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(54) Titre : PROCEDE AMELIORE POUR L'OBTENTION DE L-(-)-CARNITINE A PARTIR DE PRODUITS RESIDUELS POSSEDANT UNE CONFIGURATION INVERSE

(54) Title: IMPROVED PROCESS FOR MANUFACTURING L-(-)-CARNITINE FROM WASTE PRODUCTS HAVING OPPOSITE CONFIGURATION

(57) Abrégé/Abstract:
Manufacturing of L-(-)-carnitine from starting compounds containing an asymmetrical carbon atom having a configuration opposite to that of L-(-)-carnitine is set out utilizing as the starting compound D-(+)- carnitinamide or D-(+)-carnitinenitrile which is then converted to acyl D-(+)-carnitinenitrile or acyl D-(+)-carnitinamide followed by acid hydrolysis to D-(+)-carnitine followed by lactonizing to the lactone of L-(-)-carnitine and finally converting the resulting product to L-(-)-carnitine.

ABSTRACT

IMPROVED PROCESS FOR MANUFACTURING L-(-)-CARNITINE FROM WASTE PRODUCTS HAVING OPPOSITE CONFIGURATION

Manufacturing of L-(-)-carnitine from starting compounds containing an asymmetrical carbon atom having a configuration opposite to that of L-(-)-carnitine is set out utilizing as the starting compound D-(+)-carnitinamide or D-(+)-carnitinenitrile which is then converted to acyl D-(+)-carnitinenitrile or acyl D-(+)-carnitinamide followed by acid hydrolysis to D-(+)-carnitine followed by lactonizing to the lactone of L-(-)-carnitine and finally converting the resulting product to L-(-)-carnitine.

TITLE OF THE INVENTION

IMPROVED PROCESS FOR MANUFACTURING L-(-)-CARNITINE FROM WASTE PRODUCTS HAVING OPPOSITE CONFIGURATION

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to an improved process for manufacturing L-(-)-carnitine from starting compounds containing an asymmetrical carbon atom having a configuration opposite to that of L-(-)-carnitine. The process of the present invention overcomes the drawbacks of conventional processes which first convert a starting compound into an achiral intermediate, generally crotonobetaine or gamma-butyrobetaine, and then convert the achiral intermediate to L-(-)-carnitine. The process of the present invention uses D-(+)-carnitinamide or D-(+)-carnitinenitrile as preferred starting compounds.

Discussion of the Background

Carnitine contains a single center of asymmetry and therefore exists as two enantiomers, designated D-(+)-carnitine and L-(-)-carnitine. Of these, only L-(-)-carnitine is found in living organisms, where it functions as a vehicle for transporting fatty acids across mitochondrial membranes. Whilst L-(-)-carnitine is the physiologically-active enantiomer, racemic D,L-carnitine has conventionally been used as a therapeutic agent. It is now recognized, however, that D-(+)-carnitine is a competitive

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inhibitor of carnitine acyltransferases, and that it diminishes the level of L-(-)-carnitine in myocardium and skeletal muscle.

It is therefore essential that only L-(-)-carnitine be administered to patients undergoing haemodialysis treatment or treatment for cardiac or lipid metabolism disorders. The same requirement applies to the therapeutic utilization of acyl derivatives of carnitine for treating disorders of the cerebral metabolism, peripheral neuropathies, peripheral vascular diseases and the like. These disorders are typically treated with acetyl L-(-)-carnitine and propionyl L-(-)-carnitine, which are obtained by acylating L-(-)-carnitine.

Various chemical procedures have been proposed for the industrial-scale production of carnitine. Unfortunately, these procedures are not stereospecific and produce racemic mixtures of D-(+)- and L-(-)-isomers. It is thus necessary to apply resolution methods in order to separate the enantiomeric constituents of the racemate.

Typically, the D,L-racemic mixture is reacted with an optically active acid (e.g. D-(-)-tartaric acid, D-(+)-camphorsulfonic acid, (+)-dibenzoyl-D-(-)-tartaric acid, N-acetyl-L-(+)-glutamic acid and D-(+)-camphoric acid) to obtain two diastereoisomers which can be separated from each other. In the classic process disclosed in U.S. Patent 4,254,053, D-(+)-camphoric acid is used as the resolution

agent of a racemic mixture of D,L-carnitinamide, obtaining D-(+)-carnitinamide as a by-product, and L-(-)-carnitinamide which, by hydrolysis, gives L-(-)-carnitine.

However, these resolution procedures are complex and costly, and in all cases result in the production of equimolar quantities of L-(-)-carnitine and D-(+)-carnitine or a precursor thereof as by-product, having configuration opposite to that of L-(-)-carnitine. Several microbiological processes have recently been proposed for producing L-(-)-carnitine *via* stereospecific transformation of achiral derivatives obtained from the huge amounts of D-(+)-carnitine (or of a precursor thereof, such as D-(+)-carnitinamide) which are generated as by-products in the industrial production of L-(-)-carnitine.

These processes are generally predicated upon the stereospecific hydration of crotonobetaine to L-(-)-carnitine, and differ principally by virtue of the particular microorganism employed to accomplish the biotransformation of interest. See, for example, the processes disclosed in: EP 0 12 1444 (HAMARI), EP 0 122 794 (AJINOMOTO), EP 0 148 132 (SIGMA-TAU), JP 275689/87 (BIORU), JP 61067494 (SEITETSU), JP 61234794 (SEITETSU), JP 61234788 (SEITETSU), JP 61271996 (SEITETSU), JP 61271995 (SEITETSU), EP 0 410 430 (LONZA), EP 0 195 914 (LONZA), EP 0 158 194 (LONZA), and EP 0 457 735 (SIGMA-TAU).

On the other hand, JP 62044189 (SEITETSU) discloses a process for stereoselectively producing L-(-)-carnitine

starting from gamma-butyrobetaine, which is in turn obtained enzymically from crotonobetaine.

All of these processes have several drawbacks. First, D-(+)-carnitine must first be converted to an achiral compound (crotonobetaine, gamma-butyrobetaine) before it can be used as the starting compound in all of the aforesaid microbiological processes.

In addition, the microbiological procedures proposed to date have not proven practicable for manufacturing L-(-)-carnitine on an industrial scale for one or more of the following reasons:

(i) the yield of L-(-)-carnitine is extremely low;

(ii) the microorganisms must be cultivated in a costly nutritive medium;

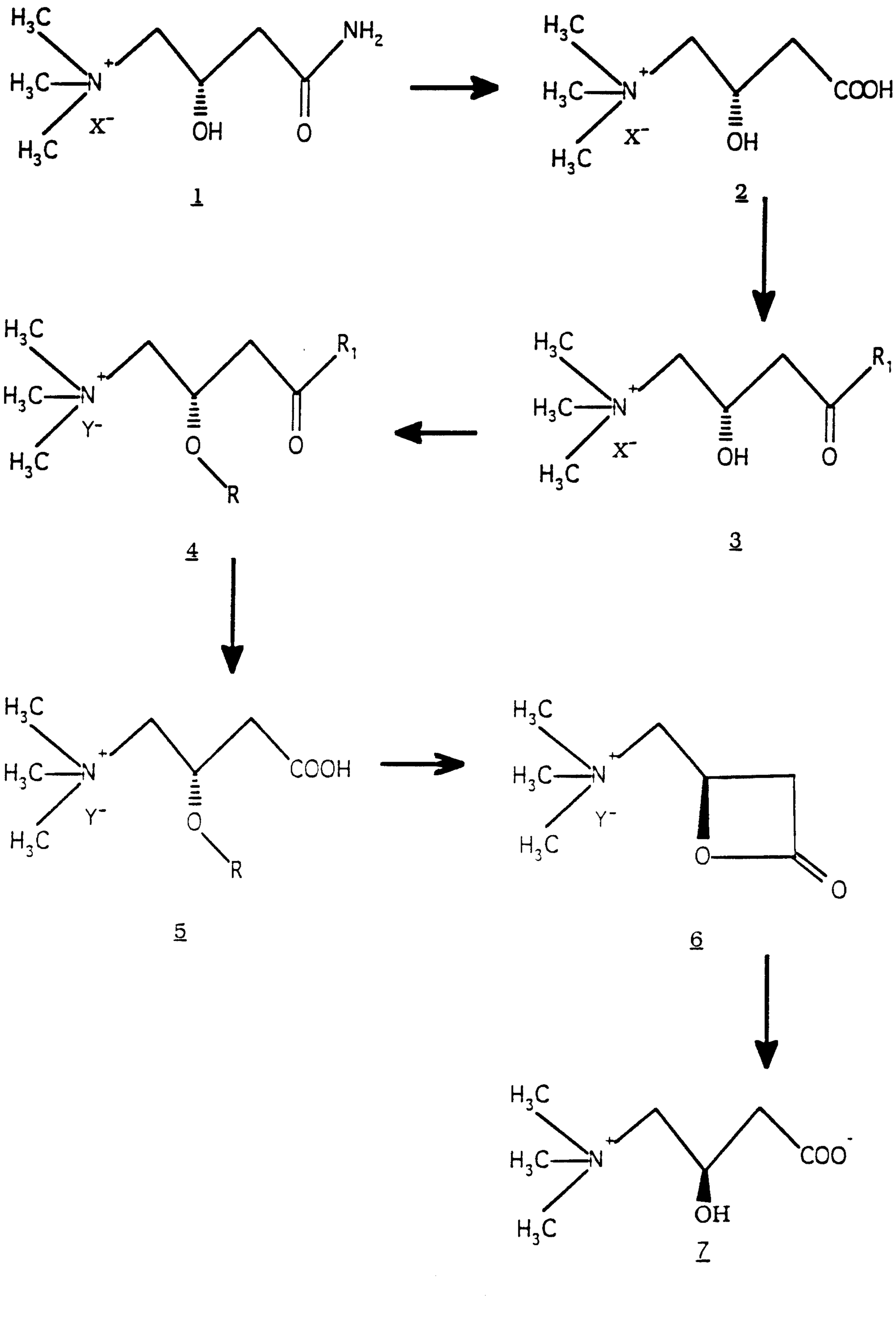
(iii) the microorganism can only tolerate low concentrations [up to 2-3% (w/v)] of crotonobetaine;

(iv) side reactions occur, such as the reduction of crotonobetaine to gamma-butyrobetaine or the oxidation of L-(-)-carnitine to 3-dehydrocarnitine. These side reactions reduce the final yield of L-(-)-carnitine.

In order to overcome all of the aforesaid drawbacks of the known processes, in the present applicant's U.S. patent 5,599,978, a process has been disclosed which allows high yields of L-(-)-carnitine to be obtained starting from a by-product having configuration opposite to that of L-(-)-carnitine (such as D-(+)-carnitinamide) with no need to first convert the starting by-product into an achiral intermediate.

This process which is illustrated in the following reaction scheme 1:

SCHEME 1



1 2 3 4 5 6 7

comprises hydrolyzing a D-(+)-carnitinamide salt 1 to D-(+)-carnitine 2 and esterifying 2 into ester 3 (via known methods) wherein R1 is preferably arylalkoxy, e.g. benzyloxy.

The ester 3 is then converted to the acyl derivative 4 wherein Y, which can be the same as X, is preferably a counterion, e.g. perchlorate, imparting solubility to 4. OR is a leaving group wherein R is preferably an alkylsulfonyl group having 1-12 carbon atoms, e.g. mesyl.

The acylation of 3 to 4 is carried out preferably in pyridine by reacting the ester 3 with an acylating agent RY wherein Y is halogen and R is an acyl group as defined above. Preferably RY is the chloride of the selected acyl group.

The ester group $-COR_1$ of 4 (R_1 =benzyloxy) is hydrogenated to carboxyl group thus giving acyl D-(+)-carnitine 5 which is converted to the lactone 6 of L-(-)-carnitine. The lactonization is suitably carried out in an aqueous basic environment: either with $NaHCO_3$ (ratio 1:1) or with an AMBERLITE™ IRA-402 basic resin activated in HCO_3^- form or with an LA2 resin. The lactone is isolated by evaporating the aqueous solution or precipitating it as a salt (for example, as tetraphenylborate or reineckate).

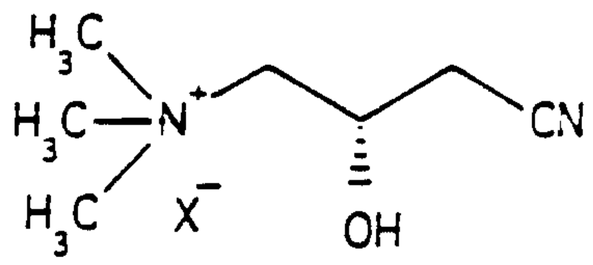
Finally, lactone 6 is suitably converted to L-(-)-carnitine inner salt 7. The lactone is dissolved in water and the resulting solution treated with a base such as $NaHCO_3$ (ratio 1:1), for 8-24 hours.

L-(-)-carnitine can suitably be purified from the salts which are formed from the X^- anion, from the excess, if any, of the acyl halogenide,

from pyridine, and the like, by chromatographing the aqueous solution on a strongly acidic resin such as IR 120, eluting with water and then with NH_4OH, or alternatively eluting first on a strongly basic resin such as AMBERLITE™ IRA 402 activated in OH form and thereafter on a weakly acid resin such as AMBERLITE™ IRC-50.

The process of the present invention which is illustrated in the following reaction scheme 2 constitutes a remarkable improvement over the previous process.

SCHEME 2



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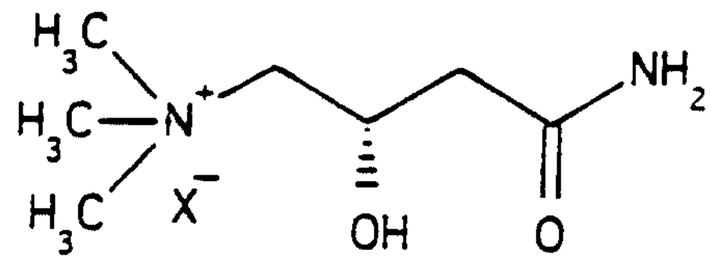

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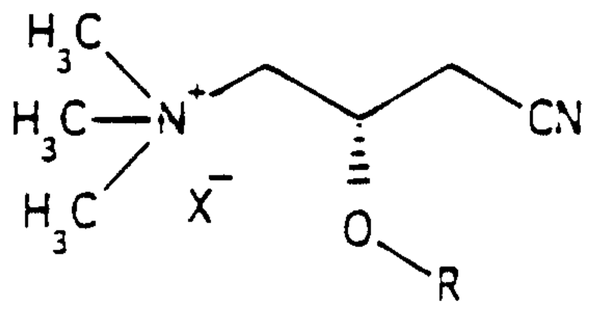

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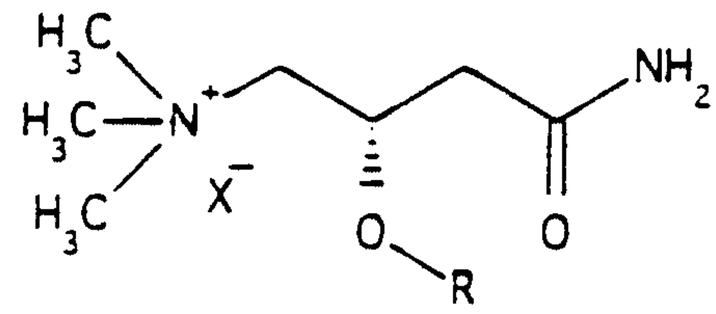

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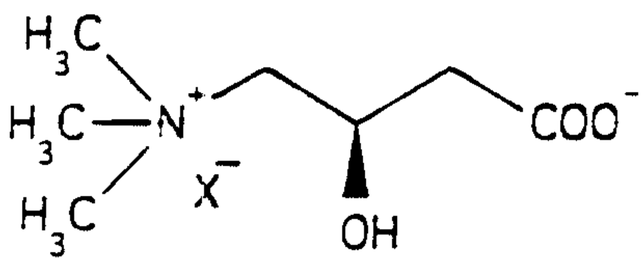

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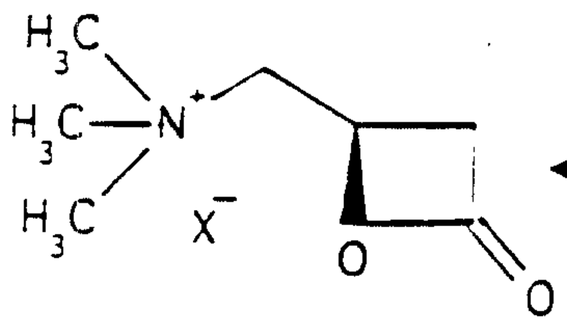

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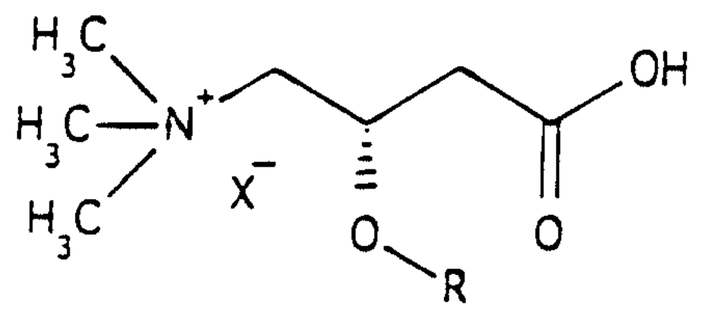

4

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With reference to the reaction scheme 2, D-(+)-carnitinenitrile 1 wherein X^- is any anion, preferably an anion imparting solubility, such as perchlorate, tetraphenylborate, alkylsulphonate wherein the alkyl group has 1-12 carbon atoms, is converted to acyl derivative 2 wherein OR is a good leaving group.

To this end, the acylation of 1 to 2 is carried out by reacting 1 with an acylating agent selected from RY wherein Y is halogen (e.g. chlorine) and the anhydride R_2O wherein R is an alkylsulfonyl group having 1-12 carbon atoms, formyl or trifluoroacetyl. Preferably, the alkylsulfonyl group is selected from methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), p-bromobenzenesulfonyl (brosyl), p-nitrobenzenesulfonyl (nosyl), trifluoro-methanesulfonyl (triflyl), nonafluoromethanesulfonyl (nonaflyl) and 2,2,2-trifluoroethanesulfonyl (tresyl). Mesyl is particularly preferred.

When RY is a chloride, the reaction takes place in pyridine or pyridine alkyl derivatives wherein the alkyl group is lower alkyl having 1-4 carbon atoms, or in other basic organic solvents such as triethylamine, or in inert anhydrous organic solvents such as acetonitrile or methylene chloride, in mixture with a base such as pyridine, lutidine, picoline or polyvinylpyridine.

The acylating agent is added at ratios ranging from 1:1 to 1:10, preferably 1:3. The resulting reaction mixture is kept under stirring at 0°C-50°C, for 1-24 hours.

The acyl D-(+)-carnitinenitrile 2 is converted via acid hydrolysis with conventional procedures to acyl D-(+)-carnitinamide 3 which can be

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directly arrived at by acylating D-(+)-carnitinamide with RY (as shown in the reaction scheme).

Hydrolysis of 2 takes place in an acid aqueous environment, at pH 0-4, at 50°C-80°C, for 10-48 hours, yielding the intermediate acyl D-(+)-carnitinamide 3 which forms acyl D-(+)-carnitine 4.

The acyl D-(+)-carnitinamide 3 is hydrolyzed to acyl D-(+)-carnitine 4 under the same conditions.

Conversion of acyl D-(+)-carnitine 4 to lactone 5 and the conversion of this latter compound to L-(-)-carnitine 6 are carried out as disclosed in the previously cited U.S. patent 5,599,978.

It should be understood that, whereas the process disclosed above has been described, for the sake of clarity, as a sequence of four distinct operating steps, the corresponding industrial process consists of two steps only. When the process of the present invention is carried out as an industrial process, the acyl D-(+)-carnitinenitrile 2 can be directly converted to L-(-)-carnitine inner salt 6 without isolating either the acyl D-(+)-carnitinamide 3 or the acyl D-(+)-carnitine 4 or the lactone 5.

In fact, the ester of acyl D-(+)-carnitinenitrile 2 is hydrolyzed in an acid environment to compound 3 and this latter to compound 4, then the resulting aqueous solution is concentrated and the concentrate is brought to pH 7-9, preferably 8-9 and kept at this pH value for 30-50 hours yielding L-(-)-carnitine. L-(-)-carnitine thus obtained is purified from any salt via treatment with acidic and basic resins.

In the following example which describes one embodiment of the process of the invention, the intermediate compounds 2, 3 and 4 were isolated so as to exhaustively characterize them from a physico-chemical standpoint.

It will be, however, apparent to any expert in organic synthesis that the industrial process comprises the following steps only:

(a) acylating the hydroxyl group of D-(+)-carnitinenitrile 1 or D-(+)-carnitinamide 1' with an acylating agent RY, wherein R has the previously defined meanings, with the resulting formation of a leaving group OR thus obtaining acyl D-(+)-carnitinenitrile 2 or acyl D-(+)-carnitinamide 3; and

(b) converting 2, or respectively 3 to L-(-)-carnitine inner salt 6.

Preparation of methanesulfonyl-D-carnitinenitrile perchlorate 2.

Methanesulfonyl chloride (14.2 g; 123 mmoles) was added over a period of 5 minutes to a solution of D-carnitinenitrile perchlorate 1 (10 g; 41 mmoles) in anhydrous pyridine (200 mL).

The solution was kept under stirring for 1 hour, then poured into an Erlenmeyer flask containing Et_2O (800 mL) under stirring. The precipitate which formed was crystallized from hot CH_3CN/iPrOH (filtering off the insoluble residue in hot CH_3CN).The crystalline product thus obtained was triturated with hot iPrOH yielding 9.4 g of compound 2.

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Yield = 71%

Differential thermal analysis = the compounds melts at 155°C.

$[\alpha]_D^{20}$ = +43° (C=1% H_2O)

TLC = silica gel Eluant = $CHCl_3$/MeOH/iPrOH/H_2O/AcOH

42 28 7 10.5 10.5

Rf= 0.58

Elementary analysis for $C_8H_{17}ClN_2O_7S$

	C%	H%	N%	Cl%
Calculated	29.26	5.34	8.73	11.05
Found	30.21	5.35	8.47	10.97

^{1}HNMR(D_2O):δ5.78-5.70(m,1H,-CHOMs),4.12-3.80(m,2H,-$CH_2N^+Me_3$),

3.42(s,3H,CH_3SO_3-),3.42-3.35(m,1H,C<u>H</u>HCOO-),3.30(s,9H,-N^+Me_3),

3.20-3.12(m,1H,-CH<u>H</u>COO-)

^{13}CNMR(D_20): δ 118.695; 71.848; 69.616; 57.138; 41.575; 25.965

IR (Kbr) = ν (cm^{-1}) 2256 (-C=N), 1351 and 1175 (CH_3SO_3-)

HPLC

Column=Nucleosil 5-SA Diameter 4.0 mm Length= 200 mm

Eluant = CH_3CN/KH_2PO_4 50 mM (65/35) pH=3.5 with H_3PO_4

Flow rate= 0.75 mL/min

Retention time= 12.80 min.

Detector= RI Waters 410

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Preparation of methanesulfonyl D-carnitinamide perchlorate 3.

Methanesulfonyl chloride (9.88 g; 86.31 mmoles) was added over a 5-minute period to a solution of D-carnitinamide perchlorate 1' (15 g; 57.54 mmoles) in anhydrous pyridine (300 mL).

The solution was kept under stirring at room temperature for 1 h and 15 minutes, then poured into an Erlenmeyer flask containing Et_2O (2.5 L) under stirring. The precipitate thus obtained was refluxed with iPrOH which was then decanted. The undissolved solid residue was further washed with iPrOH and then dried yielding 10.2 g of compound 3.

Yield= 52%

Differential thermal analysis = the compound melts at 156-158°C

$[\alpha]_D^{20}$ = +21.5° (C=1% H_2O)

TLC= silica gel Eluant= $CHCl_3$/MeOH/iPrOH/H_2O/AcOH

42 28 7 10.5 10.5

Rf= 0.52

Elementary analysis for $C_8H_{19}ClN_2O_8S$

	C%	H%	N%	Cl%	S%
Calculated	28.36	5.65	8.31	10.46	9.46
Found	28.74	5.60	7.89	10.2è	9.25

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^{1}HNMR(DMSO)d_6): δ 7.60 and 7.20(2s,2H,-$CONH_2$),5.4(m,1H,-CHOMs),

4.0-3.62(4m,2H,-$CH_2N^+Me_3$),3.35(s,3H,CH_3SO_3-),3.15(s,9H,N^+Me_3),

2.8-2.7(m,2H,-CH_2CON)

^{13}CNMR(D_2O): δ 175.272; 74.831; 70.798; 56.871; 41.521; 41.308

IR (Kbr) = ν (cm^{-1})1696 (-C= O), 1333 and 1174 (CH_3SO_3-)

HPLC

Column=Nucleosil 5-SA Diameter 4.0 mm Length= 200 mm

Eluant = CH_3CN/KH_2PO_4 50 mM (65/35) pH=3.5 with H_3PO_4

Flow rate= 0.75 mL/min

Retention time= 19.83 min.

Detector= RI Waters 410

Preparation of methanesulfonyl D-carnitine 4 from methanesulfonyl D-carnitinenitrile perchlorate 2.

A solution of methanesulfonyl D-carnitinenitrile perchlorate 2 (2 g; 6.23 mmoles) in 12N HCl (40 mL) was heated at 50°C under stirring for 36 hours.

The reaction proceeds via the formation of methanesulfonyl-D-carnitinamide 3 as shown by HPLC analysis after 2 hours from reaction beginning.

At the end of the reaction the solution was brought to dryness

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under vacuum giving an oily solid which was taken up with CH_3CN. The insoluble solid was filtered off and the filtrate poured in Et_2O; the precipitate thus obtained was isolated by decantation, washed with Et_2O and dried under vacuum yielding 2 g of the raw product 4.

Methanesulfonyl-D-carnitinamide 3.

HPLC

Column=Nucleosil 5-SA Diameter 4.0 mm Length= 200 mm

Eluant = CH_3CN/KH_2PO_4 50 mM (65/35) pH=3.5 with H_3PO_4

Flow rate= 0.75 mL/min

Retention time= 19.83 min.

Detector= RI Waters 410

Methanesulfonyl-D-carnitine 4.

HPLC

Column=Nucleosil 5-SA Diameter 4.0 mm Length= 200 mm

Eluant = CH_3CN/KH_2PO_4 50 mM (65/35) pH=3.5 with H_3PO_4

Flow rate= 0.75 mL/min

Retention time= 11.38 min.

Detector= RI Waters 410

^{1}HNMR(D_2O): δ 5.70 and 5.6(m,1H,-CHOMs),

4.06-3.75(m,2H,-$CH_2N^+Me_3$),3.33(s,3H,CH_3SO_3-),

3.27(s,9H,N^+Me_3),3.15-3.00(m,2H,-CH_2COOH)

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The product thus obtained was used as such, without further purification, in the reaction sequence disclosed in the previously cited Italian patent application RM 92 A 000195 to obtain L-carnitine inner salt.

Preparation of methanesulfonyl-D-carnitine 4 from methanesulfonyl-D-carnitinamide perchlorate 3 .

The reaction was carried out as described for the reaction starting from methanesulfonyl D-carnitinenitrile perchlorate 2.

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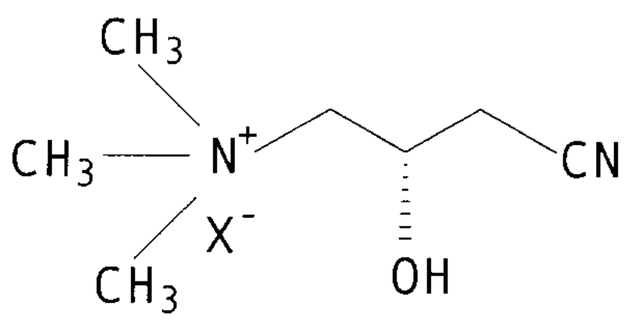
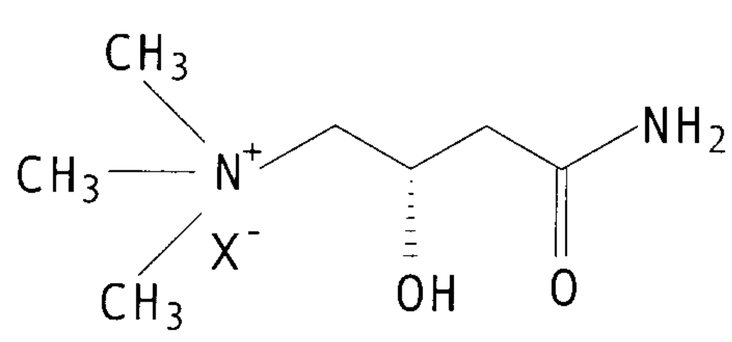
CLAIMS:

1. A process for producing L-(-)-carnitine from a precursor thereof having opposite configuration selected from D-(+)-carnitinenitrile and D-(+)-carnitinamide of the general formulae (1) and (1'), respectively:



(1)

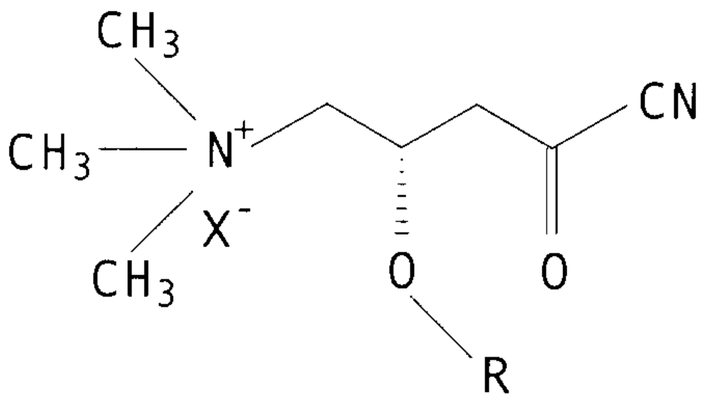
and



(1')

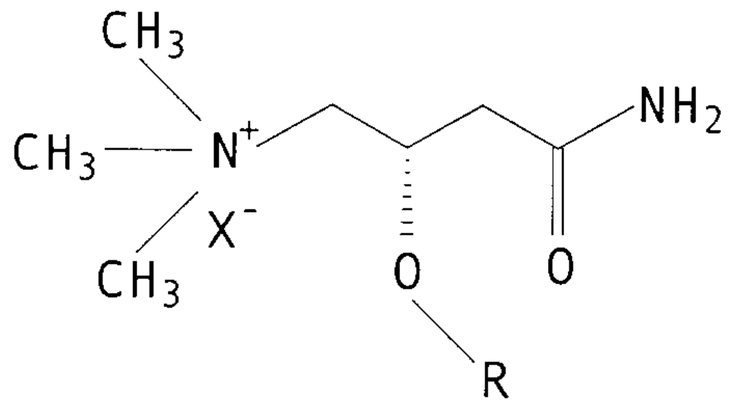
wherein X^- is a counterion, which comprises:

(a) converting the precursor of the general formulae (1) or (1') to acyl D-(+)-carnitinenitrile or acyl D-(+)-carnitinamide of the general formulae (2) or (3):



(2)

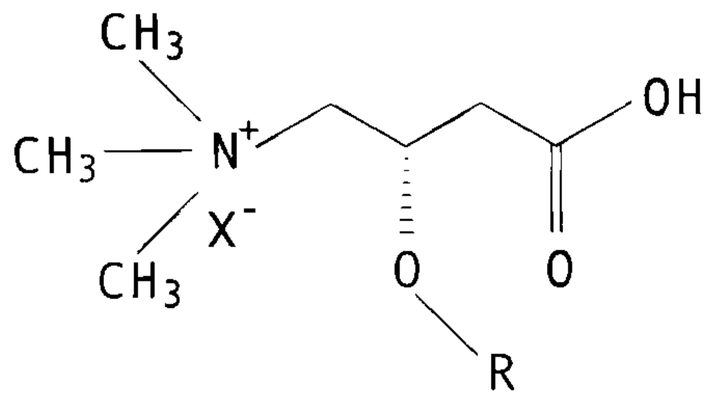
or



(3)

wherein X^- is as defined above, OR is a leaving group, and wherein R is a group selected from an alkylsulfonyl having 1-12 carbon atoms, formyl, trifluoroacetyl, p-toluenesulfonyl, p-bromobenzenesulfonyl and p-nitrobenzenesulfonyl;

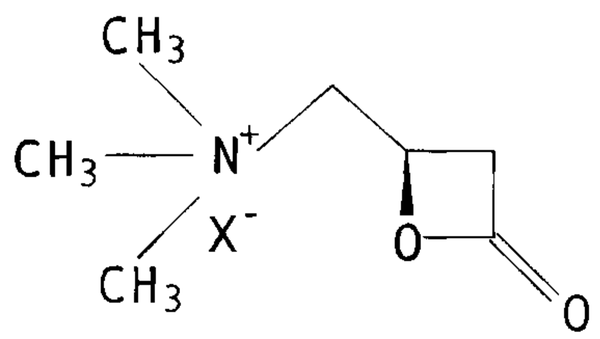
(b) converting the acyl D-(+)-carnitinenitrile of the general formula (2) or the acyl D-(+)-carnitinamide of the general formula (3) via acid hydrolysis to acyl D-(+)-carnitine of the general formula (4):



(4)

wherein X^- and R are as defined above;

(c) lactonizing the acyl D-(+)-carnitine of the general formula (4) to the lactone of L-(-)-carnitine of the general formula (5):

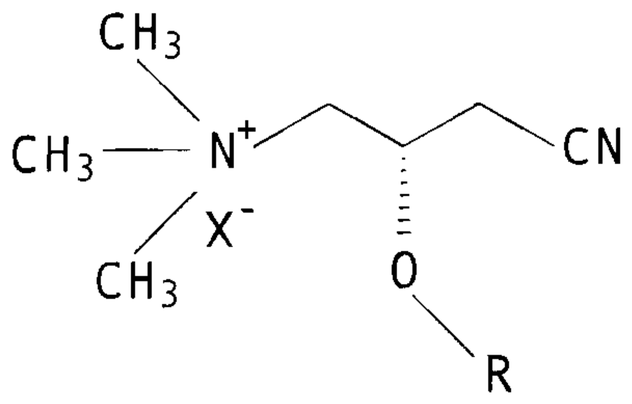


(5)

wherein X^- is as defined above, by treating the acyl D-(+)-carnitine of the general formula (4) in a basic environment; and

(d) converting the lactone of the general formula (5) to L-(-)-carnitine by treating the lactone of the general formula (5) in a basic solution and isolating L-(-)-carnitine inner salt by chromatography of the solution on a resin.

2. The process of claim 1, wherein when the precursor is of the general formula (1), the conversion of step (a) comprises acylating the precursor of the general formula (1) to acyl D-(+)-carnitinenitrile of the formula (2):



(2)

wherein X^- and R are as defined in claim 1, by reacting the precursor of the general formula (1) with an acylating agent of the general formula RY, wherein R is as defined in claim 1, and Y is a halogen, in pyridine or C_1-C_4-alkylpyridine, or in a basic or inert anhydrous organic solvent, wherein the acylating agent is added at a ratio ranging from 1:1 to 1:10, and the reaction is effected at 0°C-50°C for 1-24 hours.

3. The process of claim 2, wherein Y is chlorine.

4. The process of claim 2 or 3, wherein the acylating agent is added at a ratio of 1:3.

5. The process of claim 1, wherein when the precursor is of the general formula (1'), the conversion of step (a) comprises directly acylating the precursor of general formula (1') to the acyl D-(+)-carnitinamide of the general formula (3) by reacting the precursor of the general formula (1') with an acylating agent of the general formula RY, wherein R is as defined in claim 1, and Y is as defined in claim 2 or 3, in pyridine or C_1-C_4-alkylpyridine, or in a basic or inert anhydrous organic solvent, wherein the acylating agent is added at a ratio as defined in claim 2 or 4, and the reaction is effected at 0°C-50°C for 1-24 hours.

6. The process of any one of claims 1 to 5, wherein steps (b), (c) and (d) are carried out as a single step, without isolating intermediate compounds of the general formulae (2), (3), (4) and (5).

7. The process of any one of claims 1 to 6, wherein:

X^- is a halide, sulphate, phosphate, perchlorate, metaperiodate, tetraphenylborate, or alkylsulfonate having 1-12 carbon atoms; and

R is methanesulfonyl, p-toluenesulfonyl, p-bromobenzenesulfonyl, p-nitrobenzenesulfonyl, trifluoromethanesulfonyl, nonafluoromethanesulfonyl or 2,2,2-trifluoroethanesulfonyl.

8. The process of claim 7, wherein X^- is Cl^-.

9. The process of claim 7 or 8, wherein R is methanesulfonyl.

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