and Zn^0 (m.p. = 419.6 °C).

LC-MS/MS conditions

The mass spectra were obtained in a LC-MS/MS system, formed by a triple quadrupole mass spectrometry quattro micro model (Micromass) coupled to a high performance liquid chromatography HP1100 model (Agilent). Prednicarbate and its first decomposition product were dissolved in acetonitrile ($1 \text{ } \mu\text{g mL}^{-1}$) and analyzed at a constant flow rate of 0.22 mL min^{-1} of methanol:ammonium acetate 10 mM (97.5:2.5, v/v). In order to ionize the target compound, electrospray ionization (ESI) in positive mode (+) was used. ESI (+) conditions: source temperature at 100 °C, desolvation temperature at 300 °C, cone gas flow and desolvation gas flow were, respectively, 4 and 400 L h^{-1} . The capillary and cone voltage were 3.06 and 23.57 kV, respectively. The argon pressure used for dissociation was 4.51 mbar, and the collision energy (CE) used in order to determine the ion products was 10 eV.

NMR spectroscopy

The results of the proton magnetic resonance ($^1\text{H-NMR}$), of the carbon magnetic resonance ($^{13}\text{C-NMR}$) and of the Distortionless Enhancement by Polarization Transfer 135 in $^{13}\text{C-NMR}$ (DEPT-135) were obtained for the prednicarbate and for the product originated at first step of thermal decomposition for such substance. NMR experiments were performed in DRX 500 model equipment, Avance series (Bruker), using deuterated chloroform (CDCl_3) as solvent. Typical 1D proton experiments were performed over the 0–8 parts per million (ppm), spectral range of 500 MHz. Carbon experiments were performed over the range 0–220 ppm with proton decoupling employed, 125 MHz. DEPT-135 experiments were acquired using the same carbon spectral, 125 MHz.

IR spectroscopy

FTIR spectra of prednicarbate and of its thermal decomposition product were recorded at room temperature in the $4,000\text{--}400 \text{ cm}^{-1}$ range in KBr pellets.

Results and discussion

Thermal behavior of prednicarbate

TG/DTG curves of the prednicarbate sample (Figs. 2, 3) showed no mass loss up to 200 °C. Above this temperature four mass loss steps were observed. The DSC curve (Fig. 2) showed an endothermic event in the 175–196 °C temperature range, however, the TG/DTG curves showed no mass loss in this temperature interval. The sharp endothermic peak corresponded to melting process of prednicarbate ($T_{\text{onset}} = 182.7$ °C; $T_{\text{peak}} = 186.2$ °C; $\Delta H_{\text{fus}} = 73.3$ J g⁻¹). The second thermal event observed in the DSC curve was endothermic and began immediately after the melting process and it corresponds to the first step of thermal decomposition of the drug. This first mass loss event occurred in the 207–291 °C ($T_{\text{peak DTG}} = 264$ °C) temperature range and it represents 20.7% (Δm_1) of mass loss. The third event observed in the DSC curve is an exothermic one ($T_{\text{peak}} = 332.5$ °C) and it corresponds to the second step of thermal decomposition observed in

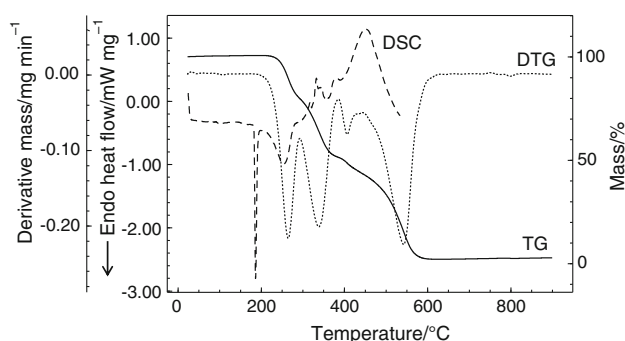


Fig. 2 DSC and TG/DTG curves of prednicarbate in dynamic N₂ atmosphere

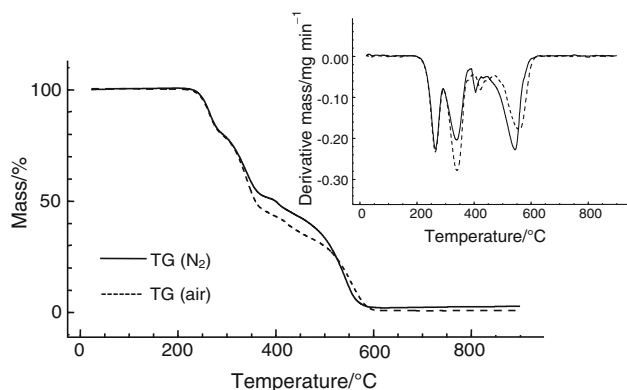


Fig. 3 TG/DTG curves of prednicarbate in dynamic N₂ and air atmospheres

TG/DTG curves ($\Delta m_2 = 28.4\%$, $T_{\text{peak DTG}} = 339$ °C). The third and fourth mass loss steps ($\Delta m_3 = 7.6\%$ and $\Delta m_4 = 42.6\%$, respectively) correspond to the secondary thermal decomposition (Fig. 2).

Characterization of product at first step of thermal decomposition

LC-MS/MS

Prednicarbate was analyzed by LC-MS/MS in positive mode (ESI). The presence of a major positive ion with m/z 489 in mass spectrum of prednicarbate (Fig. 4) indicated the presence of the drug molecule. The ions with m/z 471, 381, 307 and 115 were originated by the break of the ion m/z 489 in the ion source, since they were also present in the product ion mass spectrum of m/z 489.

The mass spectrum of the substance obtained at the first decomposition step of prednicarbate by TG (after isothermal treatment at 264 °C for 30 min, under dynamic N₂ atmosphere), showed m/z 399 as the major ion (Fig. 5). The other ions in the mass spectrum of the primary decomposition product less than 399 are originated by the break of ion m/z 399 in the ion source, because these ions were also presented in the product ion mass spectrum of m/z 399.

The mass spectrum of chemical specie from the first step of thermal decomposition for the prednicarbate originated in dynamic air atmosphere and products ion mass spectrum of m/z 399 (Fig. 6) showed that at this step the same chemical specie was formed both in air and in N₂.

The results acquired by LC-MS/MS to the first thermal decomposition step of the prednicarbate showed a compound with 90 units of molecular mass less than for the original molecule.

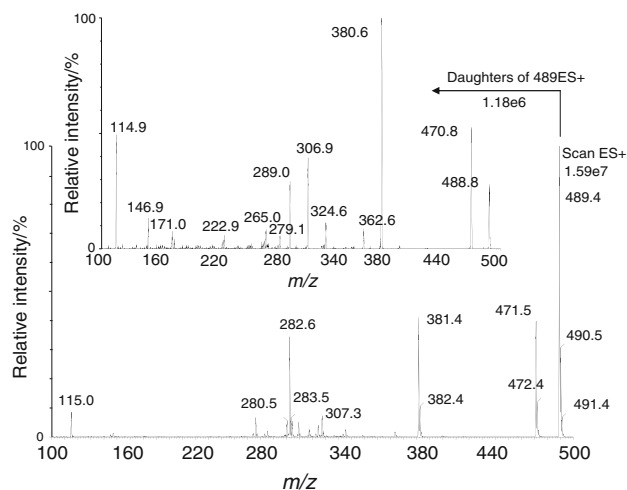


Fig. 4 Scan mode mass spectra of prednicarbate sample and product ion scan mass spectra of protonated at m/z 489

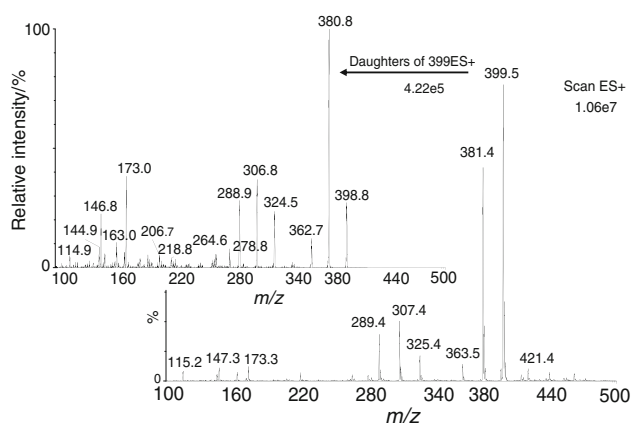


Fig. 5 Scan mode mass spectrum of product at first step of thermal decomposition of prednicarbate isolated in dynamic atmosphere of N_2 and product ion scan mass spectrum of protonated at m/z 399 (CE 10 eV)

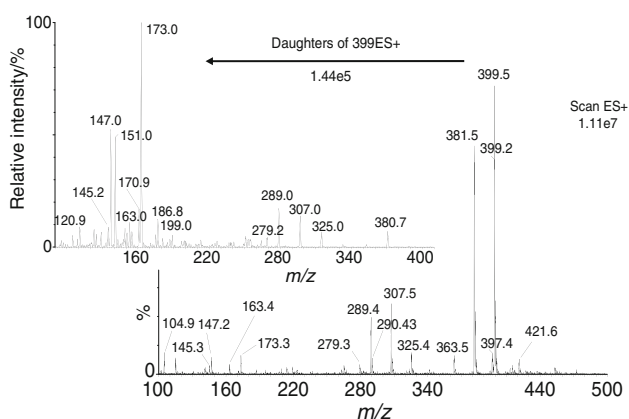


Fig. 6 Scan mode mass spectrum of product at first step of thermal decomposition of prednicarbate isolated in dynamic air atmosphere and product ion scan mass spectrum of protonated at m/z 399 (CE 10 eV)

NMR

The 1H -NMR, ^{13}C -NMR and DEPT-135 analysis were performed for the prednicarbate (Table 1) and for the product at first step of thermal decomposition in dynamic N_2 atmosphere. The evaluation of results for structural elucidation of originated product of thermal decomposition was done from the comparison between the spectra of two compounds, and also with information obtained from literature for the corticosteroid compounds [18, 19].

The 1H -NMR spectrum of the primary decomposition product of prednicarbate in N_2 was evaluated in a comparative way (Fig. 7). The disappearance of one triplet (1.29 ppm, 3H) and one multiplet (4.16 ppm, 2H) showed the elimination of hydrogens bonded to C_{26} and C_{27} , respectively. The appearance of one sign at 6.74 ppm in the

Table 1 NMR spectral data of the prednicarbate^a in $CDCl_3$

Carbon atom number	DEPT-135	^{13}C -NMR δ_C /ppm	1H -NMR δ_H /ppm
1	CH	156.08	7.27 t ($J = 10.0$ Hz; 1.0 Hz)
2	CH	127.77	6.27 dd ($J = 10.0$ Hz; 1.8 Hz)
3	N/A	186.45	N/A
4	CH	122.39	6.02 t ($J = 1.8$ Hz; 1.0 Hz)
5	N/A	169.84	N/A
6	CH_2	27.07	H α 2.34 dd H β 2.57 ddd
7	CH_2	30.38	H α 1.14 H β 2.11
8	CH	31.11	2.15 m
9	CH	54.85	1.17
10	N/A	43.93	N/A
11	CH	69.75	4.51
12	CH_2	39.52	H α 2.14 H β 1.87
13	N/A	47.50	N/A
14	CH	51.58	1.67 m
15	CH_2	23.93	H α 1.83 m H β 1.49 m
16	CH_2	33.72	H α 1.20 H β 2.85 ddd
17	N/A	96.50	N/A
18	CH_3	16.28	1.02 s
19	CH_3	21.01	1.45 s
20	N/A	198.83	N/A
21	CH_2	67.01	4.91; 4.68 dd ($J = 70.0$ Hz)
22	N/A	174.01	N/A
23	CH_2	31.85	2.47 ddd
24	CH_3	8.95	1.18 t
25	N/A	154.22	N/A
26	CH_2	64.67	4.16 m
27	CH_3	14.11	1.29 t

Chemical shifts (δ) are given in ppm; multiplicities and coupling constants (J) in Hz

N/A: not applicable— J and quaternary carbon (DEPT-135 and 1H -NMR)

Multiplicity is indicated as s (singlet), d (doublet), t (triplet) or m (multiplet)

^a δ_H /ppm of the OH groups are not included

spectrum of the thermal decomposition compound refers to hydrogen bonded to C_{16} (1H).

The comparison of ^{13}C -NMR and DEPT-135 spectra of prednicarbate and of product at the first decomposition step (Fig. 8) indicated that there are three carbons less in the

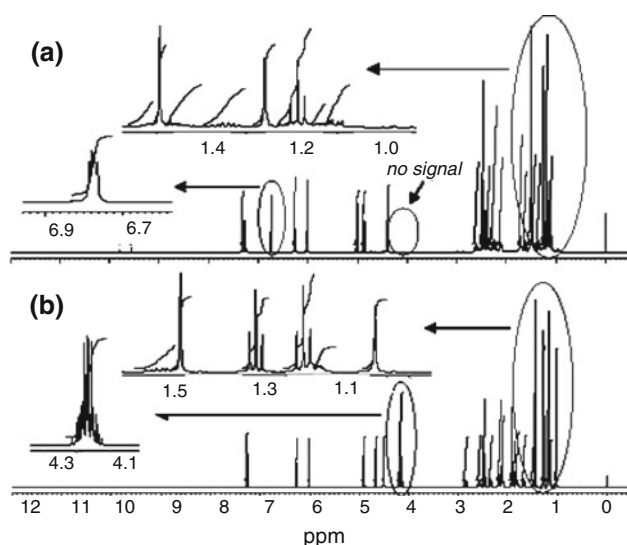
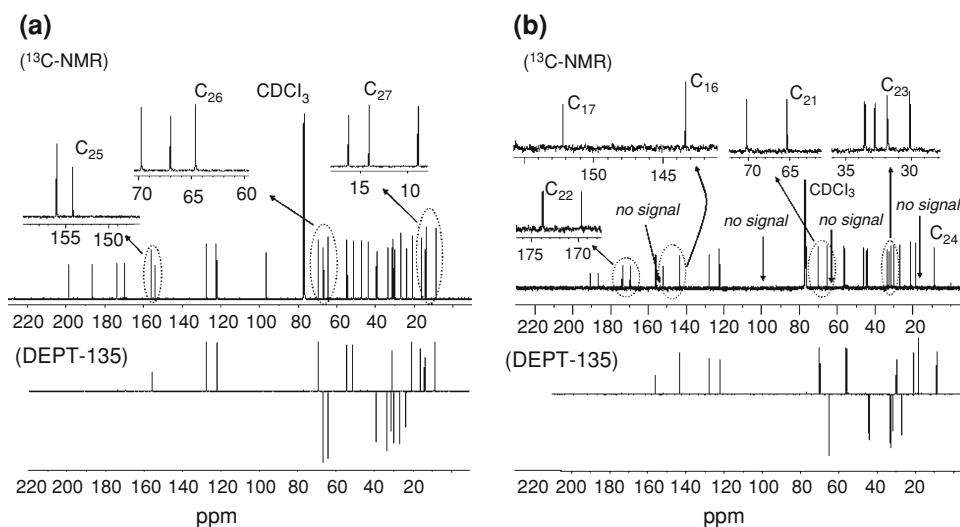


Fig. 7 Comparison of the ^1H -NMR spectra (500 MHz) in CDCl_3 solution of the product at first step of thermal decomposition of prednicarbamate isolated in N_2 atmosphere (a) and prednicarbamate (b)

new compound. In the spectra of the thermal decomposition compound, the disappearance of signs at 14.1 ppm (C_{27} , CH_3), 64.5 ppm (C_{26} , CH_2), and 154.2 ppm (C_{25} , C_{4°) was observed. The characteristic signs of C_{21} (65.36 ppm, CH_2), C_{22} (173.85 ppm, C_{4°), C_{23} (31.86 ppm, CH_2), and C_{24} (9.01 ppm, CH_3) remained the same in the spectrum of the thermal decomposition compound. The chemical shift characteristic of C_{17} for the prednicarbamate spectrum (96.50 ppm, C_{4°) was not observed in the spectrum of the thermal decomposition product. A new sign, however, was observed at 152.3 ppm (C_{4°). This sign corresponds to the C_{17} , a new sp^2 -hybridized carbon in the thermal decomposition compound. This modification occurred due to the elimination of one of the lateral chains bonded to this carbon atom. The originated double bond

Fig. 8 Comparison of the ^{13}C -NMR spectra and DEPT-135 spectra in CDCl_3 solution of the prednicarbamate (a) and product at first step of thermal decomposition of the prednicarbamate isolated in N_2 atmosphere (b)



between C_{17} and C_{16} can also be demonstrated from the chemical shift observed for the C_{16} , which occurred in the spectrum of the thermal decomposition compound at 143.4 ppm (CH).

IR spectroscopy

The IR spectra of prednicarbamate (Fig. 9a) and the product of the first thermal decomposition step, obtained in dynamic N_2 (Fig. 9b) and air (Fig. 9c) atmospheres, were evaluated in a comparative way. The IR spectrum of prednicarbamate showed absorption band assigned to $\text{C}=\text{O}$ group ($\nu_{\text{C}=\text{O}}$) at $1,752\text{ cm}^{-1}$ that is characteristic of the ester (C_{22}) and carbonate (C_{25}) groups. The absorption bands at $1,282$ and $1,083\text{ cm}^{-1}$ were observed in the same spectrum, respectively, are assigned to asymmetric stretching of $\text{C}-\text{O}$ ($\nu_{\text{as}}\text{ C}-\text{O}$) in carbonate group and symmetric stretching of $\text{C}-\text{O}$ ($\nu_{\text{s}}\text{ C}-\text{O}$) in ester group.

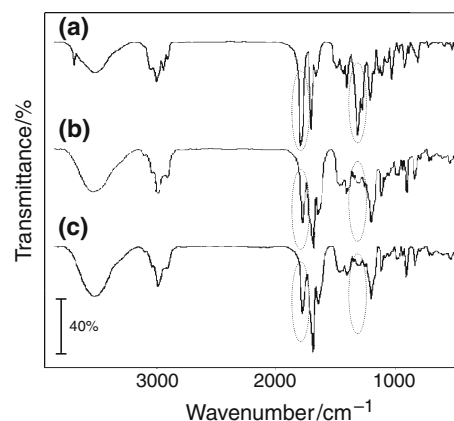
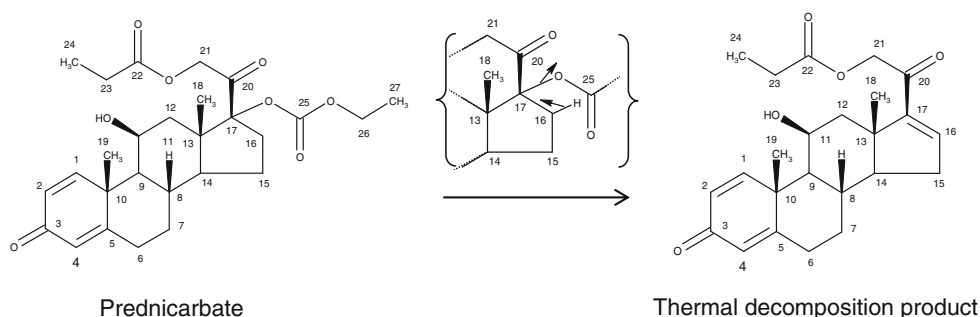


Fig. 9 IR spectra of prednicarbamate (a) and product at first step of thermal decomposition of the prednicarbamate isolated in N_2 (b) and air (c) atmospheres

Fig. 10 First step of thermal decomposition of prednicarbate by thermogravimetry ($T_{\text{peak DTG}} = 264\text{ }^{\circ}\text{C}$)



The presence of stretching band with smaller intensity at $1,749\text{ cm}^{-1}$ and the presence of stretching band at $1,083\text{ cm}^{-1}$ in the IR spectrum of the product at first step of thermal decomposition of the prednicarbate in dynamic N_2 and air atmospheres, indicated that the ester group remained in these molecules. On the other hand, the disappearance of stretching band at $1,280\text{ cm}^{-1}$ and the presence of stretching band with less intensity at $1,750\text{ cm}^{-1}$ indicated that the elimination of the carbonate group occurred at first step of the thermal decomposition of prednicarbate.

Formation of product at first step of thermal decomposition

The results obtained by LC-MS/MS, NMR, and IR showed that the product formed in the first step of thermal decomposition is less by 90 molecular mass units than the prednicarbate, due to the elimination of one of the chemical groups bonded to C_{17} (Fig. 10).

Conclusions

The thermal analysis has been widely employed in pharmaceutical areas, becoming an important tool in the study of drugs. In the present work, the first mass loss event of prednicarbate was observed between 207 and $291\text{ }^{\circ}\text{C}$ ($T_{\text{peak DTG}} 264\text{ }^{\circ}\text{C}$). This thermal event resulted in a thermal decomposition compound less by 90 molecular mass units than for the prednicarbate, according to mass spectrometry results. The use of NMR and IR techniques made possible to characterize the structure details of this decomposition compound. With the association of results from these analytical techniques it was possible to determine that the thermal decomposition compound was originated by the elimination of carbonate group bonded to C_{17} , and subsequent formation of double bond between C_{16} and C_{17} .

Therefore, TG can be considered as an important technique to obtain the thermal decomposition product of prednicarbate and, when associated to other analytical techniques in order to elucidate the molecular structure,

such as LC-MS/MS, NMR, and IR, it is possible to use this product as a reference substance.

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