193. L-Carnitine. Novel Synthesis and Determination of the Optical Purity

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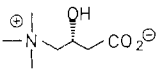
A new route to L-carnitine (**1**) based on the resolution of the trimethylammonium derivative **5** is described. The enantiomeric purity of **1** is determined by ^{1}H-NMR of its *O*-acetyl hydrochloride **11** using [Eu(hfc)$_3$] as chiral shift reagent. The optical rotation of **1** with an enantiomeric purity $\geq 99\%$ is $[\alpha]_D^{25} = -31.3°$ (c = 10, H_2O).

Introduction. – L-Carnitine (= (*R*)-3-hydroxy-4-(trimethylammonio)butanoate; **1**) was first isolated from meat extract in 1905 [1]. Its structure was established by chemical synthesis in 1927 [2]. During the 1950's, the H_2O-soluble compound carnitine was shown to be an essential growth factor for the meal worm, *Tenebrio molitor,* and hence was called vitamin B_T, with T standing for *Tenebrio* [3]. The physiological function of L-carnitine is to transport long-chain fatty acids through the mitochondrial membrane, thereby enabling their oxidation [3–5]. L-Carnitine has an important role in energy metabolism and is involved in regulating the level of blood lipids, and it is used in infant and in sport nutrition [6] [7]. As a drug, L-carnitine is utilized to increase cardiac output, to improve myocardial function, and to treat carnitine deficiency, especially after hemodialysis [7–11].



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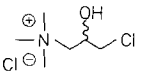

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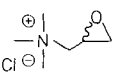

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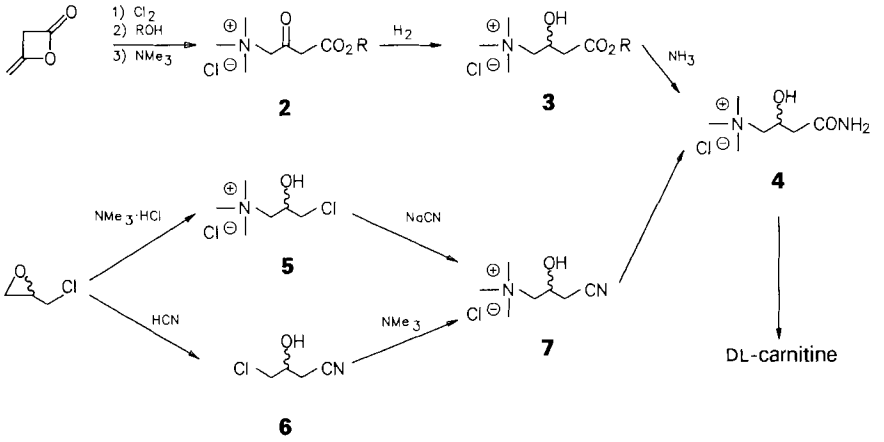
There are a number of straightforward chemical methods for the synthesis of DL-carnitine. Most of them start from diketene (*e.g.*, →**2**→**3**→**4**) or epichlorohydrin (*e.g.*, →**5** or **6**→**7**; *Scheme 1*) [12] [13]. Many syntheses of L-carnitine have consequently been developed by resolution of the racemic product or its precursors **3**, **4**, and **7** [13]. Thus, DL-ammonium chloride **4** has been separated using (+)-D-camphor-10-sulfonic acid [14], DL-ammonium chloride **7** separated using (+)-D-camphor-10-sulfonic acid or (–)-dibenzoyl-L-tartaric acid [15–17], DL-carnitine separated using mandelic acid [18] or (–)-dibenzoyl-L-tartaric acid [19] [20], and DL-carnitine [21] or *O*-acyl-DL-carnitine [22] [23] separated enzymatically.

L-Carnitine was also prepared from chiral compounds such as (+)-L-ascorbic acid [24], D-mannitol [25], (*R*)-4-chloro-3-hydroxybutyrate [26–29], 4-hydroxy-L-proline [30], or (–)-β-pinene [31]. The transformation of the achiral γ-butyrobetaine [32–34] and *trans*-crotonobetaine [35–37] into L-carnitine by isolated enzymes or microorganisms has also been intensively investigated.

Scheme 1



The discovery of the adverse effects of the unnatural D-carnitine [29] [38] and of the questionable effectiveness of the racemic mixture in angina-pectoris therapy [39], coupled with the recent growing interest in therapeutic application of L-carnitine, has prompted us to investigate a practical and economical synthesis for this compound. The known syntheses starting from chiral precursors as well as those evaluated by us are cumbersome and economically unfeasible on an industrial scale. On the other hand, despite the numerous difficulties involved in the resolution of racemic mixtures, we thought it worthwhile to undertake research efforts in this field. We justified our belief with the fact that known resolutions had only been performed on late intermediates or on the final product, while economy stipulates that this step be executed at the earliest stage possible. Secondly, the racemic trimethylammonium derivatives **5** and **8** were commercially available at low price and known as intermediates for racemic carnitine.

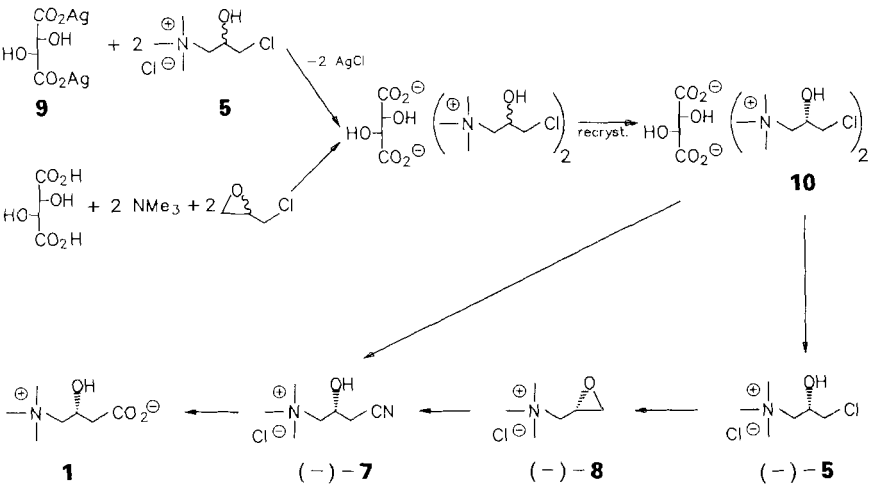
Since optical measurements have been the only means to determine the enantiomeric purity of L-carnitine, we developed a direct method which can be used as reference to the determination of the optical rotation.

Synthesis. – To check the possibility of resolving racemic mixtures **5**, 2 equiv. of the quaternary ammonium chloride **5** were added to a suspension of the di-silver salt **9** of (+)-L-tartaric acid (= (*R*, *R*)-tartaric acid) [40]. After removal of the AgCl and recrystallization, the L-tartrate **10** was obtained in good yield (33.6%) and enantiomeric purity [41] *(Scheme 2)*. In contrast to previous resolutions, this first step can surprisingly be performed with (+)-L-tartaric acid. An additional economic benefit of this process is that both carboxyl groups take part in the resolution.

(3-Chloro-2-hydroxypropyl)ammonium salts similar to **5** are produced industrially by reaction of epichlorohydrin with trimethylammonium salts [42] [43]. This indicates a way to avoid the use of the silver tartrate **9**. In fact, the reaction of the bis(trimethylammonium) (+)-L-tartrate with epichlorohydrin gives directly the tartrate **10** in a yield of 33.2%.

Treatment of the tartrate **10** with $Ca(CN)_2$ gives the insoluble Ca-tartrate and the (*R*)-salt (–)-**7** *(Scheme 2)* previously prepared by *Strack via* resolution [15] [16]. Follow-

Scheme 2



ing a known procedure [44], the acidic alcoholysis of (−)-**7**, followed by treatment with an ion-exchange resin, yields L-carnitine (**1**) in good yields and optical purity.

Alternatively, the tartrate **10** is converted to the chloride (−)-**5** by treatment with $CaCl_2$, recovering tartaric acid as its calcium salt. The chloride (−)-**5** is then transformed into the glycidyl salt (−)-**8** by base treatment and then into (−)-**7** by reaction with acetone cyanohydrin [45]. Using a procedure developed for the racemic material [42], L-carnitine (**1**) is also obtained by reacting (−)-**5** with NaCN and hydrolysing (−)-**7** with HCl. In this case, **1** can be prepared without isolation of the intermediated (−)-**8** and (−)-**7**.

Determination of the Optical Purity. – The specific optical rotation has hitherto been used to determine the enantiomeric (optical) purity of L-carnitine (**1**). The procedure lacks correlation with an absolute method and, therefore, can not be calibrated. As our product exhibited a higher rotation value than stated by *Strack* ($[\alpha]_D^{22} = -30.9°$ (c = 10, H_2O) [15]), we looked for a calibration method. The known biochemical determination methods being too unreliable [46] [47] and our attempts to separate the enantiomers by liquid chromatography having been fruitless, we used a modification of a NMR method recently reported [48]. Carnitine was first transformed into *O*-acetyl-carnitine hydrochloride with AcCl [49]. The ^{1}H-NMR spectrum (CD_3OD) of the resulting crude material was measured in the presence of 1.1 equiv. of [Eu(hfc)$_3$][1]) as chiral shift reagent. Two distinct peaks appear for the Me_3N^+ group, at 3.22 ppm for the (−)-*O*-acetyl-L-carnitine hydrochloride (**11**) and at 3.28 ppm for its (+)-D-isomer. As little as 0.5% of (+)-D-isomer can be discerned *(Fig. 1)*. This allows a confident correlation with measurements of optical-rotation values *(Fig. 2)*. Thus, L-carnitine with an enantiomeric purity ≥ 99.0% (by ^{1}H-NMR of **11**) and a chemical purity ≥ 99.8% (titration, HPLC) has the following

[1]) [Eu(hfc)$_3$] = tris[3-(heptafluoropropylhydroxymethylidene)-*d*-camphorato]europium(III).

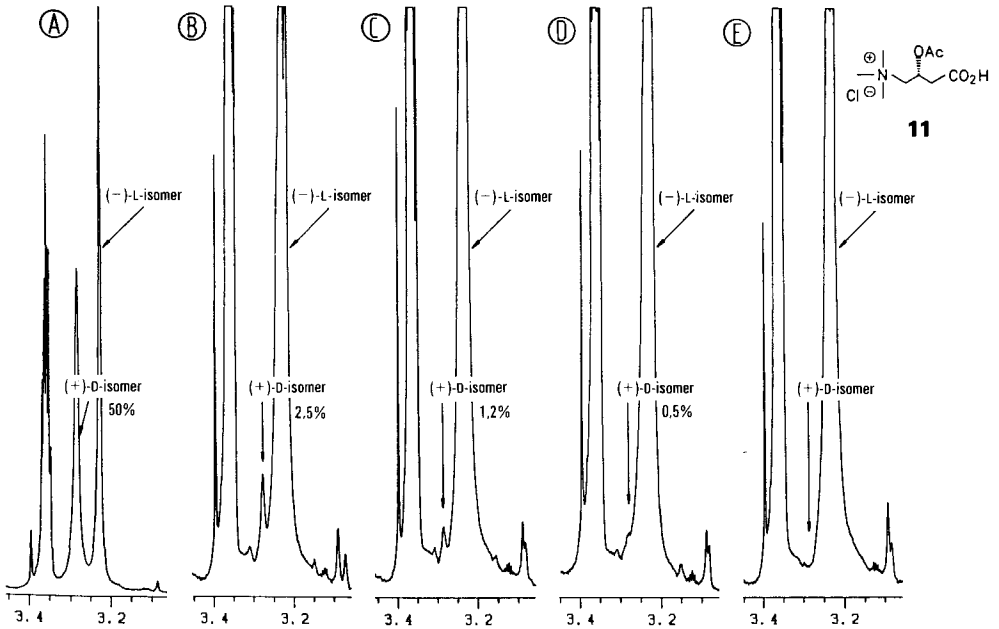


Fig. 1. *^{1}H-NMR* (300 MHz) *of* O-*acetylcarnitine hydrochloride* (10 mg) *in* CD_3OD (1 ml) *with [Eu(hfc)$_3$] as shift reagent.* Internal reference 0.01% TMS; A: *O*-acetyl-DL-carnitine·HCl (DL-**11**; enlargement factor × 0.1); B: L-**11** + 5% DL-**11**; C: L-**11** + 2.4% DL-**11**; D: L-**11** + 1% DL-**11**; E: L-**11** from *LONZA*'s L-carnitine.

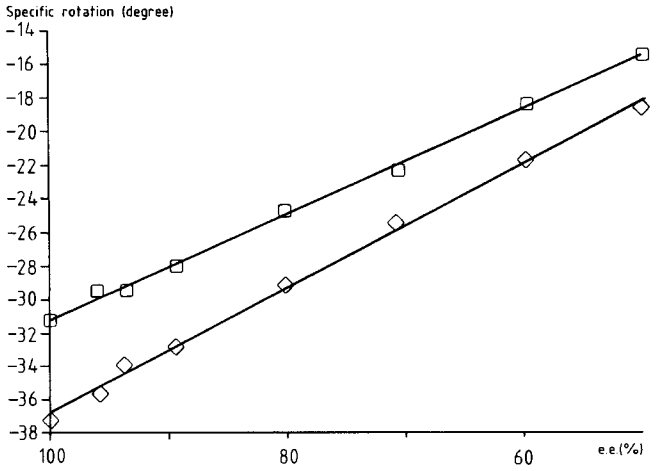


Fig. 2. *Correlation of the specific rotation* $[\alpha]_D^{25}$ (□) *and* $[\alpha]_{546}^{25}$ (◇) *with the enantiomeric excess* (e.e.) *of* L-*carnitine.* $[\alpha]_D^{25} = -31.3°$ and $[\alpha]_{546}^{25} = -37.0°$ (c = 10, H_2O) for L-carnitine with an enantiomeric purity ≥ 99.0%.

optical rotations: $[\alpha]_D^{25} = -31.3°$ (c = 10, H_2O) and $[\alpha]_{546}^{25} = -37.0°$ (c = 10, H_2O). As shown in *Fig. 3*, the optical rotation is dependent on the temperature.

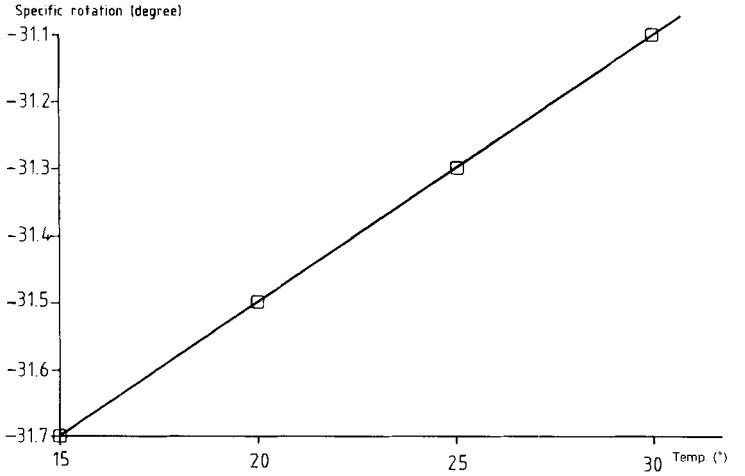


Fig. 3. *Correlation of* $[\alpha]_D$ of L-*carnitine* (**1**) *with temperature*

The realization of this work was made possible thanks to the collaboration of the R&D and the analytical departments of *Lonza*, Visp. We thank particularly Dr. *A. Gerhard* and Mr. *J. Jovanovic* for the NMR spectra and measurements of the specific rotations and Mr. *R. Cicciarelli* for his helpful contribution in solving our analytical problems.

Experimental Part

General. Every reaction step was followed and all products analysed by HPLC using a *Whatman-Partisil-SCX* (10 μm) column and either UV (210 nm) or conductivity for detection. M.p.: *Büchi-520* apparatus; uncorrected. Specific rotations: *Perkin-Elmer-241* spectrophotometer, 1-dm cell at 589 or 546 nm. IR spectra (KBr): *Nicolet FTIR 20 SXB* spectrometer; in cm^{-1}. ^{1}H-NMR spectra: *Nicolet NMC-1280* (300 MHz) spectrometer; chemical shifts in (ppm) rel. to TMS as internal reference; [Eu(hfc)$_3$] from *Fluka*.

(S)-Bis[(3-chloro-2-hydroxypropyl)trimethylammonium] L-*Tartrate* (**10**). 1) To a suspension of 46.25 g (127 mmol) of di-silver L-tartrate (**9**) [40] in 350 ml of H_2O was added a soln. of 47.8 g (254 mmol) of **5** in 200 ml H_2O. The mixture was stirred for 4 h at r.t. and filtered. The AgCl (36.2 g, 99.5%) was washed with MeOH and Et_2O, the solvent evaporated, and the residue dried for 5 h at 40°/1 Torr yielding 61.4 g (106.3%) of residue. This crude mixture was dissolved in 80 ml of hot MeOH and **10** crystallized by addition of 260 ml of acetone to give 19.35 g (33.6%) of crude **10**, m.p. 148–9°, $[\alpha]_D^{25} = -5.9°$ (c = 1, H_2O). Optically pure **10** (13.2 g, 22.9%) was obtained after 3 recrystallizations from MeOH/acetone. M.p. 152–3°. $[\alpha]_D^{25} = -10.7°$ (c = 1, H_2O). IR: 3420*s*, 3200*s*, 3010*m*, 2980*w*, 2940*w*, 1600*s*, 1480*m*, 1420*m*, 1400*m*, 1325*m*, 1225*w*, 1150*w*, 1100*m*, 1075*m*, 990*w*, 960*m*, 920*m*, 785*m*, 720*m*. Anal. calc. for $C_{16}H_{34}Cl_2N_2O_8$ (453.36): C 42.39, H 7.56, Cl 15.64, N 6.18; found: C 42.17, H 7.66, Cl 15.32, N 6.01.

2) To a soln. of 30.8 g (0.2 mol) of (+)-L-tartaric acid in 50 ml of MeOH were added 91.0 g (0.4 mol) of a NMe_3 soln. (26% in MeOH). The soln. was stirred for a further 40 min at r.t., and 37.8 g (0.4 mol) of epichlorohydrin were added within 5 h. The soln. was stirred for a further 38 h at r.t. and warmed up to 60°. Then 280 ml of acetone were added to precipitate crude **10** (31.6 g, 33.2%) which was recrystallized in MeOH/acetone: 24.7 g (27.2%) of **10**. M.p. 150–151°. $[\alpha]_D^{25} = -10.2$ (c = 1, H_2O).

((R)-3-Cyano-2-hydroxypropyl)trimethylammonium Chloride ((−)-**7**). To a suspension of 3.85 g (50 mmol) of $Ca(OH)_2$ in 35 ml H_2O were added 11.85 g (110 mmol) of a HCN soln. (25.1% in H_2O). The yellow suspension was stirred for a further 30 min at r.t., and 22.85 g (50 mmol) of **10** were added within 5 min (pH of the soln. *ca.* 10). The Ca-tartrate·$4H_2O$ precipitated, and the mixture was stirred for 3 h at 55° (bath temp.). After cooling to 4°, the precipitate was filtered, washed twice with 10 ml of MeOH and dried: 13.4 g (103%) of Ca-tartrate·$4H_2O$. The filtrate was decolorized with *Norit* and evaporated at 50°/20 Torr. The residue (22.4 g) was suspended in 50 ml of

EtOH for 1 h at 70° (bath temp.). After cooling at 4°, the crystals were filtered by suction, washed with EtOH, and dried: 15.8 g (88.5%) of (−)-**7**. M.p. 249° (dec.; [15]: 261°). $[\alpha]_D^{25} = -25.9°$ (c = 10, H_2O; [15]: $[\alpha]_D^{25} = -26.2°$ (c = 10, H_2O)). IR: 3280*s*, 3197*s*, 3020*m*, 2980*m*, 2960*m*, 2240*m*, 1490*m*, 1477*s*, 1410*m*, 1405*m*, 1365*w*, 1345*w*, 1305*w*, 1230*m*, 1090*s*, 1003*w*, 973*s*, 960*s*, 935*s*, 777*w*, 687*m*, 628*w*. ^{1}H-NMR ((D_6)DMSO): 2.80 (*dd*, J = 17, 6.2, H–C(3)); 2.91 (*dd*, J = 17, 5, H–C(3)); 3.27 (*s*, NMe_3); 3.52 (*dd*, J = 13, 3, H–C(1)); 3.59 (*dd*, J = 13, 9, H–C(1)); 4.69 (*dddd*, J = 9, 6.2, 5, 3, H–C(2)). Anal. calc. for $C_7H_{15}ClN_2O$ (178.67): C 47.06, H 8.46, Cl 19.84, N 15.68; found: C 47.03, H 8.12, Cl 19.62, N 15.58.

((S)-3-Chloro-2-hydroxypropyl)trimethylammonium Chloride (−)-**5**. To a soln. of 18.2 g (40 mmol) of **10** in 35 ml H_2O was added dropwise at r.t. within 8 min a soln. of 5.9 g (40 mmol) of $CaCl_2 \cdot 2H_2O$ in 6 ml of H_2O. The Ca-tartrate·$4H_2O$ precipitated, and the mixture was stirred for 1 h at r.t. After cooling to 4°, the precipitate was filtered, washed twice with 10 ml of MeOH, and dried: 10.35 g (94.5%) of Ca-tartrate·$4H_2O$. The filtrate was evaporated at 50°/20 Torr and the residue (17.7 g) suspended in 26 ml of abs. EtOH for 2 h at 70° (bath temp.). After cooling to 4°, the crystals were filtered, washed twice with EtOH, and dried: 11.8 g (78.6%) of (−)-**5**. M.p. 212–214°. $[\alpha]_D^{25} = -30.2°$ (c = 1, H_2O). IR: 3200*s*, 3050*w*, 3020*m*, 2980*w*, 2960*w*, 2920*w*, 1400*w*, 1355*m*, 1335*w*, 1310*m*, 1260*w*, 1150*m*, 1100*s*, 970*s*, 930*m*, 790*s*, 720*s*. ^{1}H-NMR ((D_6)DMSO): 3.20 (*s*, NMe_3); 3.42 (*dd*, J = 13, 9, H–C(1)); 3.52 (*dd*, J = 13, 1, H–C(1)); 3.65 (*dd*, J = 6.5, 5.5, H–C(3)); 3.66 (*dd*, J = 6.5, 4.5, H–C(3)); 4.38 (*ddddd*, J = 9, 5.5, 4.5, 4.5, 1, H–C(2)); 6.42 (*d*, J = 4.5, OH). Anal. calc. for $C_6H_{15}Cl_2NO$ (188.10): C 38.31, H 8.04, Cl 37.69, N 7.45; found: C 38.20, H 8.09, Cl 37.40, N 7.31.

((S)-2,3-Epoxypropyl)trimethylammonium Chloride (−)-**8**. To a soln. of 9.5 g (50 mmol) of (−)-**5** in 35 ml of MeOH was added dropwise at r.t. within 10 min a soln. of 5.8 g (50 ml) of K(*t*-BuO) in 20 ml of MeOH. The KCl precipitated, and the mixture was stirred for a further 3 h at r.t. The solid was filtered, washed twice with 5 ml of EtOH and dried: 3.95 g (103%) of KCl. The filtrate was evaporated at 40°/20 Torr and the residue (9.15 g, 119%) shaken with 50 ml of $CHCl_3$. The insoluble material (0.05 g) was filtered, the solvent evaporated and the residue dried: 7.5 g (98%) of (−)-**8**. M.p. 119–121°. $[\alpha]_D^{25} = -27.1°$ (c = 1, H_2O). IR: 3440*s*, 3030*m*, 2940*w*, 1630*m*, 1485*m*, 1420*w*, 1270*w*, 980*m*, 935*m*, 900*m*, 870*m*. ^{1}H-NMR ((D_6)DMSO): 2.70 (*dd*, J = 5, 2, H–C(3)); 2.94 (*dd*, J = 5, 4.5, H–C(3)); 3.18 (*dd*, J = 13.5, 8, H–C(1)); 3.23 (*s*, NMe_3); 3.55 (*dddd*, J = 8, 4.5, 2, 2, H–C(2)); 4.00 (*dd*, J = 13.5, 2, H–C(1)).

The epoxide (−)-**8** gave (−)-**7** by treatment with 1.05 equiv. of acetone cyanohydrin in H_2O (3 h, r.t.), following a known procedure for the preparation of DL-carnitine nitrile [45]. After recrystallization from EtOH (95%), the final yield of (−)-**7** was 81.6%. M.p. 256° (dec.). $[\alpha]_D^{25} = -25.8°$ (c = 1, H_2O).

L-*Carnitine* (**1**). a) *From (−)-***7**. To a soln. of 10.1 g (55 mmol) of (−)-**7** in 140 ml of MeOH were added 57 g of gas. HCl within 2 h at 65° (bath temp.). The mixture was stirred for 2 h at 65°, while NH_4Cl precipitated. After cooling to 5° and filtration, the mother liquor was evaporated. The crude product was dissolved in H_2O, treated with *Dowex 1 × 4* (OH^-), the soln. evaporated, and the residue recrystallized from i-BuOH yielding 7.3 g (82.3%) of **1**. $[\alpha]_D^{25} = -31.3°$ (c = 10, H_2O). IR: 3450*s*, 3080*s*, 2880*m*, 1685*m*, 1595*s*, 1475*m*, 1150*m*, 1108*s*, 1150*m*, 980*w*, 965*m*, 945*s*, 918*w*, 900*w*, 875*m*, 810*m*. ^{1}H-NMR ((D_6)DMSO): 1.77 (*dd*, J = 15, 8.5, H–C(2)); 1.15 (*dd*, J = 15, 4, H–C(2)); 3.11 (*s*, NMe_3); 3.22 (*m*, H–C(4)); 3.45 (*m*, H–C(4)); 4.17 (*m*, H–C(3)).

b) *From (−)-***5**. To a soln. of 10.3 g (55 mmol) of (−)-**5** in 9.6 ml of MeOH and 0.4 ml of H_2O was added a soln. of 3.0 g (60 mmol) of NaCN in 8 ml of H_2O within 12 min at 55°. After stirring for 1 further h at 65°, 125 ml of MeOH were added and 68 g of gas. HCl introduced within 2 h at 65–70°. The mixture was stirred for 1.5 h at 70°, while NH_4Cl precipitated. The same workup as above afforded 7.45 g (83.6%) of pure **1**. $[\alpha]_D^{25} = -31.3°$ (c = 10, H_2O).

O-*Acetyl*-L-*carnitine Hydrochloride* (= *((R)-2-Acetoxy-3-carboxypropyl)trimethylammonium Chloride*; **11**). A mixture of 1.0 g (12.7 mmol) of AcCl and 6.3 ml of AcOH was stirred for 3 h at 80°. At 80°, **1** (1.02 g, 6 mmol) was added and the soln. stirred for a further 60 min. Excess AcOH and AcCl were evaporated to give 1.54 g of crude **11**. The enantiomeric purity was determined without further purification. An anal. sample was prepared by recrystallization from i-PrOH. $[\alpha]_D^{25} = -28.0°$ (c = 10, H_2O; [15]: $[\alpha]_D = -27.7°$ (c = 10, H_2O)). IR: 3425*m*, 2980*m*, 2835*m*, 1740*s*, 1725*s*, 1485*m*, 1422*m*, 1402*w*, 1382*m*, 1290*m*, 1240*s*, 1212*s*, 1185*s*, 1145*w*, 1130*w*, 1082*m*, 1055*m*, 1020*m*, 970*m*, 950*w*, 926*m*. ^{1}H-NMR (CD_3OD): 2.11 (*s*, OAc); 2.74 (*dd*, J = 17, 7, H–C(3)); 2.81 (*dd*, J = 17, 5, H–C(3)); 3.23 (*s*, NMe_3); 3.72 (*dd*, J = 14, 0.7, H–C(1)); 3.88 (*dd*, J = 14, 8.5, H–C(1)); 5.61 (*m*, H–C(2)). Anal. calc. for $C_9H_{18}ClNO_4$ (239.70): C 45.10, H 7.57, Cl 14.79, N 5.84; found: C 44.85, H 7.90, Cl 14.80, N 6.08.

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