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Entrainment resolution of carnitinamide chloride ☆

Marco Pallavicini [a,*], Cristiano Bolchi [a], Matteo Binda [a], Rossana Ferrara [a], Laura Fumagalli [a], Oreste Piccolo [b], Ermanno Valoti [a]

[a] *Istituto di Chimica Farmaceutica e Tossicologica "Pietro Pratesi", Università di Milano, via Mangiagalli 25, I-20133 Milano, Italy*
[b] *Studio di Consulenza Scientifica, via Bornò 5, I-23896 Sirtori (LC), Italy*

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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 stereotechnology to reach it. We have efficiently resolved carnitinamide chloride by preferential crystallization from methanol according to simple entrainment procedure. The previous transformation of the chloride into other salts or the use of specific solvent systems was not required for successful resolution, as in the case of the reported entrainment of carnitinamide precursor, carnitine nitrile.

1. Introduction

Over the last 30 years, many significant nutritional and medical applications have been found for (*R*)-carnitine (*R*)-**1**, thus increasing its commercial demand and increasing the efforts of both fundamental and applied research to improve its production. Asymmetric chemical syntheses, biotransformation of achiral precursors, diastereomeric salts resolutions, enzymatic resolutions and transformations of chiral pool substances have been investigated to develop advantageous methods for the industrial scale production of (*R*)-**1**.[2] Crotonobetaine and 4-butyrobetaine biotransformation[3] and racemic carnitinamide **2** D-camphorate resolution via crystallization[4] may be cited as examples of methods scaled up and currently applied in industry. These successes have not, however, relaxed the interest in new preparative procedures for (*R*)-**1**, which still remains a challenging target as demonstrated by the recently patented stereoselective enzymatic hydrolyses of (±)-4-chloro-3-hydroxybutyric acid alkyl ester, synthesized from racemic epichlorohydrin,[5] and of (±)-3-acyloxy-γ-butyrolactone, synthesized from racemic 3-hydroxy-γ-butyrolactone,[6] or the lipase-catalyzed stereoselctive acetylation of 3-hydroxy-4-tosyloxybutanenitrile.[7] Compared with other stereotechnologies, racemate resolutions, in particular the chemical ones, suffer from two major drawbacks: the cost of the non-catalytic chiral auxiliary, the resolving agent, which often needs to be recovered, and the reuse of the undesired enantiomer. Such problems can be overcome or minimized when the racemate is an inexpensive material, directly resolvable by preferential crystallization, without the resolving agent, thanks to its conglomerate nature.[8] This is the case for carnitine nitrile **3**, a carnitine precursor, whose chloride is a conglomerate, readily available by a one-pot conversion of inexpensive racemic epichlorohydrin.[9] A 1968 patent claims the direct resolution of (±)-**3** chloride by crystallization from methanol or water or aqueous methanol.[10] However, the efficiencies of such procedures are poor, giving the pure enantiomer recovered at each crystallization in 4–11% of the total enantiomer initially present in the racemate supersaturated solution. Fourteen years later, Jacques and Collet reported that the perchlorate and the hydrogen oxalate of (±)-**3** are also conglomerates and patented the entrainment resolution of these salts by crystallization from water or 70/30 methoxyethanol–water mixture.[11] The efficiencies of the new procedures are higher, in particular those of the mono-oxalate from water, which allows more than 20% of the total enantiomer to be separated at each crystallization. Furthermore, the same authors attempted the entrainment resolution of chloride (±)-**3** again, accomplishing a series of alternate preferential crystallizations of the two enantiomers from 70/30 methoxyethanol–water, but, as reported in the previous patent, the precipitates have a low enantiomeric purity (~50%) and the pure enantiomer recovered at each crystallization averages to a modest 9% of the total enantiomer initially present in the racemate supersaturated solution. Therefore, it is not surprising that the direct resolution of (±)-**3** has not found industrial application in spite of the general convenience of entrainment procedures. In fact, chloride (±)-**3** is readily available, but not efficiently resolved by this method[10,11] and, conversely, mono-oxalate (±)-**3** better resolved, but is not readily available, since its preparation requires the intermediary conversion of chloride into hydroxide by ion-exchange chromatography, treatment with oxalic acid, complete water removal and crystallization of the racemate from methoxyethanol.[11]

☆ See Ref. 1.

* Corresponding author. Tel.: +39 2 50319336; fax: +39 2 50319335.
E-mail address: marco.pallavicini@unimi.it (M. Pallavicini).

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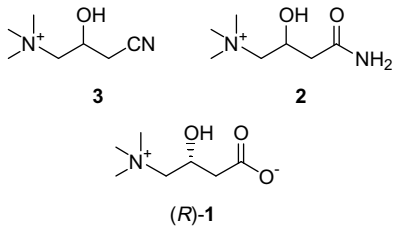


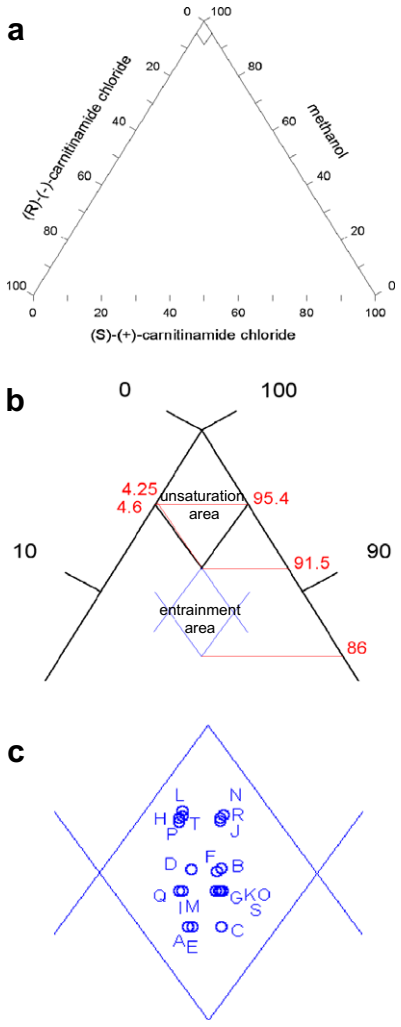
Recently, we have characterized the enantiomer systems formed by some inorganic salts of carnitine amide **2** proving that the chloride is a conglomerate, the nitrate is a racemic compound and the sulfate is a solid solution.[12] The chloride of **2** is an immediate carnitine precursor, easily prepared from **3** chloride by hydrolysis[13] and thus, similar to chloride **3**, an inexpensive racemic material. This makes (±)-**2** chloride a good candidate to resolution by preferential crystallization making the entrainment resolution of (±)-**2** an attractive method for preparing (*R*)-**1**. Herein, we report a simple and efficient procedure to obtain chlorides (*R*)-**2** and (*S*)-**2** by direct crystallization from methanolic supersaturated solutions of the racemate.

Table 1
Experimental solubility (grams of solute per 100 g of solution) of the chlorides of (±)-**2** and of (*R*)-**2**

Solvent	*T* (°C)	Chloride (±)-**2**	Chloride (*R*)-**2**
Water	22		56
Methanol	22	6.8	3.6
Methanol	30	8.5	4.6
Ethanol	22		<0.3

2. Results and discussion

On the basis of the previously demonstrated conglomerate nature of chloride **2**, we decided to attempt its resolution according to an entrainment procedure, which involves the preferential precipitation of one enantiomer from a supersaturated solution of the racemate enriched with a slight excess of the same enantiomer. Before performing the crystallization experiments, we measured the solubility of (±)-**2** chloride and of (*R*)-**2** chloride in water, in methanol and in ethanol. The results, reported in Table 1, show that methanol, thanks to the moderate dissolving capability of **2**, is a crystallization solvent preferable to water and ethanol, where the



		% (*S*)-**2** chloride	% (*R*)-**2** chloride	% MeOH
1st cycle	A	5.85	6.44	87.71
	B	5.79	5.40	88.81
	C	6.34	5.94	87.72
	D	5.36	5.85	88.78
2nd cycle	E	5.90	6.38	87.72
	F	5.75	5.49	88.76
	G	5.92	5.68	88.40
	H	4.72	5.49	89.79
3rd cycle	I	5.42	6.18	88.40
	J	5.32	4.97	89.71
	K	5.97	5.63	88.40
	L	4.67	5.43	89.90
4th cycle	M	5.42	6.18	88.40
	N	5.32	4.87	89.80
	O	6.02	5.58	88.40
	P	4.72	5.60	89.68
5th cycle	Q	5.37	6.23	88.40
	R	5.30	4.94	89.76
	S	5.98	5.62	88.40
	T	4.68	5.56	89.76

Figure 1. (a) Solubility diagram of chloride **2** in methanol at 30 °C. The concentrations of the components are expressed as weight percentages. (b) Unsaturation area (black quadrilateral) and area usable for the entrainment (blue parallelogram) in the solubility diagram of **2** chloride. (c) Entrainment area with the ternary compositions describing the five cycles of resolution of (±)-**2** chloride (see Table 2). The points A-E-I-M-Q are the ternary compositions of the (*R*)-enriched supersaturated solutions of (±)-**2** chloride, the points C-G-K-O-S those of the (*S*)-enriched supersaturated solutions of (±)-**2** chloride, the points B-F-J-N-R those of the mother liquors remaining from the crystallization of (*R*)-**2** chloride and the points D-H-L-P-T those of the mother liquors remaining from the crystallization of (*S*)-**2** chloride. The respective terns of values (weight percentages) are listed in the side table.

Table 2
Resolution of (±)-**2** chloride by entrainment

Cycle	Precipitation time[a]	Chloride **2** added (mg)		Recovery of resolved chloride **2**[b] (mg)		α_D^{30} of the mother liquors
		(±)	(*R*)	(*R*)	(*S*)	
1	12′ (A→B)	4000	200	420 (79.6% ee)		+0.086
	14′ (C→D)	420			410 (73.2% ee)	−0.074
2	15′ (E→F)	410		400 (63.8% ee)		+0.090
	46′ (G→H)	400			560 (64.8% ee)	−0.125
3	31′ (I→J)	560		530 (75.1% ee)		+0.100
	75′ (K→L)	530			605 (65.3% ee)	−0.108
4	42′ (M→N)	605		570 (76.3% ee)		+0.120
	80′ (O→P)	570			520 (91.2% ee)	−0.150
5	56′ (Q→R)	520		541 (80.3% ee)		+0.102
	105′ (S→T)	541			510 (79.2% ee)	−0.131
Total		8556	200	2461[c] (75.5% ee)	2605[d] (74.3% ee)	
Residue[e]		3510				

[a] After the third crystallization (E→F), methanol was increased from 30 to 32 g. Between brackets, the ternary composition of the solution before and after the precipitation (see Table in Fig. 1).
[b] Between brackets, the enantiomeric purity resulting from the percent ratio of the observed specific rotation to the specific rotation {$[\alpha]_D^{20} = -24.5$ (*c* 1, methanol)} of pure chloride (*R*)-**2**.
[c] 1800 mg (100% ee) after recrystallization from methanol.
[d] 1874 mg (98.1% ee) after recrystallization from methanol.
[e] Recovered, with 9.6% ee [(*R*) enriched], by concentration of the mother liquors remaining from the last crystallization.

solubility of the chloride is too high and too low, respectively. In methanol, we found a 1.8 ratio between the solubilities, expressed as a solute/solution weight percent, of (±)-**2** and (*R*)-**2**. Such a value, lower than 2, is typical for conglomerates, whose molecules are dissociable in solution as is the case for those of chloride **2**.[14]

The ternary phase diagram of (*R*)-**2** chloride/(*S*)-**2** chloride/methanol was constructed using values of 8.5% and 4.6% solubility for chloride (±)-**2** and chloride (*R*)-**2**, respectively, in methanol at 30 °C (see Fig. 1a) At this temperature, the weight percentage of methanol for a saturated solution of chloride (±)-**2** was 91.5, while it was 95.4 for a saturated solution of chloride (*R*)-**2**. The resultant unsaturation area is not represented by a parallelogram, but by a quadrilateral whose two lower sides, constituting the solubility curve, are not parallel to the sides of the triangle and intersect to form an angle little wider than 60° (α <2) (see Fig. 1b).

In this triangular phase diagram, we defined the region in which resolution by entrainment is favourable, that is, suitable conditions of supersaturation for an efficient resolution by preferential crystallization. A 14 wt % of chloride (±)-**2**, that is 1.6 times its solubility in methanol at 30 °C, was empirically established as a concentration corresponding to a sufficiently high, but nevertheless even metastable supersaturation, since without seeding, no crystallization took place in a methanolic solution with such a concentration over several days at this temperature. Therefore, the point corresponding to 7–7–86% ternary composition of (*R*)-**2** chloride-(*S*)-**2** chloride–methanol was fixed as a supersaturation limit and used to graphically delimitate the area of the ternary diagram useful for entrainment (see the blue parallelogram in Fig. 1b). Within this area, we drew five consecutive crystallization cycles. Figure 1c shows the ternary compositions (points A-T) assumed by the system over the course of the cycles.

In the first cycle, chloride (±)-**2** (4 g), enriched with a slight excess of chloride (*R*)-**2** (0.2 g), was dissolved in boiling methanol (30 g) (see ternary composition A in Fig. 1c) and slowly cooled to 33 °C while stirring. The resulting supersaturated solution was seeded with crystals of (*R*)-**2** chloride, cooled further to 30–32 °C and, after 12 min, filtered to isolate a laevorotatory precipitate, containing an excess of (*R*)-**2** chloride, which was 1.7 times the initial (*R*)-enrichment. The filtered mother liquors, now having a composition B (see Fig. 1c) and rotation changed to a positive value because of the (*S*)-enrichment, were added with chloride (±)-**2** to restore the initial supersaturation (see ternary composition C in Fig. 1c); the procedure was repeated for the (*S*)-enantiomer. Again, we isolated a crystalline, but now destrorotatory precipitate, which contained an excess of chloride (*S*)-**2** more than double the (*S*)-enrichment of the filtered mother liquors of the previous crystallization, while the filtered (*R*)-enriched mother liquors of this second crystallization had now assumed composition D (see Fig. 1c) and showed a negative value for the rotation. After the third crystallization, the amount of methanol was increased from 30 to 32 g raising the crystallization time from 12–15 min to about 60 min for the subsequent seven resolutions. After five cycles, we obtained 2.46 g of (*R*)-**2** chloride with 75.5% enantiomeric purity and 2.61 g of (*S*)-**2** chloride with 74.3% enantiomeric purity (Table 2). Finally, these two quantities were recrystallized from methanol to yield about 1.8 g of each enantiomer, 9 times the initial investment in chloride (*R*)-**2**, with >98% enantiomeric purity. The mother liquors remaining from the last crystallization were laevorotatory and, after concentration, afforded 3.51 g of chloride **2** enriched in the levo enantiomer with 9.6% enantiomeric purity. These values are consistent with those of the mixture initially subjected to resolution, that is, 4.2 g and 4.8% enantiomeric purity [4.0 g of chloride (±)-**2** + 0.2 g of chloride (*R*)-**2**].

3. Conclusion

The unreported resolution of chloride (±)-**2** by preferential crystallization according to the entrainment method was tried and developed successfully. The main advantages of such a new procedure, in addition to the typical ones of entrainment (very simple operations and no chiral auxiliaries), can be summarized as follows: (i) the only necessary materials are methanol and the easily available, inexpensive and very stable chloride of (±)-**2**; (ii) transformation of this latter into other salts, such as mono-oxalate or perchlorate, or specific solvent systems is not required for successful resolution, as in the case of the carnitine nitrile entrainment; (iii) the resolution efficiency (chemical yield x enantiomeric excess) is absolutely good (approx. 20% medium recovery of total enantiomer at each crystallization) and highly reproducible.

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