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Melting point phase diagrams of free base and hydrochloride salts of bevantolol, pindolol and propranolol

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Summary

The crystalline nature of β-adrenergic antagonist racemates was characterized by differential scanning calorimetry. Melting point phase diagrams were prepared for the free base and hydrochloride salt forms of bevantolol, pindolol and propranolol. The free base form of bevantolol and propranolol behaved as a racemic compound with pseudoracemate character in the vicinity of the racemic mixture. Eutectics were found near the pure enantiomers and at the racemic mixture. The hydrochloride salt forms of these drugs were classified as conglomerates, possessing a eutectic in the diagram only at the racemic mixture. The diagram for free base pindolol revealed a pseudoracemate; a diagram for its hydrochloride salt was not feasible. Calculated initial and final melting temperatures adequately described experimental results for conglomerate, racemic compound and pseudoracemate examples.

Introduction

β-Adrenergic receptor antagonists have been used mainly in the treatment and management of cardiac irregularities such as angina pectoris (Prichard, 1974), hypertension (Connolly et al., 1976) and cardiac arrhythmias (Dollery et al., 1969). Chiral β-adrenergic antagonists are marketed as a racemic mixture which consists of equal moles of the (+)- and (−)-enantiomers. A racemic mixture is commonly given the prefix (±)- in the literature. It has been reported that for most β-adrenergic antagonists the desired activity resides in the (−)-enantiomer (Himori et al., 1979; Tsuhihashi et al., 1990). There have been reports of significant differences in metabolic pathways between the enantiomers (Dayer et al., 1984; Walle et al., 1984; Dayer et al., 1985). In light of the stereoselectivity of the target receptor sites and the metabolic pathways, the administration of the inactive or less active enantiomer will not substantially increase the desired pharmacological response but may unnecessarily increase the toxicity and adverse side effects.

Roozeboom (1899) classified a binary crys-

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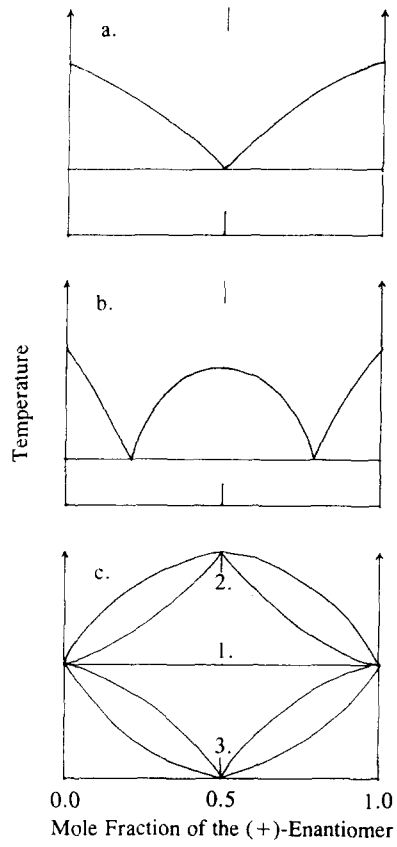

Fig. 1. Melting point phase diagram classifications. (a) Conglomerate, (b) racemic compound and (c) pseudoracemate [(1) ideal, (2) with a maximum or (3) with a minimum melting point].

talline mixture on the basis of its melting point phase diagram as one of three basic types. In terms of binary mixtures of enantiomers, a conglomerate is composed of discrete regions where only one enantiomer exists and its phase diagram has the appearance of diagram a in Fig. 1. The second, and the most common, type is the racemic compound or true racemate where the two enantiomers can be present in a well-defined crystal unit which is not equimolar. The excess enantiomer is present as an impurity. The relative composition of the crystal unit is easily determined from the eutectics in the phase diagram. Though diagram b in Fig. 1 is an example of a melting point phase diagram for a racemic compound, the melting points of the enantiomers and the racemic mixture, as well as the location of the eutectics, may substantially alter the appearance. The final crystal form is the pseudoracemate which is found as a result of a solid solution between the two species which coexist in a relatively random fashion in the crystal lattice. A solid solution is rare in nature (Jacques et al., 1981; Brittain, 1990) and its melting point phase diagram may or may not exhibit a maximum or minimum melting point as in diagram c of Fig. 1. Only conglomerates and pseudoracemates have a eutectic at the racemic mixture composition and, for this reason, chiral crystalline drugs which are racemic mixtures should possess a narrow melting range only if they fall into one of those two classifications.

Characterizing the crystalline nature can lead to resolution methods that are reasonable and logical. Resolution of an enantiomer on a preparative scale can be a tedious and expensive process if the racemic mixture is a true racemate or a pseudoracemate because it will require the use of chiral reagents, chiral chromatography or derivatization procedures (Rheinbolt and Kircheisen, 1926; Leclercq et al., 1976; Oonk et al., 1977; Brittain, 1990; Dwivedi et al., 1990). Conglomerates, on the other hand, can be resolved on an industrial basis by the 'entrainment' method which involves introducing seed crystals of the desired enantiomer into a cooling saturated solution of the racemic mixture and harvesting the crystals that grow on that template (Jacques et al., 1981).

It has been proposed that conglomerates result far more frequently from the salt form than the free base form of base drugs (Jacques et al., 1981; Brittain, 1990). The first objective of this study was to test this hypothesis by characterizing the crystalline nature of three β-adrenergic antagonists, bevantolol, pindolol and propranolol, in the free base and hydrochloride salt form. Pindolol is unique among the β-adrenergic antagonists of the 3-(aryloxy)-1-(alkylamino)-2-propanol type in that it possesses an indole group (see Fig. 2) which increases its polarity in comparison to bevantolol or propranolol. A second objective of this study was to examine the crystalline nature of these structurally similar compounds in light of this difference in polarity.

Materials and Methods

The racemic mixture and pure enantiomers of propranolol hydrochloride were a gift from Wyeth-Ayerst (Princeton, NJ) or were purchased from Sigma Chemical Co. (St. Louis, MO). Parke-Davis (Ann Arbor, MI) provided (+)-, (−)-bevantolol and (±)-bevantolol hydrochloride. (±)-, *R*-(+)- and *S*-(−)-pindolol, each in the free base form, were gifts from Sandoz Research Institute (East Hanover, NJ). The enantiomers and racemic mixtures were used without further purification. Hydrogen chloride gas was obtained from Air Products and Chemicals, Inc. (Allentown, PA). The water used in this study was distilled and deionized.

Free base drug was prepared by adding dilute sodium hydroxide to an aqueous solution of the salt form followed by extraction with methylene chloride. Evaporation of the solvent yielded the desired free base. Hydrochloride salts of the racemic mixture and individual enantiomers were prepared by dissolving the free base in methylene chloride and bubbling hydrogen chloride gas

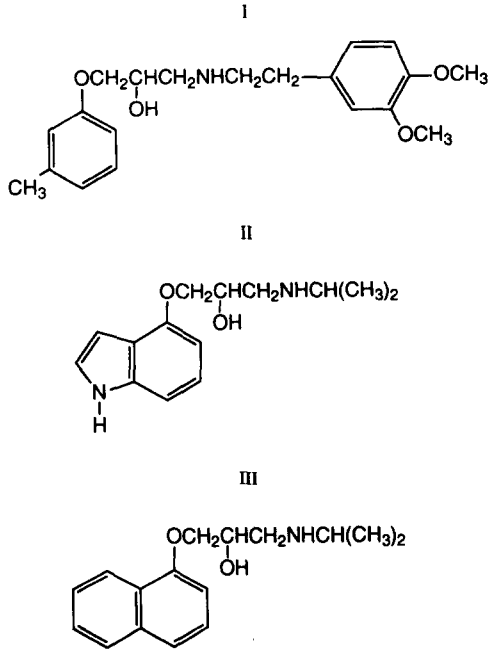

Fig. 2. Chemical structures for (I) bevantolol, (II) pindolol and (III) propranolol.

through the solution. Evaporation of the solvent allowed harvesting of the desired salt crystals. As an alternative method for pindolol, a few drops of concentrated HCl were added to a 50:50 95% ethanol/methylene chloride solution of (±)-pindolol. White crystals appeared after storage at freezer temperature. The liquid phase was decanted off and the crystals were allowed to stand at room temperature for a few minutes during which time they turned a pale yellow color and appeared wet. When these crystals were vigorously stirred, off-white crystals were obtained. These crystals were carefully sealed in a flask with Parafilm® and stored at −15°C until thermal analysis could be performed.

Enthalpies of fusion, melting points and purities of pure enantiomers and racemic mixtures as well as the initial and final melting points of samples with different mole fractions of the (+)-enantiomer were determined by thermal analysis. Differential scanning calorimetry (DSC) was performed under a nitrogen atmosphere using a Dupont Thermal Analyst 2000 (DSC 10 differential scanning calorimeter) which was equipped with a Perkin-Elmer Graphic Plotter 2.

One of three methods was used to prepare samples in the range $0.5 < X_{(+)} < 1.0$. Since only milligram quantities of the pure enantiomers of pindolol, bevantolol or bevantolol hydrochloride were available, the requisite amount of pure enantiomer was added to a weighed sample of racemic mixture. The sample was melted in the calorimeter and allowed to cool slowly; the recrystallized sample was used to determine the melting range. Sample preparation for propranolol hydrochloride involved mixing carefully weighed amounts of enantiomer and racemic mixture into a test tube, melting the sample, mixing with a glass rod and allowing it to cool. For free base propranolol samples, an aqueous solution of weighed (+)- and (±)-propranolol hydrochloride was alkalinized and extracted with methylene chloride. The sample was recovered by evaporation of the organic solvent.

Accurately weighed samples in the range of 1–4 mg were placed in an aluminum pan and a lid was crimped on. An empty aluminum pan and lid served as the reference. The reference and

sample were equilibrated at the starting temperature for 2 min and then heated at a rate of 10 degree/min until the sample had completely melted. The calorimeter was calibrated with an indium standard before analyzing any samples. Initial and final melting points were taken as the peak temperatures from the thermograms and were corrected for the leading edge angle of the indium standard. In the event that only one peak was evident, the peak temperature was assumed to be a final temperature. Purities were determined by a published method (Kirkwood and Oppenheim, 1961). Simultaneous thermogravimetric and DSC analysis was performed at a 2 degree/min rate under an argon atmosphere using a PL Thermal Systems STA 625. Sample preparation was carried out as before with the exceptions that larger samples of 5–10 mg were used and the pans were not covered.

Theoretical curves for the conglomerate and racemic compound melting point phase diagrams were calculated using literature equations (Jacques et al., 1981). Theoretical final melting points for a conglomerate, T_f, can be calculated for the range $0.5 \leq X_{(+)} \leq 1.0$ using:

$$\ln X = \frac{\Delta H_{fA}}{R}\left(\frac{1}{T_{fA}} - \frac{1}{T_f}\right) \quad (1)$$

where X is the mole fraction of the (+)-enantiomer in the sample; T_{fA} and ΔH_{fA} are the absolute melting point and the enthalpy of fusion of the pure enantiomer. The final melting points from the eutectic to the pure enantiomer for a racemic compound can be estimated using the same equation. Between the racemic mixture and the eutectic point of a racemic compound the appropriate equation is:

$$\ln 4X(1-X) = \frac{2\Delta H_{fR}}{R}\left(\frac{1}{T_{fR}} - \frac{1}{T_f}\right) \quad (2)$$

where T_{fR} and ΔH_{fR} are the absolute melting point and the enthalpy of fusion of the racemic mixture.

For a pseudoracemate, initial and final melting temperatures were calculated assuming that the melt of the binary mixture was an ideal solution, but the crystalline mixture exists as a solid solution possessing excess free energy, ΔG^E, that can be expressed as a function of the mole fraction of the pure enantiomer (Oonk et al., 1977):

$$\Delta G^E = AX^2(1-X)^2 + BX(1-X) \quad (3)$$

where A and B are constants. The excess free energy could be used to estimate initial melting points:

$$T_i = T_{fA} + \frac{\Delta G^E}{\Delta S_{fA}} \quad (4)$$

where ΔS_{fA} is the entropy of fusion for the pure enantiomer. Curves based on Eqns 3 and 4 were fitted to the experimental data using Enzfitter (Elsevier-BIOSOFT, Cambridge, U.K.) to generate the values of A and B. An equal free energy curve, which bisects the initial and final temperature range at each mole fraction, as described elsewhere (Oonk et al., 1977), is used to reflect the initial melting points to estimate the final melting points.

TABLE 1

Purities, melting points and enthalpies of fusion for the enantiomers and racemic mixture of bevantolol, pindolol and propranolol

Sample	Purity (%)	T_f (°C)	ΔH_f (kJ/mol)
(±)-Bevantolol HCl	98.2	134.5	40.5
(+)-Bevantolol HCl	99.4	154.6	46.7
(−)-Bevantolol HCl	98.1	155.0	45.6
(±)-Bevantolol	98.1	87.4	45.9
(+)-Bevantolol	99.9	75.1	47.2
(−)-Bevantolol	99.8	75.1	43.2
(±)-Propranolol HCl	99.2	163.4	42.8
(+)-Propranolol HCl	99.9	194.6	36.9
(−)-Propranolol HCl	99.2	194.7	35.1
(±)-Propranolol	99.2	92.9	38.1
(+)-Propranolol	99.8	70.9	33.4
(−)-Propranolol	99.9	71.4	34.2
(±)-Pindolol	99.4	169.7	57.9
(+)-Pindolol	98.5	93.0	25.7
(−)-Pindolol	98.2	92.0	25.7

Results and Discussion

The purity, melting point and enthalpy of fusion results are presented in Table 1. The melting points and enthalpies of fusion for the respective enantiomers are close and preparation of a melting point phase diagram from data only in the range $0.5 \leq X_{(+)} \leq 1.0$ was justified. Initial and final melting points and theoretical curves are presented in Figs 3–5. Theoretical curves adequately describe the data.

The diagrams for the hydrochloride salt forms of bevantolol and propranolol are well-behaved conglomerate plots with consistent initial melting points and a single eutectic at the racemic mixture. The final melting points for the free base forms of the β-adrenergic antagonists followed the pattern for a racemic compound. Eutectics were found only near the pure enantiomers when $X_{(+)}$ is 0.94 or 0.063 for bevantolol and 0.95 or 0.046 for propranolol. The consistent initial melting points for samples with high enantiomeric content confirmed the racemic compound character, but in the vicinity of the racemic mixture, the initial melting point increases and the racemate proved to be a eutectic mixture. This pseudoracemate character near the racemic mixture of a binary mixture that would otherwise be classified as a racemic compound, though rare, has been reported (Jacques et al., 1981).

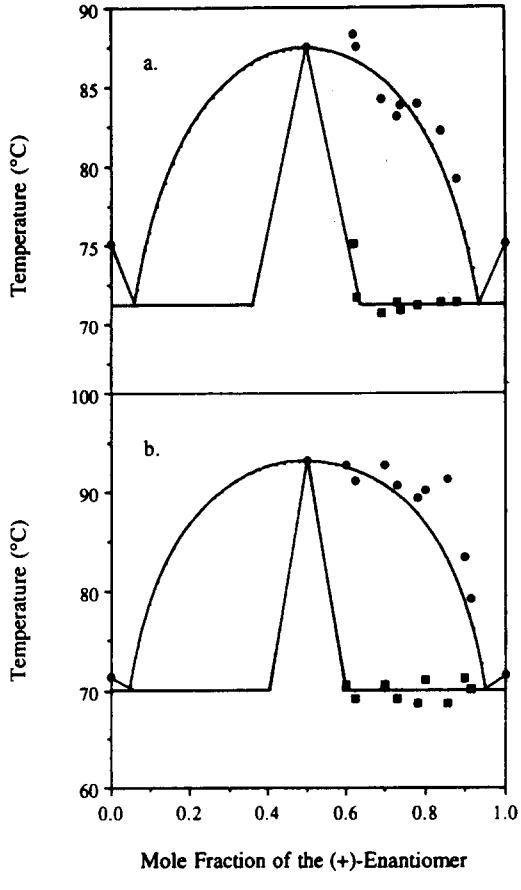

Fig. 3. Melting point phase diagrams for (a) bevantolol and (b) propranolol. Squares and circles denote initial and final melting points, respectively. Solid lines are theoretical curves.

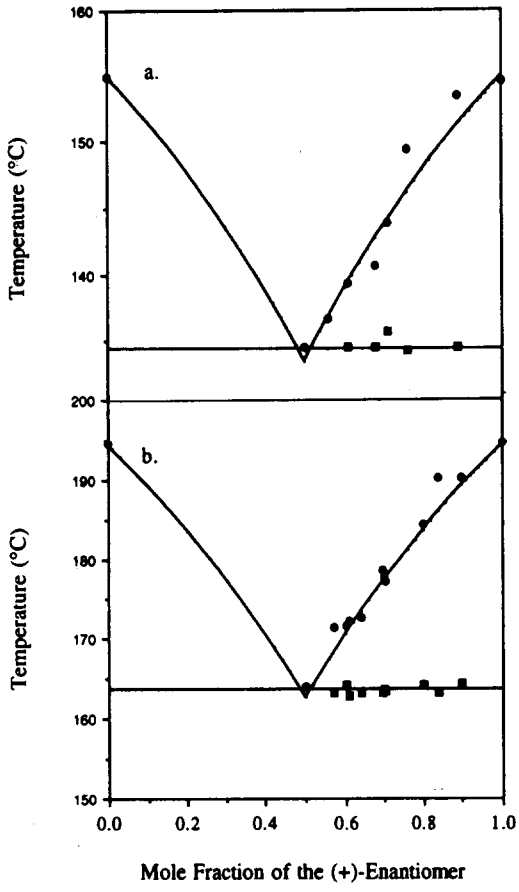

Fig. 4. Melting point phase diagrams for (a) bevantolol HCl and (b) propranolol HCl. Squares and circles denote initial and final melting points, respectively. Solid lines are theoretical curves.

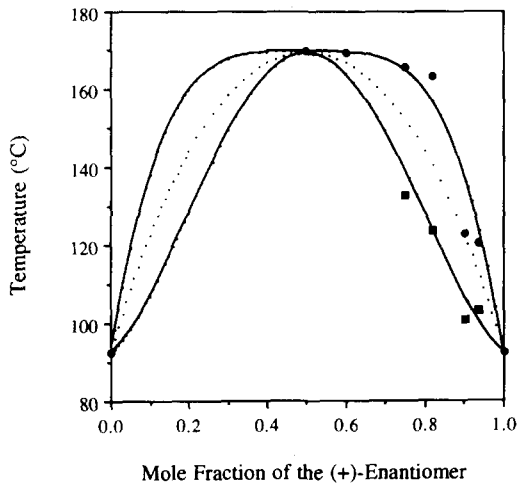

Fig. 5. The melting point phase diagram for pindolol. Squares and circles denote initial and final melting points, respectively. Solid lines are theoretical curves; the dotted line is the equal free energy curve.

The appearance of the melting point diagram for free base pindolol is characteristic of a pseudoracemate with a maximum melting point. The more polar nature of pindolol apparently allows a greater extent and magnitude of interactions in the crystalline binary mixture, resulting in a pseudoracemate, not a racemic compound, nature. It is interesting to note that all three different crystalline natures can be found in such structurally similar compounds.

A melting point phase diagram was not feasible for the hydrochloride salt of pindolol due to its sensitivity to acidic conditions. A black mixture resulting from the original synthesis method for pindolol hydrochloride was believed to be due to oxidation of the indole ring and possible polymerization. The alternative method provided milder acidic conditions and yet the yellow color in the reaction mixture is still indicative of degradation. The wet appearance was considered evidence of the hygroscopic nature of the harvested crystals. The appearance of off-white crystals simply by stirring the wet, yellow crystals may be due to formation of a hydrate.

To investigate the possibility of hydrate formation, the crystals were subjected to thermogravimetric analysis which revealed loss of mass in the 25–100°C and again in the 120–160°C range corresponding to the endothermic peaks in the simultaneous DSC thermogram (see Fig. 6). The total loss of mass, for example, 0.56 mg (6.4%) from an 8.76 mg sample, agrees with the loss of mass assuming a monohydrate (6.8%). It is appar-

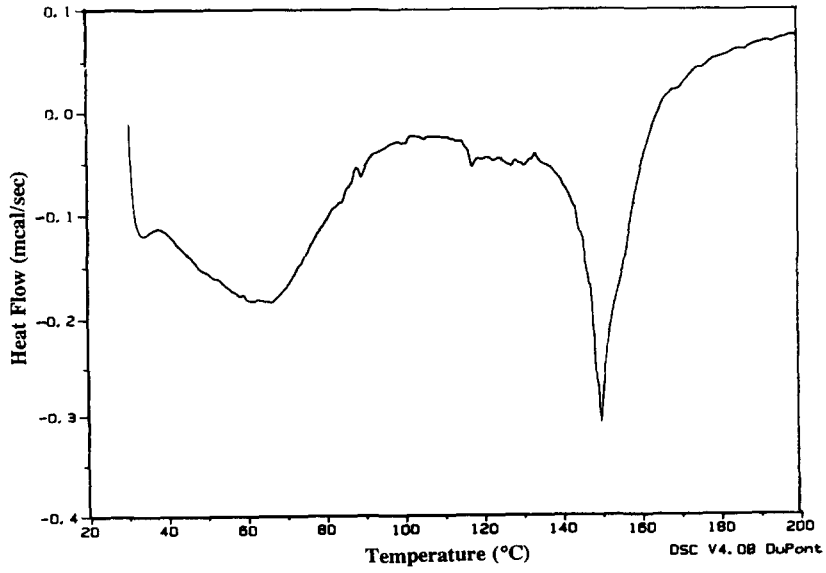

Fig. 6. DSC thermogram of the suspected monohydrate of pindolol HCl.

ent that the water would be associated with the pindolol crystal structure at two types of sites. The peak centered at 147°C corresponds to melting of the sample, The irregular shape to this peak may have been due to impurities or degradation products, or resulted from the loss of water. At higher temperatures, the baseline drift was due to decomposition.

Conclusions

The melting point phase diagrams for bevantolol and propranolol revealed that the free base form was a racemic compound with pseudoracemate character in the vicinity of the racemic mixture; the hydrochloride salt was a conglomerate. The conglomerate character of these salts may allow relatively facile resolution by the method of entrainment On the other hand, due to the racemic compound and pseudoracemate nature of the free base form of these racemates, extensive effort would be required to accomplish resolution of the free base enantiomers. The diagram for pindolol has the classic appearance of a pseudoracemate with a maximum melting point. Initial observations of the thermal behavior of the hydrochloride salt of (±)-pindolol indicated that a hydrate form may exist which has two types of water of hydration. Synthesis of a stable hydrochloride salt form of pindolol was not successful, presumably due to the acid catalyzed oxidation of the indole ring. In this relatively small group of structurally similar compounds, all three classifications of binary crystalline mixtures are exhibited.

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