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Enantiomer systems of carnitinamide inorganic salts: introductory studies to a successful entrainment resolution

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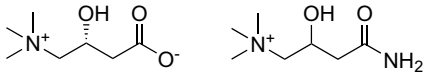
Abstract—The ready availability of (*R*)-carnitinamide, an immediate synthetic precursor of (*R*)-carnitine, is an ambitious goal and resolutions, due to the very low cost of racemic carnitinamide, can be the most convenient technology to achieve it. Before developing a new advantageous resolution of carnitinamide chloride by entrainment, we characterized the enantiomer systems formed by the chloride, nitrate and sulfate of carnitinamide, mainly by DSC and IR analyses, proving that a different type of racemate is produced by each of these salts: a conglomerate by the chloride, a racemic compound by the nitrate and a solid solution, a very rare type of enantiomer system, by the sulfate.

1. Introduction

Due to its important role in the biochemical pathways for β-oxidation of fatty acids and in other metabolic functions, (*R*)-carnitine (*R*)-**1** has found many significant applications in therapy and in nutrition. In recent years, the increasing demand for this enantiopure compound has led to the development of a number of procedures for its preparation, based on different approaches, such as asymmetric chemical synthesis, biotransformation of achiral precursors, resolution through diastereomeric derivatives, enzymatic resolution or the use of non-racemic chiral starting materials. However, to the best of our knowledge, only two of these methods have been scaled up and are currently being applied to industrial production: the biotransformation of 4-butyrobetaine[1] and the resolution of carnitinamide **2**, a very inexpensive carnitine precursor, with D-camphoric acid, followed by the hydrolysis of the amide functional group.[2] In 2006, we patented a simple and efficient entrainment resolution of chloride **2** after studying the nature of the racemates of three readily available salts, such as nitrate, chloride and sulfate.[3] Herein, we report the results of those investigations, which interestingly allowed us to recognize the three known different forms of the racemate, namely that of the racemic compound and the less frequent ones of a conglomerate and pseudoracemate, in the nitrate, chloride and sulfate of **2**, respectively.



(*R*)-**1** **2**

2. Results and discussion

Nitrate **2** was prepared from the readily available chloride, the most common salt of **2**, resulting from HCl treatment of cyanocarnitine,[4] in turn obtained as a chloride, from epichlorohydrin, trimethylamine and hydrogen cyanide.[5] Conversion of the chloride into the nitrate was accomplished by treatment of an aqueous solution of chloride **2** with stoichiometric silver nitrate. After the removal of silver chloride precipitate, nitrate **2** was isolated by concentration of the filtrate and, after prolonged drying under high vacuum, characterized by ^{1}H NMR and elemental analyses. The same procedure was applied to chloride (*R*)-**2** obtaining nitrate (*R*)-**2**. The melting point of this latter compound (133.7 °C) proved to be about 10 ° higher

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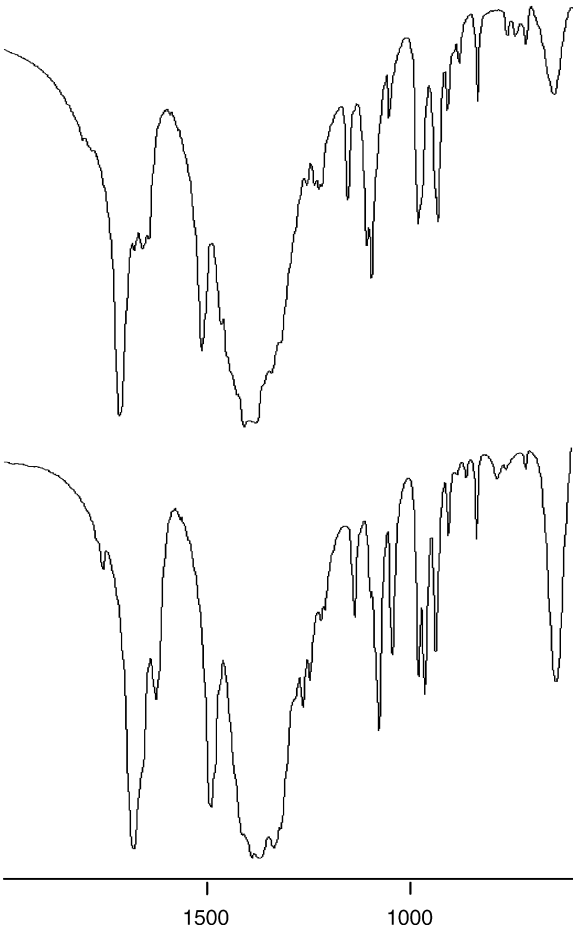

Figure 1. IR spectra of (*R*)-carnitinamide nitrate (upper) and of racemic carnitinamide nitrate (lower) in the crystalline phase.

than that of the corresponding racemate (123.6 °C). Moreover, the respective IR spectra turned out to be different (see Fig. 1).

Such data suggested that nitrate **2** forms a racemic compound melting at a lower temperature than the enantiomers. This was confirmed by DSC analyses of nitrate **2**, nitrate (*R*)-**2** and a number of their mixtures, which allowed us to construct the binary phase diagram, depicted in Figure 2 for mole fractions of (*R*)-**2** nitrate ranging from 0.5 to 1. The melting profiles of the differently proportioned **2** nitrate/(*R*)-**2** nitrate mixtures were characterized by the presence of two peaks, the first, between 120 and 122 °C, representing the fusion of the eutectic, a nearly 1/1 mixture of the racemic compound and (*R*)-enantiomer, the second, representing the fusion of the excess of racemic compound or (*R*)-enantiomer over the eutectic composition at temperatures increasing with such an excess. As can be seen in the same figure, the experimental values fit well with the theoretical ones (solid curves), calculated on the basis of the melting point of nitrate (*R*)-**2** and of its heat of fusion (122.2 J/g) by the Schröder–van Laar equation and of the melting point of nitrate **2** and of its heat of fusion (175.8 J/g) by the Prigogine–Defay equation.

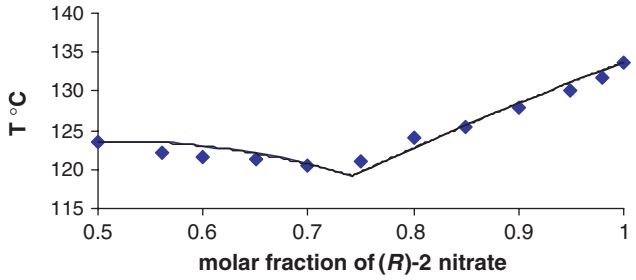

Figure 2. Binary melting-point phase diagram for carnitinamide nitrate. The solid curve represents the values calculated on the basis of the Prigogine–Defay and the Schröder–van Laar equations.

The two curves intersect at the 0.742 molar fraction of nitrate (*R*)-**2**. Such a theoretical value of eutectic composition is consistent with the experimental observation that, unique among the DSC curves recorded for the 10 differently proportioned nitrate **2**/nitrate (*R*)-**2** mixtures, that of a sample with 0.75 mole fraction of (*R*)-**2** nitrate [1/1 mixture of **2** and nitrates (*R*)-**2**] shows only one sharp melting peak at 120.3 °C.

In the case of chloride **2**, the nature of a 1/1 mechanical mixture of enantiomers or conglomerate was suggested by the melting points reported by Kato and Hosein in 1968,[6] notably higher for the enantiomers (238 °C) than for the racemate (206 °C), and successively demonstrated by the identical infrared spectra of the chlorides of (*R*)-**2**, (*S*)-**2** and **2** described by Lorenz in 1980.[7] On the basis of these indications, we performed thermal analyses of chloride **2**, chloride (*R*)-**2** and their four different mixtures. By DSC analysis, the melting points of chloride (*R*)-**2** and of the racemate were estimated to be 241.6 and 210.1 °C, respectively. However, the heat of fusion of the pure enantiomer could not be evaluated, because of thermal decomposition that started before the fusion was complete. This hampered the calculation of the theoretical binary phase diagram by the Schröder–van Laar equation. The DSC curves of the four mixtures showed two clearly detectable fusions, the former of the eutectic, consisting in the racemic mixture, at temperatures ranging between 210 and 211 °C, the latter of the exceeding (*R*)-enantiomer at higher temperatures, increasing with the enantiomeric excess. As shown in Figure 3, plotting the approximate extrapolated offset temperatures of the higher melting peak of the four mixtures and the melting temperatures of the racemic mixture and of the pure (*R*)-enantiomer against mole fraction of this latter resulted in an experimental binary phase diagram, which is consistent with the claimed nature of the conglomerate for chloride **2** on the basis of IR analyses.[7] Furthermore, determining of relative solubilities of the racemic mixture and the pure enantiomer, which indicated a ratio little lower than 2, conforms to the solubility rules formulated for conglomerate systems, produced by ionic compounds, which state that the solubility of a racemate is $\sqrt{2}$ times the solubility of the corresponding enantiomer.[8]

Finally, we examined the neutral sulfate of **2** and (*R*)-**2**, prepared by treatment of the corresponding chlorides with

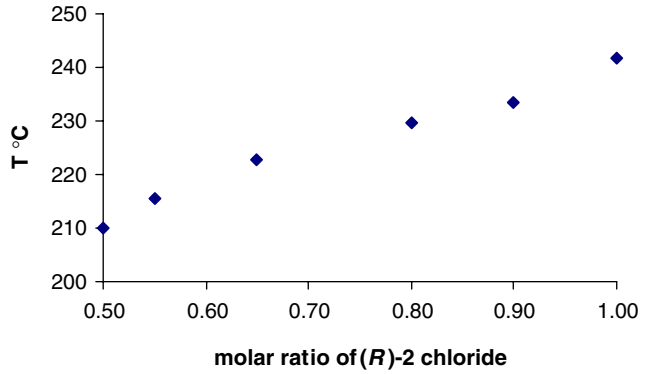

Figure 3. Experimental binary phase diagram of carnitinamide chloride. Onset temperature of the unique fusion peak is reported for the racemate and the (*R*)-enantiomer, while extrapolated offset temperature of the second melting peak is reported for the four mixtures.

an equivalent silver sulfate in water, removal of the precipitated silver chloride, concentration of the filtrate, drying of the resultant residue to give the theoretical amount (1.06 g of the neutral sulfate from 1 g of chloride) and crystallization from methanol. After characterization by elemental analysis and ^{1}H NMR, we performed DSC and IR analyses. The conglomerate nature was immediately excluded on the basis of the higher melting point for the racemate (220.3 °C) than for the (*R*)-enantiomer (204.3 °C), as well as that of the racemic compound, since the IR spectra of **2** and (*R*)-**2** sulfates in the crystalline phase proved perfectly superimposable (see Fig. 4). Therefore, in order to determine, which type of phase diagram would be the most likely for the carnitinamide sulfate system, DSC measurements were made for a series of sulfate **2**/sulfate (*R*)-**2** mixtures with molar fractions of (*R*)-**2** sulfate ranging between 0.5 and 1, prepared by dissolving the two components in methanol and evaporating the resulting solution to dryness. In the DSC traces of such mixtures, we observed only one melting peak, the onset temperature of which changed with composition, decreasing with the enantiomeric excess. This is all the more reason to exclude the occurrence of a conglomerate or of a racemic compound, which both give phase diagrams of eutectic type. The extrapolated onset temperatures and the peak maxima, corrected for the shift of the peak temperature due to the heating of the sample,[9] were plotted against composition to give the diagram shown in Figure 5. It is evident that the two series of experimental points line up drawing solidus and liquidus curves of a pseudoracemate with positive deviations from ideality, in other words with a maximum. Mixed crystals are formed at all compositions and such a transformation of the system from a racemic mixture or conglomerate into solid solution by replacement of the monoanion nitrate or chloride with a dianion sulfate is likely to be related to the possibility of salifying, in the only case of sulfate, the same acid molecule with two homochiral or heterochiral ammonium ions indifferently. As in the case of the chloride, decomposition partially overlapped fusion hindering fusion heat's determination. Consequently, experimental solidus and liquidus points are only reported in the binary melting-point phase diagram, where their trend is better visualized by two tendency lines. In the same diagram, the DSC melting scans are also depicted to graphically show how solidus and liquidus points were derived from DSC traces.

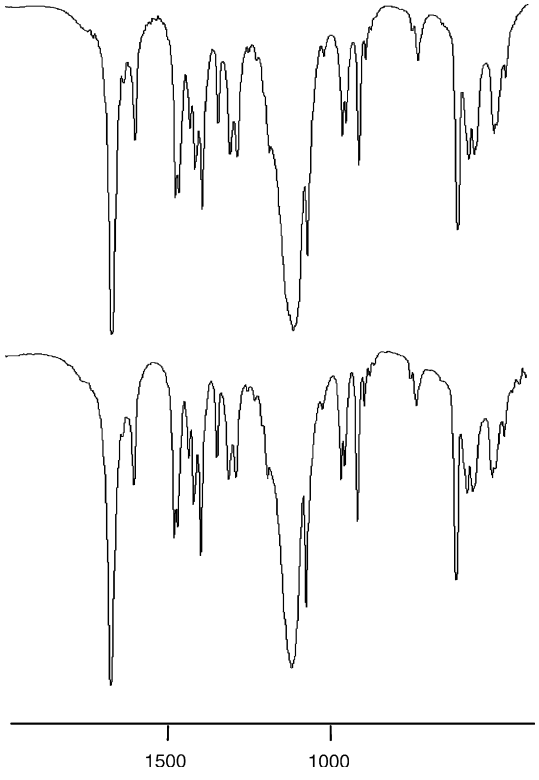

Figure 4. IR spectra of (*R*)-carnitinamide sulfate (upper) and of racemic carnitinamide sulfate (lower) in the crystalline phase.

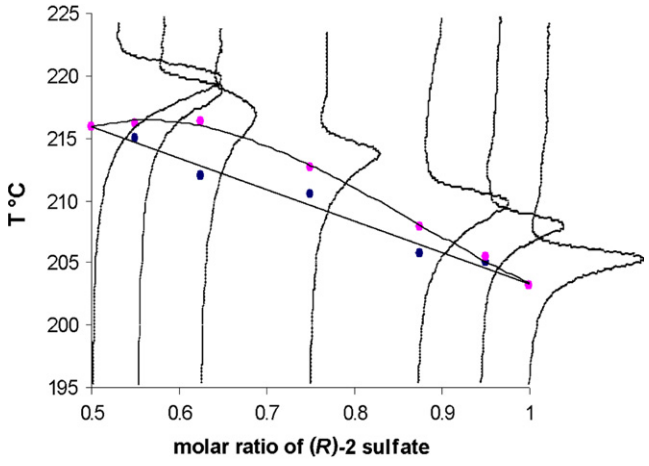

Figure 5. DSC melting scans of mixtures of carnitinamide sulfate enantiomers, with 0.5–1 molar ratio of (*R*)-enantiomer, and derived solidus (blue circles) and liquidus (purple circles) points with respective tendency lines. Solidus points are the extrapolated peak onset temperatures, while liquidus points the peak maximum temperatures corrected according to Ref. 9.

3. Conclusion

In conclusion, we have characterized the racemates of three different salts of carnitinamide, the immediate synthetic precursor of carnitine. The investigation was aimed at

developing a new process to obtain (*R*)-carnitine, based on the resolution of carnitinamide enantiomers by the advantageous method of preferential crystallization (entrainment); which succeeded in leading to an efficient, robust and economic resolution procedure of carnitinamide chloride, as we have recently claimed in a patent.[3] The theoretical aspects of the research turned out to be as interesting as the practical ones. In fact, we could (a) substantiate the statement that carnitinamide chloride forms a conglomerate by DSC and solubility analyses, (b) construct the binary phase diagram of carnitinamide nitrate, demonstrating that it is a racemic compound and locating its eutectic and (c) recognize carnitinamide sulfate as a solid solution, a very rare type of racemate, of which only 19 examples are listed in the inventory of Jacques.[10] With three different inorganic counterions, the same quaternary ammonium gives the three known types of enantiomer systems: a singular and didascalic case.

4. Experimental

^{1}H NMR spectra were recorded on a Varian Gemini 300 (300 MHz) instrument. Optical rotations were measured in a 1 dm cell of 1 mL capacity using a Perkin–Elmer 241 polarimeter. IR spectra were recorded with a FT-IR Paragon 1000 PC Perkin–Elmer spectrometer. The melting points of racemates and pure enantiomers were determined by DSC analysis, taking the extrapolated peak onset temperature. For the non-racemic enantiomer mixtures, the temperature of the maximum of the second peak was plotted as a melting point in the binary phase diagram of carnitinamide nitrate and the extrapolated offset temperature of the second peak in that of carnitinamide chloride, respectively. For the non-racemic enantiomer mixtures of carnitinamide sulfate, exhibiting a single fusion peak, the extrapolated onset temperatures and the peak maxima, corrected for the shift of the peak temperature due to the heating of the sample,[9] were plotted as solidus and liquidus points, respectively, in the binary phase diagram. The DSC curves were recorded and integrated with the aid of a TA Instruments DSC 2010 apparatus. For DSC analyses, samples of 2–5 mg were run in crimped aluminium pans. The enantiomer mixtures were prepared, in the case of carnitinamide chloride and of carnitinamide nitrate, by mixing the solid racemate with the solid (*R*)-enantiomer, while in the case of carnitinamide sulfate, dissolving the (*R*)-enantiomer and the racemate in methanol and concentrating the resultant solutions to dryness. All the analyses were performed with a heating rate of 5 °C min^{-1}.

Racemic carnitinamide chloride **2** was prepared according to the literature methods,[4,5] while a sample of (*R*)-**2** was kindly provided by Sigma-Tau S.p.A. (Pomezia, Italy). Conversion to carnitinamide nitrate and the sulfate was accomplished by treatment of water solution of the chloride, racemate or (*R*)-isomer, with equivalent aqueous silver nitrate and silver sulfate, respectively, removal of precipitated silver chloride by filtration and concentration of the filtrate. **2** Nitrate: mp 123.6 °C; ^{1}H NMR (D_2O) δ 2.41 (d, 2H, $J = 6.3$ Hz), 3.11 (s, 9H), 3.30–3.41 (m, 2H), 4.55 (m, 1H); ^{1}H NMR (DMSO-d_6) δ 2.19 (dd, 1H, $J = 15.0$, 6.1 Hz), 2.27 (dd, 1H, $J = 15.0$, 7.0 Hz), 3.10 (s, 9H), 3.30 (d, 2H, $J = 5.8$ Hz), 4.40 (m, 1H), 5.52 (d, 1H, $J = 5.5$ Hz), 6.98 (br s, 1H), 7.43 (br s, 1H). Anal. Calcd for $C_7H_{17}N_3O_5$ (223.23): C, 37.66; H, 7.68; N, 18.82: Found: C, 37.86; H, 7.76; N, 18.78. (*R*)-**2** Nitrate: mp 133.7 °C; $[\alpha]_D^{25} = -23.4$ (*c* 1, MeOH). ^{1}H NMR spectra identical to those of the racemic nitrate. **2** Sulfate: mp 220.3 °C. Anal. Calcd for $C_7H_{17}N_2O_4S_{0.5}$ (209.25): C, 40.18; H, 8.19; N, 13.39; S, 7.63. Found: C, 40.06; H, 8.32; N, 13.18; S, 7.66. (*R*)-**2** Sulfate: mp 204.3 °C; $[\alpha]_D^{25} = -17.9$ (*c* 1, MeOH). ^{1}H NMR (D_2O) spectra of both sulfates (racemate and *R* enantiomer) identical to that of the racemic nitrate in D_2O.

Acknowledgements

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