Thermal behaviour and binary phase diagram of (S)-(+)-4,4'-(1-methyl-1,2-ethandiyl)-bis-(2,6-piper-azinedione) (dexrazoxane), a cardioprotective agent, and of its (R)-(−)-enantiomer

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Abstract: The binary phase diagram of (S)-(+)-4,4'-(1-methyl-1,2-ethandiyl)-bis-(2,6-piperazinedione), **1** (dexrazoxane), a cardioprotective agent, and of its (R)-(−)-enantiomer, **2**, has been investigated by differential scanning calorimetry (DSC); the equimolecular mixture of **1** and **2** corresponds to a racemic compound (racemate) whose melting point is higher than that of the enantiomers. Thermal behaviour (DSC) is examined and discussed in comparison with the data obtained by other physical methods (IR spectroscopy and X-ray powder diffraction).

Keywords: *(S)-(+)-4,4'-(1-methyl-1,2-ethandiyl)-bis-(2,6-piperazinedione); binary phase diagram; enantiomeric purity; differential scanning calorimetry; IR spectroscopy; X-ray powder diffraction; polarimetry.*

Introduction

The relevance of stereochemical aspects for biologically active substances has been recently reappraised, particularly in the case of enantiomeric relationships [1, 2]. In the solid state, intercrystalline forces between molecules are highly specific and sensitive also to minor changes in molecular geometry. Therefore, although a molecule of the (+)-enantiomer bears the same relationship to another molecule of the (+)-form as a molecule of the (−)-enantiomer bears to another (−)-molecule, the interrelation between (+)- and (−)-molecules is different [3]. As a consequence, in the solid state, deviations from ideal behaviour are frequently observed, e.g. when the compounds are subjected to heating and melting. The different cases experimentally encountered are: racemic mixtures, racemic compounds and racemic solid solutions [3]. In the present paper are reported the results of differential scanning calorimetry (DSC) experiments for the determination of the binary phase diagram involving (S)-(+)-4,4'-(1-methyl-1,2-ethandiyl)-bis-(2,6-piperazine-dione), compound (dexrazoxane), **1**, and its R-enantiomer, **2**. **1** is a drug used as cardioprotective agent in the clinical treatment of cancer. Its synthesis,
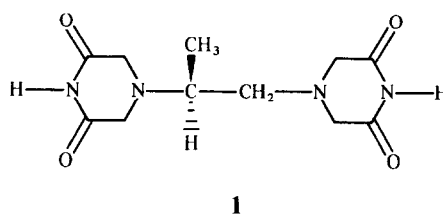
Scheme

physico-chemical properties and biological activity have been described [4, 5]. This study was performed in order to establish to which of the above mentioned cases 4,4'-(1-methyl-1,2-ethandiyl)-bis-(2,6-piperazinedione) belongs and to check its enantiomeric purity. Other physical methods (IR spectroscopy and X-ray powder diffraction) have been also applied.

Materials and Methods

The S-enantiomer (dextrorotatory, **1**) is a monomorphic crystalline substance with $[\alpha]_D^{20}$ + 48.7° (c 1.0, 0.1 M citrate buffer, pH 2.5). RP-HPLC analysis displayed a single peak (UV detection at 210 nm). By DSC a well-shaped melting peak at 194.4°C has been observed (Fig. 1) corresponding to a molar purity of 99.8%, the remaining being assumed to be the R-enantiomer. Thermal parameters

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are given in Table 1. The IR spectrum (KBr pellet) is shown in Fig. 2. Calc. for $C_{11}H_{16}N_4O_2$: C, 49.25; H, 6.01; N, 20.89. Found: C, 49.21; H, 6.06; N, 20.93.

The *R*-enantiomer (laevorotatory, **2**) is also chemically pure (RP-HPLC single peak at 210 nm), with $[\alpha]_D^{20} - 48.2°$ in the same con-

ditions as for **1**; by DSC molar purity is 99.7%, the remaining being assumed to be the *S*-enantiomer. Found: C, 49.06; H, 6.00; N, 20.78. The enantiomeric purity of both **1** and **2**, measured by chiral HPLC separation on a column using ovomucoid protein chemically bonded to the surface of a silica stationary
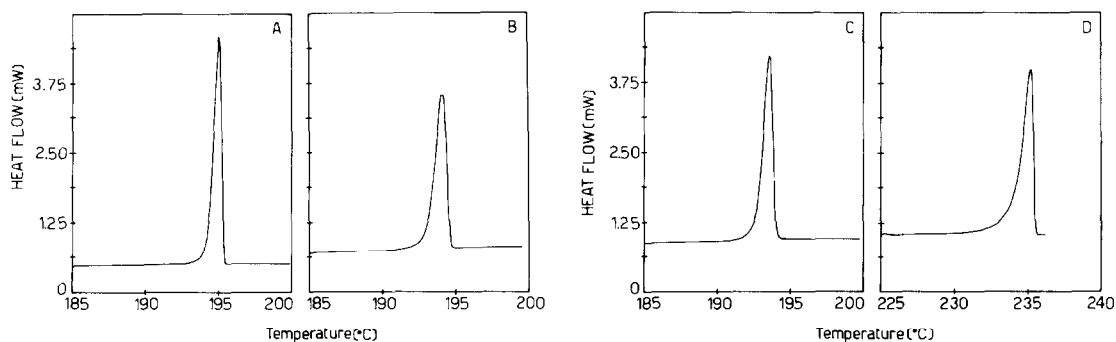

Figure 1
DSC thermograms (heating rate $1.2°C\ min^{-1}$) of the *S*-enantiomer (A) and of dextrorotatory mixtures with the *S/R* ratios: (B) 98.0/2.0, (C, eutectic) 96.2/3.8, (D, racemate) 50.0/50.0.

Table 1
Thermal parameters of the enantiomer and of the racemate (heating rate $1.2°C\ min^{-1}$)

Compound	Calorimetric purity (%)	Melting point* (°C)	Melting enthalpy* (cal mole^{-1})	Melting entropy (cal mole^{-1} K^{-1})	Free energy of formation† (cal mole^{-1})
S-enantiomer (1)	99.8	194.4 ± 0.1	9041 ± 45	19.3	—
Racemate	99.4	234.2 ± 0.1	10750 ± 34	21.2	−1489

*95% confidence limits for five determinations.
†Calculated at the melting temperature of the enantiomer.
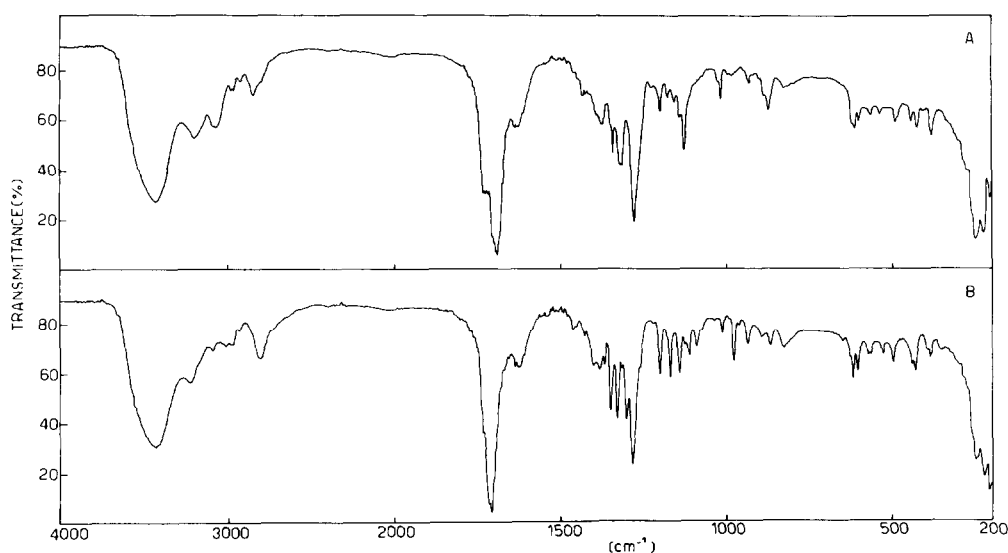

Figure 2
IR spectra of: (A) *S*-enantiomer and (B) racemate.

phase support (J. Tu, Adria Laboratories, Columbus, Ohio, personal communication), showed purities to be higher than 99.0%.

(S,R)-4,4'-(1-Methyl-1,2-ethandiyl)-bis-(2,6-piperazinedione) (racemate)

On the basis of the obtained melting point diagram (Fig. 3), the equimolecular mixture of the enantiomers is a racemic compound (racemate) corresponding to a crystalline monomorphic substance. Thermal parameters are given in Table 1. After melting (234.2°C), the racemate is not thermally stable as indicated by thermogravimetry (Perkin–Elmer TGA-7, Delta Series). By RP-HPLC a purity of 99.5% is determined on the basis of the percentage area counts at 210 nm; DSC molar purity is 99.4%. The IR spectrum (KBr) is shown in Fig. 2.

Sample preparation

Dextrorotatory mixtures of known amounts (Table 2) of the two enantiomers were
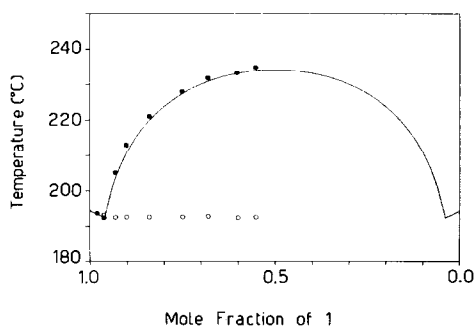
Figure 3
Binary phase diagram of 4,4'-(1-methyl-1,2-ethandiyl)-bis-(2,6-piperazinedione). Experimental melting points are indicated by filled circles. The eutectic melting points are indicated by circles.

prepared by mixing exactly weighed amounts of the racemate and of the S-enantiomer in an agate mortar and by gently grinding in the presence of a small quantity of acetone; subsequently, the mixtures were dried under vacuum at 40°C; by effect of grinding and drying, complete evaporation of acetone occurred and an homogeneous mixture of enantiomers was obtained [6]. Optically active mixtures have been checked for composition by specific rotation measurements (see below).

DSC measurements

The S-enantiomer, the racemate, and the mixtures with intermediate compositon (see previous paragraph) have been thermally analysed by using a Perkin–Elmer DSC-7 (Delta Series) differential scanning calorimeter. For the calculation of the melting point diagram (Fig. 3) we applied the equations of Schröder–Van Laar in its simplified form (equation 1) [7] and of Prigogine–Defay (equation 2) [8], making use of the enthalpy and melting point values experimentally obtained (see above):

$$\ln x = \frac{\Delta H_A^f}{R} \left(\frac{1}{T_A^f} - \frac{1}{T^f} \right) \qquad (1)$$

where x is the mole fraction of the more abundant enantiomer $(0.5 < x < 1.0)$ of a mixture whose melting terminates at T^f (K); ΔH_A^f (in cal mole^{-1}) and T_A^f (K) are the enthalpy of fusion and melting point of the pure enantiomers, respectively, and R is the gas constant ($R = 1.9872$ cal mole^{-1} K^{-1}).

$$\ln 4x \, (1 - x) = \frac{2 \, \Delta H_R^f}{R} \left(\frac{1}{T_R^f} - \frac{1}{T^f} \right) \qquad (2)$$

Table 2
Measured and calculated melting points (m.p.) and composition of mixtures of enantiomers

Mole fraction (X)	Calculated m.p. (°C)	Measured m.p. (°C)	Measured eutectic m.p. (°C)
0.980	193.4	194.1	—*
0.962†	192.5	192.8	192.8
0.930	204.1	205.0	192.8
0.900	211.0	212.4	192.6
0.840	219.8	220.6	192.5
0.750	227.4	227.9	192.6
0.680	230.9	231.6	192.7
0.600	233.2	233.3	192.2
0.550	234.0	234.7	192.3

*Value not given because of the proximity to the melting of the enantiomer.
†Eutectic composition.

in which x represents the mole fraction of one of the enantiomers in the mixture whose melting point (end of fusion) is T^f (K); T_R^f (K) and ΔH_R^f (cal mole^{-1}) are, respectively, the melting point and the enthalpy of fusion of the racemic compound.

For racemic compounds, equation (1) is applied to the portion of the curve between the melting points of the pure enantiomer and of the eutectic, and equation (2) to the portion between the melting points of the eutectic and of the racemate.

Representative DSC traces of the dextrorotatory mixtures with S/R ratios in the range between 90.0/10.0 and 55.0/45.0 are shown in Fig. 4.

The experimental melting point values are in agreement with those obtainable from the calculated curve (Table 2).

The racemate corresponds to higher melting enthalpy (ΔH_m) and entropy (ΔS_m) values than the pure enantiomers, according to the more frequently encountered behaviour [9]. The minimum ΔH_m and ΔS_m values (8778.1 cal mole^{-1} and 18.9 cal mole^{-1} K^{-1}, respectively) for the 96.2/3.8 eutectic mixture are in agreement with the presence of a minimum in the phase diagram.

Optical rotation measurements

The optical rotation values have been determined by using a Perkin–Elmer 241 polarimeter. As the specific rotation for the 99.8% pure S-enantiomer is $+48.7°$ (c 1.0, 0.1 M citrate buffer, pH 2.5), the linear relationship between specific rotation and percentage enantiomeric purity is:

$$[\alpha]_D^{20} = 0.489 \times \text{percentage enantiomeric purity.} \tag{3}$$

The relationship allows an evaluation of the enantiomeric purity for mixtures containing only **1** and **2** on the basis of polarimetric measurements.

Infrared spectra

The IR spectra (recorded as KBr pellets on a Perkin–Elmer 457 spectrophotometer) of the racemate and of the S-enantiomer are given in Fig. 2. They differ appreciably both in the regions corresponding to the vibration modes of the functional groups and in those related to the morphology of the solid state.
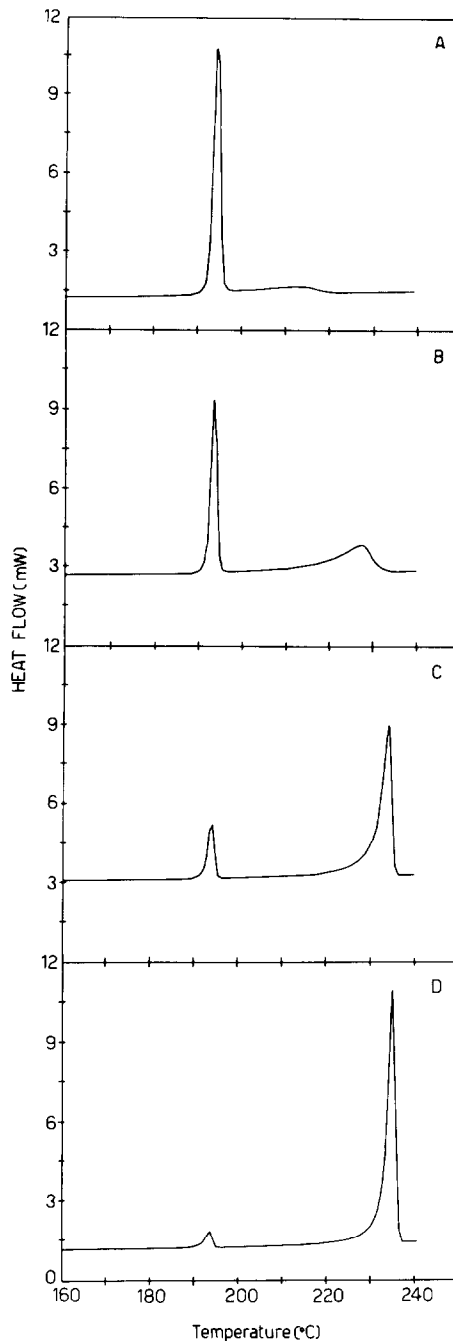

Figure 4
DSC thermograms (heating rate 10.0°C min^{-1}) of dextrorotatory mixtures with the S/R ratios: (A) 90/10, (B) 75/25, (C) 60/40, (D) 55/45.

X-Ray powder diffraction measurements

The racemate, the eutectic and the S-enantiomer were examined by the X-ray powder diffraction technique and the relevant diffractograms (obtained by using a Siemens D-500 diffractometer, with a copper–K_α radiation) are given in Fig. 5 and discussed later.
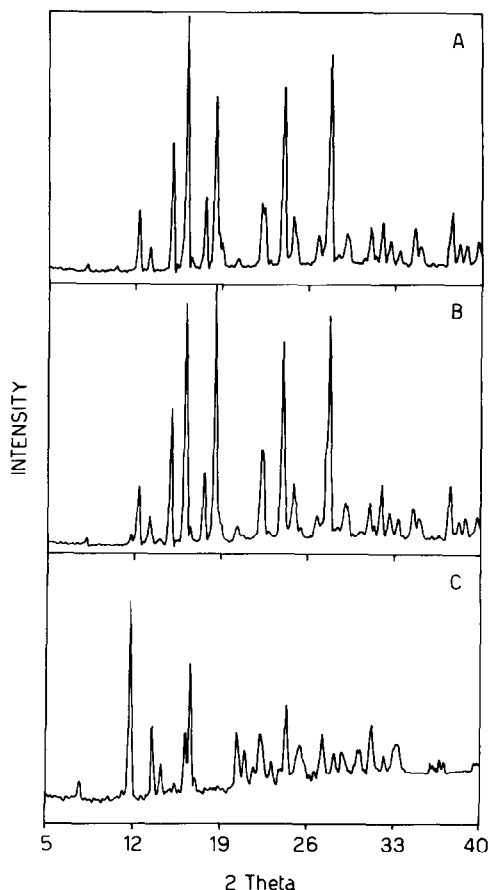
Figure 5
X-ray powder diffractograms of: (A) *S*-enantiomer (B) eutectic (C) racemate.

RP-HPLC

The RP-HPLC analyses have been performed by using an Hewlett–Packard 1090 chromatograph fitted with a μ-Bondapak C 18 column (300 × 3.9 mm i.d.); isocratic elution with the mobile phase acetonitrile–phosphoric acid pH 2.0 (3:97, v/v), at 40°C. Flow rate: 1.5 ml min⁻¹; UV detection at 210 nm.

Results and Discussion

The binary phase diagram of 4,4′-(1-methyl-1,2-ethandiyl)-bis-(2,6-piperazinedione), shown in Fig. 3, is characterized by the presence of a racemic compound, with a well defined maximal melting point (234.2°C) that is higher than that of the pure enantiomers. The eutectic composition corresponds to an *S/R* ratio 96.2/3.8 with a calculated melting point value of 192.5°C (experimental value determined by DSC = 192.8°C). It is known that at this temperature the composition of the solid

phase is identical with that of the liquid phase at equilibrium. The shape of the phase diagram and the pattern of the thermograms are in agreement with the existence of a racemic compound. For this type of racemic modification, the molecules of one enantiomer have greater affinity for those of the opposite enantiomer than for their own kind. As a consequence, opposite enantiomers pair up in the unit cell of the crystal which thus contain an equal number of molecules of the two antipodes [3]. The high value of the 'i' ratio (21.9), obtained according to Petterson [10], is indicative of an outstanding tendency towards the formation of the racemic compound and of its stability. This behaviour is confirmed by the value of the free energy of formation of the racemate from the crystalline enantiomers at their melting temperature ($\Delta G^{\phi}_{T_A}$), which is −1489 cal mole⁻¹. The value has been calculated according to the relationship reported by Jacques *et al.* [11], in which the term containing heat capacities has been omitted. This term, effectively negligible [11], cannot be determined because of decomposition phenomena, observed by TGA, after melting of the racemate. The shape of the melting point diagrams changes according to the relative values of the melting points of the enantiomers and of the racemate. When, as in the case here examined, the racemic compound melts 'at a temperature remarkably higher than that of the enantiomers, the position of the eutectic points may be difficult to be appreciated because they are very near to the borders of the diagram. In fact, a mixture with *S/R* ratio, e.g. equal to 90/10 (Table 2) displays the melting point of the eutectic at a temperature only slightly lower than that of the enantiomers and is followed by an extended endotherm due to the melting of the predominant component [Fig. 4(A)].

As it is known [3], the composition at the eutectic point corresponds to the maximal solubility value.

The phase diagram shown in Fig. 3 is comparable, among those described in the literature, with that of α-(1-naphthoxy)-propionic acid [12].

In the region of high enantiomeric purity, the thermograms [Fig. 1(A)–(C)] were obtained by operating at low heating rate (1.2°C min⁻¹) in order to allow a more accurate evaluation of the melting point decrease. The shape and the position of the melting endotherms are in agreement with the expectations

based on the composition of the corresponding samples. In fact, the melting peaks of the S-enantiomer and of the eutectic are tighter than that of the mixture with S/R ratio 98.0/2.0 for which the presence of a substantial amount of eutectic gave rise to broadening. At a higher heating rate ($10°C$ min^{-1}), a pattern quite similar to that of the pure enantiomer is observed. However, in the range between 100% and the eutectic composition, the determination of the enantiomeric purity is not completely reliable because of the difficulty in distinguishing mixtures with end fusion temperatures in an interval of 2°C or less, so that the direct method [13] is not suitable. Similar limitations apply also to the region in proximity of the racemate composition.

The thermograms of the mixtures with S/R ratios in the range 90.0/10.0–55.0/45.0 (Fig. 4) clearly show the melting of the eutectic mixture.

The values of the end fusion temperature for all the examined mixtures (Table 2) are in satisfactory agreement (±1.5°C) with those calculated by means of the equations (1) and (2).

The interpretation of the binary phase diagram has been substantiated by the examination of the IR and X-ray powder diffraction spectra.

The differences between the IR spectra of the racemate and of the S-enantiomer (Fig. 2) cannot be straightforwardly interpreted, however, the higher symmetry of the C=O absorptions ($1750–1650$ cm^{-1} region) for the racemate seems to indicate a more regular distribution of the molecules in the crystal lattice than for the enantiomer.

The examination of the X-ray powder diffractograms shows a substantial difference between the enantiomer and the racemate (Fig. 5), the latter appearing as a completely different crystalline phase. As far as the eutectic is concerned, its pattern is similar to that of the enantiomer, in agreement with its composition [14].

In conclusion, the thermal behaviour of the racemate, of the pure enantiomers, and of the enantiomeric mixtures examined by the DSC technique compare well with the results obtained by means of the other physical methods. The substantial physical difference between the racemate and the enantiomers in the solid state may be attributed to a different distribution of the molecules in the crystals, as inferred also by the IR spectra.

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