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THERMOANALYTICAL AND SPECTRAL STUDY OF EFAROXAN HYDROCHLORIDE

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Abstract

The physicochemical characterization of the solid-state enantiomers and racemate of efaroxan hydrochloride ($C_{13}H_{17}N_2O^+Cl^-$, M=252.5 g mol^{-1}) was performed by thermoanalytical methods (differential scanning calorimetry, thermogravimetry and thermomicroscopy) and spectral methods (infrared spectrometry and X-ray diffractometry).

The efaroxan enantiomers and racemate were shown to be unstable near the melting point. At the beginning of the decomposition, a loss of hydrogen chloride was observed. However when sealed pans were used, the compounds decomposed at higher temperature, allowing a precise evaluation of the melting enthalpies by means of differential scanning calorimetry. The nature of the racemate and its thermal stability were assessed by evaluating its free formation enthalpy. An enantiotropic solid–solid transformation (II→I)was noted for the racemate; the reverse process (I→II) follows zero-order kinetics.

Keywords: DSC, enantiomer, IR, powder X-ray diffractometry, racemate, TG, thermomicroscopy

Introduction

The development of a new chiral drug today requires a toxicological study of the optical antipodes. Only the optical antipode with the weaker toxicity should be a candidate for future development. When optical antipodes display identical toxicity, the racemic compound should be developed. Efaroxan, (±)-2-[2-(2-ethyl-2,3-dihydro-2-benzofuranyl)]-2-imidazoline (CAS Registry Number 99197-32-0), is a potent and highly selective α-2-adrenoreceptor antagonist. Efaroxan and its derivatives have been studied by Pierre Fabre laboratories for

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use in neurodegenerative diseases and their progression, and in cognitive deficits, particularly Parkinson's and Alzheimer's diseases [1].

Efaroxan possesses an asymmetric C atom on the dihydrobenzofuranyl ring [C(2)] and therefore exhibits two enantiomers.

The *R*(+) enantiomer is the active form and previous studies have determined its absolute configuration [2].

The chirality here involves identical physicochemical properties of two optical antipodes, e.g. melting point, boiling point, solubilities in non-chiral solvents, density, refractive index and nuclear magnetic resonance spectrum, whereas optical rotation can distinguish between them. An equimolar mixture of the enantiomers is called a racemic mixture or racemate and must fit into one of the following three categories [3]:

– a conglomerate: mechanical crystalline mixture of the two enantiomers;

– a racemic compound: the two enantiomers are present in equal quantities in a well-defined arrangement inside a crystalline system;

– a pseudoracemate: the two enantiomers form a solid solution.

In the work presented here, we studied the thermal stabilities of both enantiomers and used spectral and thermal methods to categorize the racemate and describe its polymorphism. A complementary and theoretical thermodynamic study was applied to evaluate its stability by calculating the free formation enthalpy ΔG^{Φ}.

This thermodynamic function characterizes the state of association of the (*R*) and (*S*) enantiomers in the solid racemate and may be used to anticipate whether optical resolution by crystallization with seeding or preferential crystallization can take place [4, 5].

Generally, these methods are less expensive than asymmetrical synthesis or the formation and separation of diastereoisomers.

Materials and methods

Materials

Enantiomers: the enantiomeric resolution of the (+) and (–)-efaroxan enantiomers from the racemate was performed by fractional crystallization in methanol of the (+) and (–)-dibenzoyl tartrate salts.

Forms I and II of the racemate: form II was the initial form of the racemate, whereas form I was obtained by thermal treatment of form II. The conditions required to obtain form I were dependent upon the process subsequently used for characterization:

– differential scanning calorimetry: 5 min at 210°C,
– X-ray diffractometry: 5 min at 185°C,
– infrared spectrometry: 5 min at 200°C.

Methods

Thermogravimetry

TG curves were obtained on a DuPont de Nemours 2000 thermal analyser system. The samples (15 to 20 mg) were deposited in a platinum pan. TG curves were recorded under a nitrogen gas flow (10 ml min^{-1}) and over the temperature range from 50 to 400°C at a heating rate of 10°C min^{-1}.

Differential scanning calorimetry

DSC profiles were obtained on a Mettler FP 800 thermal analyser fitted with an Epson HX 20 microcomputer and an FP 80 HT temperature controller. The samples (3 to 5 mg) were analysed in 40 µl sealed pans under a nitrogen gas flow (10 ml min^{-1}). Thermal behaviour was studied at a heating rate of 5°C min^{-1}, and heat capacities were determined with sapphire as reference. The average values and standard deviations were calculated on five samples.

Thermomicroscopy

A Leitz SM POL microscope connected to an FP52 hot stage and a Mettler FP5 temperature controller was used for microscopy investigations. Observations were videotaped with a Sony DXC 101P color video camera attached directly to the microscope. A few mg of sample was placed between the slide and coverslip and heated at a rate of 10°C min^{-1} from 100 to 200°C.

X-ray diffractometry

Powder X-ray diffraction patterns were measured on a Philips model PW 1730 powder X-ray diffractometer fitted with a CGR horizontal type goniometer with monochromatized CuK_α (λ=1.54051 Å). The instrument was set at a voltage of 40 kV and a current of 20 mA.

- Angle range investigated: 3 to 25 theta degrees (°θ),
- time of acquisition per point: 500 ms,
- number of scans: 5.

The transformation of form I to form II was sufficiently slow to allow form I to be studied by X-ray diffractometry at room temperature.

Infrared spectrometry

IR spectra were obtained on a Perkin-Elmer 1600 Fourier transform IR spectrometer fitted with a diffuse reflectance accessory. The Kubelka-Munk correction was applied to the diffuse reflectance data to create absorption-like spectra. Samples were examined as KBr-triturated (1%, *w/w*).

Kinetic study of the polymorphic transformation I→II

Form I was prepared by thermal treatment of form II (5 min at 200°C). The sample was stored at room temperature for a fixed time period (t). During this period, form I reverted back to form II. The percentage of form I remaining depended on the time (t) during storage, and it was determined by measuring the quantity of heat (ΔH_t) generated by the transformation II↔I.

If, at time t=0, the transformation enthalpy is ΔH_0, the given fraction of form I is given by the following relation:

$$\alpha = \frac{\Delta H_t}{\Delta H_0} \tag{1}$$

Results and discussion

Study of efaroxan enantiomers

Thermogravimetry (Fig. 1a) indicated the start of mass loss at 155°C, before melting. This corresponded to sublimation of the sample, as confirmed by ther-

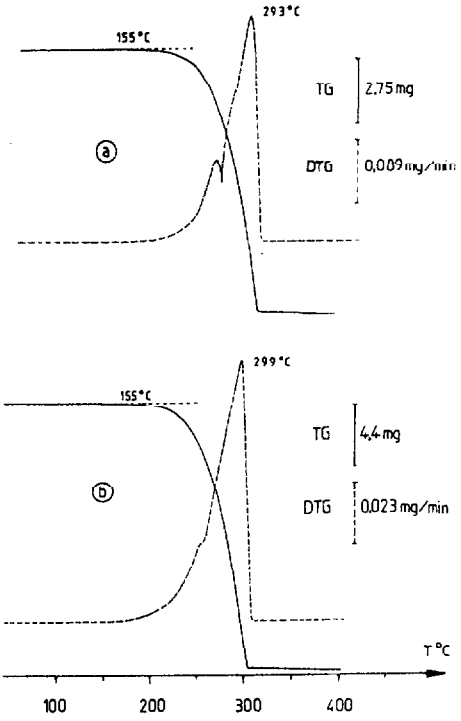

Fig. 1 TG — and DTG --- curves after heating at a rate of 10°C min^{-1}. a – enantiomers; b – racemate

momicroscopy (white deposit on the edges of the blade). The molecule began to decompose at about 235°C, with a peak at 293°C (v=31.2 mg min^{-1}).

This decomposition takes place in several stages, the first of which corresponds to a mass loss of 14% due to the removal of hydrogen chloride (% calculated=14.4%).

The DSC curve obtained under conditions similar to those used during thermogravimetry situated the fusion in the decomposition region of the molecule (T_R^f=245.1±0.3°C). Under these conditions, the measured enthalpy does not correspond to the fusion.

However, the use of hermetic crucibles shifted the decomposition to higher temperatures, and the fusion enthalpy can be precisely determined in this way: ΔH_R^f=30.2±0.8 kJ mol^{-1}. Figure 2a corresponds to the DSC curves recorded under conditions similar to those used during thermogravimetry. The Figure shows superposition of the two phenoma, fusion appearing as a narrow peak, and decomposition providing a substantial drift of the baseline. When sealed pans were used (Fig. 2b), only fusion of the sample was observed.

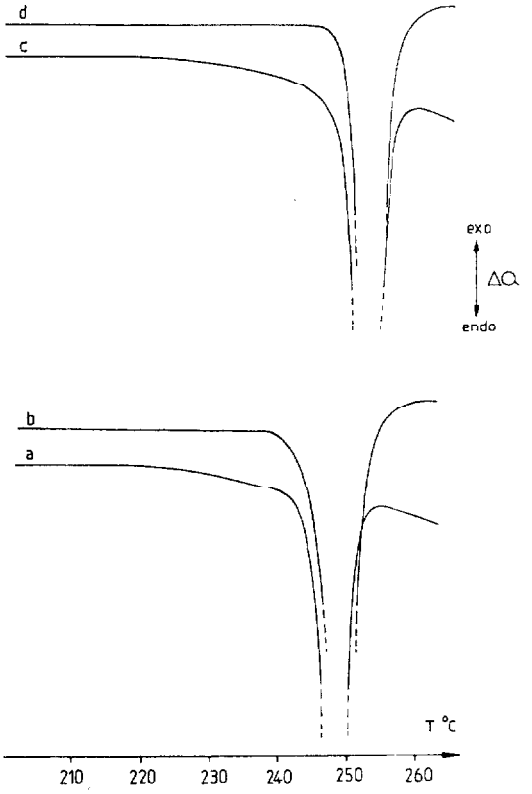

Fig. 2 DSC curves. a – enantiomer, non hermetic crucible; b – enantiomer, hermetic crucible; c – racemic, non hermetic crucible; d – racemic, hermetic crucible

The IR spectrum (Fig. 3a), recorded in the diffuse reflectance mode demonstrated several regions:

- 3200–3100 cm^{-1}: vinyl CH,
- 3050–3000 cm^{-1}: aliphatic CH: C_{sp3}-H, C_{sp2}-H,
- 1600–1590 cm^{-1}: aromatic C=C, cyclic imine: =N–,
- 1500–1450 cm^{-1}: C–H bending of the $-CH_2$ group,
- 1370–1340 cm^{-1}: C–H bending of the $-CH_3$ group,
- 1150–1070 cm^{-1}: C–O–C bending.

The stretching vibrations N^+–H(Cl^-) in the hydrochloride are situated between 2550–2800 cm^{-1}. Two absorption bands are observed at 2626 and 2728 cm^{-1} because the both nitrogen atoms are involved in two kinds hydrogen bonds [2]. The first is an intermolecular hydrogen bond between the nitrogen atom and the

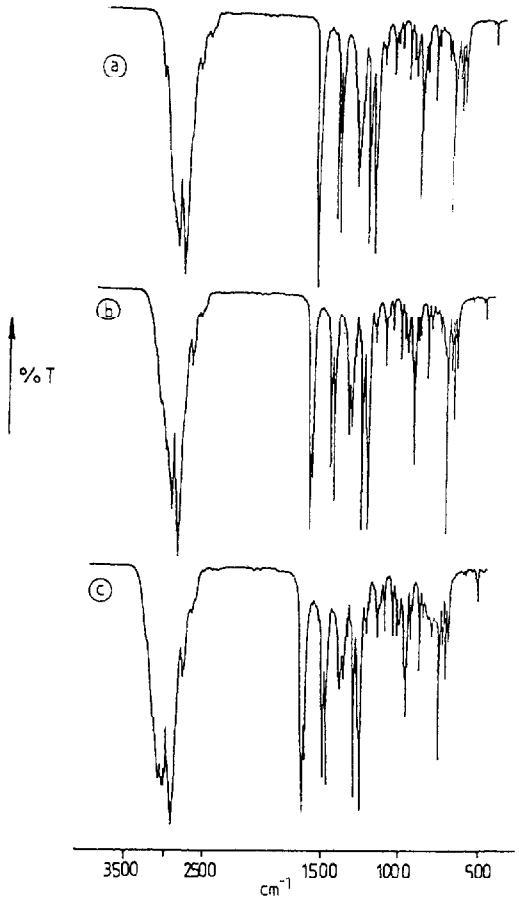

Fig. 3 IR spectra. a – enantiomers; b – racemic form II; c – racemic form I

chloride atom of the next molecule (N–H(O,Cl)) whereas the second is an intermolecular hydrogen bond between the chloride and the oxygen atoms (N–H(O,Cl)).

We can suppose that $\bar{\nu}$(N–H(Cl)) is superior to $\bar{\nu}$(N–H(O,Cl)) because the strength constant of N–H(O,Cl) is undoubtedly the weakest, so $\bar{\nu}$(N–H(Cl))= 2728 cm^{-1} and $\bar{\nu}$(N–H(O,Cl)) =2626 cm^{-1}.

Study of efaroxan racemate

Nature of racemate

It is possible to characterize the nature of a racemate by comparing its IR spectrum with those of the enantiomers. The racemates of several chiral molecules have been identified by these spectral methods [6–8].

Comparative analysis by X-ray diffractometry (Table 1) of the enantiomers and racemate (forms I and II) allows an unambiguous identification of the nature of the racemate, as the X-ray diffraction patterns of the (*R*) enantiomer and the (*RS*) racemate are different.

These two species therefore have different structures, and the racemate can not be a mechanical mixture (conglomerate) of the two enantiomers, but is a well-defined entity.

This difference in crystalline structure should be perceptible by means of IR spectrometry because the symmetry of the crystalline system and the relative arrangement of the molecules lead to particular modes of vibration.

The IR spectra of the *R* enantiomer and *RS* racemate exhibit some differences (Figs 3a,b). If we consider the IR spectra of *R*, two new bands are observed at 1253 and 1590 cm^{-1}, and the band at 3193 cm^{-1} has disappeared.

Thermoanalytical study

The TG curves are very similar to those of the enantiomers (Fig. 1b). The mass loss at 155°C is due to sublimation. The decomposition which begins at 220°C takes place in several stages, with peak decomposition at 299°C (v=4.5 mg min^{-1}).

The first stage in the decomposition process, which was attributed for the enantiomers to the loss of hydrogen chloride, is partially masked in the racemic compound by the overall decomposition.

The racemic compound melts (T^f_{RS}=247.8±0.2°C) within the decomposition region. As already seen for the enantiomers, the fusion enthalpy must be determined in hermetic crucibles so that the decomposition is shifted to higher temperatures (Figs 2c, d): ΔH^f_{RS}=31.5±0.6 kJ mol^{-1}.

Examination of the racemate and enantiomer melting temperatures can be performed to check that the racemate is not a conglomerate at its melting temperature because $T^f_{RS}>T^f_R$ [3].

Table 1 X-ray powder diffraction data

Enantiomers		Racemic form II		Racemic form I	
°θ	I/10	°θ	I/10	°θ	I/10
		4.06	51		
		5.84	11	5.80	28
		6.30	52	6.30	17
6.50	48				
6.72	59				
7.96	29	7.92	29	7.92	28
				8.12	58
		8.22	50		
8.36	50				
				8.58	22
8.70	13				
9.08	11				
9.62	24			9.66	32
		9.96	55		
		10.52	17		
10.62	31				
10.84	17	10.78	11		
				11.16	17
11.32	42	11.24	23		
11.56	24				
		11.62	23	11.66	39
11.94	100			11.90	100
		12.14	100		
12.30	31				
				12.40	59
12.58	20	12.66	26		
12.78	18				
13.08	68	13.04	93		
		13.38	16	13.32	39
13.44	52				
		14.06	16		
14.48	21				
		14.64	26	14.66	20

Table 1 Continued

Enantiomers		Racemic form II		Racemic form I	
°θ	I/10	°θ	I/10	°θ	I/10
		14.64	26	14.66	20
14.84	12				
		15.00	23		
15.98	28			15.94	16
		16.28	27		
16.84	19				
		17.18	13		
17.66	15				
20.12	16			20.06	16
		20.50	17		
21.60	10				
23.12	10				

Nature of the polymorphic transformation

Differential scanning calorimetry revealed a transformation at T_o=180.0±2°C, with an enthalpy of ΔH_o=3.8±0.1 kJ mol^{-1} (Fig. 4). Thermomicroscopy showed that the solid↔solid transformation took place at 189.8±0.8°C (heating rate: 10°C min^{-1}).

Form I is characterized by irregular square ruler crystals, which are colored in polarized light. During the process, crystals of form II are transformed into crystals of form I (prismatic aggregates).

The X-ray diffractogram pattern of form I (Table 1) demonstrates different lines at 8.12, 11.90 and 12.40°θ that are not observed for racemate form II or the enantiomers.

The IR spectra of forms I and II are quite different, in particular within the range 2900–3000 cm^{-1}.

Consequently, the transformation observed by DSC and thermomicroscopy is a solid↔solid transformation between two crystalline forms (II and I) of the racemic compound.

The transformation temperature is less than the melting point of each crystalline form and the transformation is enantiotropic [9, 10]. Form II and form I are therefore stable within their temperature ranges, and the conversion from one to the other can take place reversibly at T_o (temperature of the transformation). Experimentally, we note the temperature T_o, but under our conditions we could not observe the melting temperature of form II, whereas form I melts at 247.8±0.2°C.

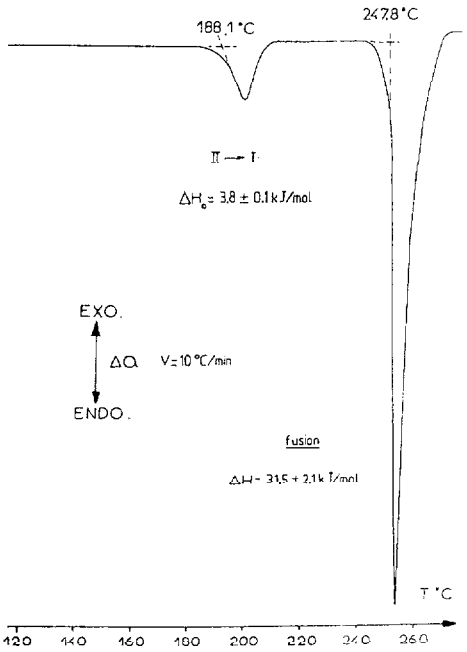

Fig. 4 DSC curve of racemic compound showing the polymorphic transition

The transformation I→II is not spontaneous, even on when slow cooling. This transformation process was the subject of a kinetic study.

Kinetic study of the transformation I→II

The mechanism of a reaction can be represented by the energy graph=f(reaction coordinates) in Fig. 5, where I corresponds to the initial state and II to the final state of the process, E_a and E'_a are the activation energies, and ΔH_o is the enthalpy of transformation. If $\Delta H_o>0$, then $\Delta H_o=E_a-E'_a$.

Determination of the activation energy

Firstly, the activation energy E_a of the transformation II→I was determined by differential scanning calorimetry, using the Kissinger law [11] represented by the following equation:

$$-\frac{E^*}{R}=\frac{d\left(\ln\frac{\beta}{T_m^2}\right)}{d\left(\frac{1}{T_m}\right)} \qquad (2)$$

where E^* is the activation energy J mol^{-1}, R is the gas constant 8.28 J mol^{-1}, β is the heating rate (K min^{-1}), and T_m is the sample temperature at which the peak differential thermal analysis deflection occurs (K).

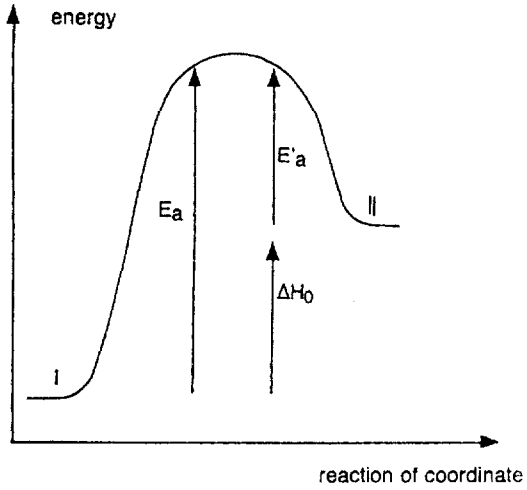

Fig. 5 Energy diagram showing the enantiotropic polymorphism

At different heating rates, we studied the shift in T_m for the transformation II→I (Table 2) and plotted

$$\ln\frac{\beta}{T_m^2} = f\left(\frac{1}{T_m}\right) \tag{3}$$

The graph is the straight line $y=30.940-1.9314\cdot10^4x$, with $r^2=0.998$. The slope of this straight line gives the activation energy, $E_a=158.3$ kJ mol^{-1}. E_a' can not be determined experimentally by the Kissinger method because the reversible transformation is not spontaneous.

Table 2 Variation of T_m with β

β/K min^{-1}	T_m/K	$1/T_m$/K^{-1}	$\ln(\beta/T_m^2)$
5	464.0	$2.1552\cdot10^{-3}$	−10.670
10	472.5	$2.1164\cdot10^{-3}$	10.013
15	475.3	$2.103\cdot10^{-3}$	−9.620
20	479.5	$2.0855\cdot10^{-3}$	−9.350

However, if the mechanism of the transformation is similar to that described by Fig. 5, then $E_a'=E_a-\Delta H_o=158.3-3.8=154.5$ kJ mol^{-1}.

Kinetic determination of the transformation I→II

The transformation rate of form I into form II is expressed by the following equation:

$$v = \frac{d|I|}{dt} = k|I|^n \tag{4}$$

where n is the reaction order.

Integration of this equation between [I] and $[I]_o$ gives

$$t = \frac{1}{k(n-1)}\left(|I|^{1-n} - |I|_o^{1-n}\right) \tag{5}$$

The half reaction time is $t_{1/2}$:

$$t\frac{1}{2} = \frac{1}{k(n-1)}\left(|I|_o^{1-n}(2^{n-1} - 1\right) \tag{6}$$

The reaction is zero order, and this gives

$$kt = |I| \quad |I|_o \quad \text{and} \quad t\frac{1}{2} = \frac{k}{2} \tag{7}$$

If the rate of conversion α is taken into account, then [II]=α=kt.

Table 3 Kinetics data

t/h	ΔH_t/kJ mol^{-1}	α
0.00	0.0	0.00
54.75	6.5	0.43
63.25	7.4	0.49
72.00	9.3	0.62
95.50	12.5	0.83

Table 3 presents the time (t), the enthalpy of the transformation II→I (ΔH_t) and the rate of conversion α. The plot α=kt gives the straight line $y=-1.8743\cdot10^{-2}+8.640\cdot10^{-3}x$ with $r^2=0.991$. The reaction is zero order. The slope of this straight line gives the constant rate transformation: $k=8.64\cdot10^{-3}$ mol h^{-1}.

The half reaction times is $t_{1/2}$=57.9 h.

This result confirms the relatively slow kinetics of the transformation I→II at room temperature, which allows form I to be characterized by spectral methods.

Stability of the racemic compound and thermodynamic aspects

The stabilities of racemic compounds have been studied by various authors [12, 13]. Stability is defined by the free enthalpy of formation of the racemic

compound (ΔG^{ϕ}) at the melting temperature of the enantiomers T_R^f when $T_R^f<T_{RS}^f$, and corresponds to the following equilibrium:

$$R \text{ crystal} + S \text{ crystal} \leftrightarrow RS \text{ crystal}$$

- $\Delta G_{T_R^f}^{\phi}<0$: formation of the racemate,
- $\Delta G_{T_R^f}^{\phi}>0$: dissociation of the racemate.

The free enthalpy of formation of the racemic compound at the melting temperature of the enantiomers is given by the following relationship:

$$\Delta G_{T_R^f}^{\phi} = \Delta H_{T_R^f}^{\phi} - T_R^f \Delta S_{T_R^f}^{\phi} \tag{8}$$

$\Delta H_{T_R^f}^{\phi}$ is the enthalpy of formation of the racemic compound at T_R^f, and is given by the following expression:

$$\Delta H_{T_R^f}^{\phi} = \Delta H_R^f - \Delta H_{RS}^f + (C^l - C_{RS}^s)(T_{RS}^f - T_R^f) \tag{9}$$

ΔH_R^f is the fusion enthalpy of the enantiomer, 30.2 ±0.8 kJ mol^{-1}; ΔH_{RS}^f is the fusion enthalpy of the racemic compound, 31.5 ±0.6 kJ mol^{-1}; C_{RS}^S, C^l are the heat capacities of the racemic compound in the solid phase and the liquid phase, with $C^l-C_{RS}^S$=−26.8 ±2.1 J mol^{-1}K^{-1}, T_R^f is the melting temperature of the enantiomers, 518.1 ±0.3 K; and T_{RS}^f is the melting temperature of the racemic compound, 520.8 ±0.2 K.

Comment: Calculation of $(C^l-C_{RS}^S)$ for most racemates provides a positive value of about 105.0 J mol^{-1} K^{-1} [12]. In the present work, the negative value can be explained by the instability of the molecule in the liquid phase.

$\Delta S_{T_R^f}^{\phi}$ is the entropy of formation of the racemic compound at T_R^f, and is given by the following expression:

$$\Delta S_{T_R^f}^{\phi} = \Delta S_R^f - \Delta S_{RS}^f + R\ln 2 + (C^l - C_R^s)\ln\frac{T_{RS}^f}{T_R^f} \tag{10}$$

ΔS_R^f, ΔS_{RS}^f and Rln2 are the entropies of fusion for the enantiomers and the racemate, and the mixture entropy.

Equations (9) and (10) are applicable when heat capacities vary little with temperature. This hypothesis seems reasonable, because the temperature range considered, $T_R^f<T<T_{RS}^f$, is small: $T_{RS}^f-T_R^f$=248.1−245.1=2.7°C.

By combining equations [8], [9] and [10], we obtain the general expression for $\Delta G_{T_R^f}^{\phi}$:

$$\Delta G_{T_R^f}^{\phi} = \Delta H_{RS}^f\left(\frac{T_R^f}{T_{RS}^f} - 1\right) - T_R^f R\ln 2 + (C^l - C_{RS}^s)\left(T_{RS}^f - T_R^f - T_R^f \ln\frac{T_{RS}^f}{T_R^f}\right) \tag{11}$$

This gives the following value:

$$\Delta G^{\omega_1}_{T_R} = -3135 \pm 750 \text{ J mol}^{-1}$$

The free enthalpy of formation of the racemic compound at the melting temperature of the enantiomers is negative. The racemic compound is thermodynamically stable at the melting temperature of the enantiomers. In fact, the contribution made by the terms which include heat capacities are negligible. The stability of the efaroxan hydrochloride racemate is essentially entropic.

This result excludes optical resolution by preferential crystallization of the enantiomers because their equimolar mixture crystallizes into a stable racemic compound.

Conclusions

The present work has identified several physicochemical properties of efaroxan hydrochloride by using thermoanalytical methods such as differential scanning calorimetry, thermogravimetry and thermomicroscopy, and spectroscopic methods such as infrared spectroscopy and powder X-ray diffractometry.

The study showed that enantiomer and racemate decomposition at the melting temperature is a complex phenomenon that occurs in several stages, the first of which corresponds to the loss of hydrogen chloride. However, under special conditions, we were able to shift the decomposition to higher temperatures, and characterized the enantiomers and racemate by their enthalpies and melting temperatures.

The racemate is a racemic compound and we demonstrated that an enantiotropic transformation takes place at 180°C between two crystalline forms (forms II and I). Below this temperature, form II is the stable form of the racemate; its fusion is never observed. Form I is stable between 180°C and its melting temperature of 247.8±0.2°C.

The transformation I→II has zero-order kinetics. This transformation is sufficiently slow for form I to be observed and characterized at room temperature.

A thermodynamic study allowed characterization of the stability of the racemate by calculation of its free enthalpy of formation. The negative value obtained shows that the racemic compound is relatively stable in the solid state. This means that, of the different optical resolution methods available, the method by crystallization with seeding or preferential crystallization is probably nor achievable. A study of the binary phase diagram between the two enantiomers should confirm this result.

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