Neutron Diffraction Structure of (2*R*,3*R*)-L-(−)-[2-D]Carnitine Tetrachloroaurate, $[(CH_3)_3N\text{-}CH_2\text{-}CHOH\text{-}CHD\text{-}COOH]^+[AuCl_4]^-$: Determination of the Absolute Stereochemistry of the Crotonobetaine-to-Carnitine Transformation Catalyzed by L-Carnitine Dehydratase from *Escherichia coli*

Robert Bau,*[,1a] André Schreiber,[1a,g] Tobias Metzenthin,[1a,h] Roy S. Lu,[1a] Frank Lutz,[1a,i] Wim T. Klooster,[1b] Thomas F. Koetzle,*[,1b] Hermann Seim,[1c] Hans-Peter Kleber,[1d] Fred Brewer,[1e] and Sasha Englard*[,1f]

Contribution from the Department of Chemistry, University of Southern California, Los Angeles, California 90089, Chemistry Department, Brookhaven National Laboratory, Upton, New York 11973, Institut für Klinische Chemie und Pathobiochemie, Institut für Biochemie, Universität Leipzig, 04103 Leipzig, Germany, Departments of Molecular Pharmacology and Biochemistry, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York 10461

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Abstract: Single-crystal neutron diffraction has been used to determine the stereochemical course of the hydration of *trans*-crotonobetaine to L-(−)-carnitine by the enzyme L-carnitine dehydratase. Firstly, an X-ray analysis of the undeuterated carnitinium salt $[(CH_3)_3N\text{-}CH_2\text{-}CH(OH)\text{-}CH_2\text{-}COOH]^+[AuCl_4]^-$ confirmed that the absolute configuration at the C_3 position of L-(−)-carnitine (the CHOH group) is indeed ***R***. This was accomplished using the gold atom as an anomalously-scattering source. Then stereospecifically deuterated L-(−)-[2-D] carnitine was prepared by the hydration of *trans*-crotonobetaine in D_2O using purified L-carnitine dehydratase from *Escherichia coli*. The subsequent neutron analysis of deuterated $[(CH_3)_3N\text{-}CH_2\text{-}CH(OH)\text{-}CH_2\text{-}COOH]^+$ $[AuCl_4]^-$ revealed that the CHD group at position C_2 also had the absolute ***R*** configuration, thus establishing that *the addition of D_2O across the C═C double bond of trans-crotonobetaine proceeds by a stereospecific syn pathway*. In contrast, all other hydration−dehydration reactions examined thus far show that, when the proton added or abstracted is bonded to a carbon atom that is adjacent to a carboxylate group, the absolute stereochemistry of the resulting transformation is *anti*. Crystallographic details for $[(CH_3)_3N{-}CH_2{-}CH(OH){-}CHD{-}COOH]^+[AuCl_4]^-$ are as follows: space group $P2_12_12_1$ (orthorhombic), $a = 10.855(2)$, $b = 11.678(3)$, $c = 22.776(6)$ Å; $Z = 8$; final agreement factor for the neutron analysis at 15 K: $R(F_o) = 0.097$ based on 1140 reflections with $I > 3\sigma(I)$.

Introduction

The difference between the neutron scattering lengths of hydrogen (−3.7406 fm) and deuterium (+6.671 fm) enables these two isotopes to be readily distinguishable in a neutron-scattering experiment.[2] In particular, the opposite signs of the scattering lengths cause H to appear as a negative peak in a neutron Fourier map, while D appears as a larger positive peak. In the past few years, we have used neutron diffraction to determine the absolute configurations of several molecules that have chiral methylene groups (i.e., molecules of the type CHDRR*). Examples of molecules that we have studied include stereospecifically-deuterated malic acid (HOOC−CHD−CHOH−COOH),[3] succinic acid (HOOC−CHD−CH_2−COOH),[4] and derivatives of neopentanol $[(CH_3)_3{-}CHD{-}OH]$[5] and ethanol $[CH_3{-}CHD{-}OH]$.[6]

In the present paper we study the absolute stereochemistry of the conversion of *trans*-crotonobetaine to L-(−)-carnitine by the enzyme L-carnitine dehydratase (Scheme 1). The reverse reaction was first postulated to occur in Enterobacteriacea[7,8] and is involved in the initial step of the degradation of L-(−)-carnitine to γ-butyrobetaine in the intestine of humans.[9,10] The enzyme responsible for this transformation, L-(−)-carnitine

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(1) (a) Department of Chemistry, University of Southern California. (b) Chemistry Department, Brookhaven National Laboratory. (c) Institut für Klinische Chemie und Pathobiochemie, Universität Leipzig. (d) Institut für Biochemie, Universität Leipzig. (e) Department of Molecular Pharmacology, Yeshiva University. (f) Department of Biochemistry, Yeshiva University. (g) Present address: Agfa-Gevaert AG, Leverkusen, Germany. (h) Present address: Hoechst AG, Frankfurt, Germany. (i) Present address: Kantonales Laboratorium Aargau, Aarau, Switzerland.

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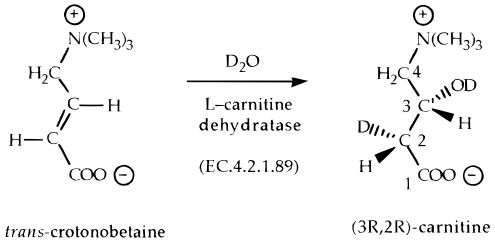
Scheme 1



trans-crotonobetaine (3R,2R)-carnitine

dehydratase from *Escherichia coli*, has been characterized[11] and sequenced.[12] Although the absolute configuration of the product L-(−)-carnitine at the C_3 site is generally accepted to be ***R***,[13] the absolute stereochemistry at position C_2 is unknown. By carrying out a single-crystal neutron diffraction study of the enzymatically synthesized product, recrystallized from H_2O as the salt L-(−)-[2-D] carnitine tetrachloroaurate, $[(CH_3)_3N\text{-}CH_2\text{-}CHOH\text{-}CHD\text{-}COOH]^+[AuCl_4]^-$, we have determined the absolute configuration of this deuterated carnitinium cation to be (3***R***,2***R***). The results are compatible with the conclusion that the L-carnitine dehydratase (systematic name: carnitine hydrolyase) adds one molecule of water stereospecifically across the double bond of crotonobetaine in a *syn* (or *cis*) fashion.

Results

Synthesis. L-(−)-[2-D] carnitine was prepared by the hydration in D_2O of *trans*-crotonobetaine using purified L-carnitine dehydratase supplemented with the enriched low-molecular weight effector (M_r < 1000 Da) essential for enzymatic activity.[11] The composition and conditions of incubation were as those described previously for the synthesis of the same isomer of monodeutero-L-carnitine with intact cells of *E. coli*.[14] The reaction mixture was incubated for a period of time that exceeded the predetermined time required to achieve both chemical and isotopic equilibration. The deuterated carnitine was separated from the remaining crotonobetaine by the method described by Loester and Seim.[15]

Crystallization. The growth of large crystals of carnitine turned out to be more difficult than expected because of its highly hygroscopic properties. The first crystallization experiments were performed with undeuterated material to ascertain the optimal growth conditions. Using either the inner salt (zwitterionic form) of L-carnitine $(CH_3)_3N^+CH_2CH(OH)CH_2COO^-$ or the protonated hydrochloride species $[(CH_3)_3NCH_2CH(OH)CH_2COOH]^+Cl^-$ in different organic solvents gave unsatisfactory results. Crystals of the inner salt appeared in some cases, for example in methanol and ethanol, but they could not be handled outside their mother−liquor because they desolvated after several minutes. The next step was to focus on the cationic form of the compound (the carnitinium ion), using different counteranions. After some effort, it was found that the tetrachloroaurate salt could be grown as long needle-like crystals. These were obtained via the reaction of equimolar amounts of the zwitterionic form of carnitine with $HAuCl_4$. Performing the latter reaction in alcoholic solvents gave air-stable needles, but initial efforts yielded crystals that were far too small even for X-ray data collection. However, slow recrystallization in a closed vial at about 0 °C, using purified water as solvent, gave relatively large, yellow columns suitable for an X-ray analysis. The same procedure was subsequently used to prepare large crystals of the deuterated form of the title compound, $[(CH_3)_3NCH_2CH(OH)CHDCOOH]^+[AuCl_4]^-$, for the neutron diffraction analysis.

X-ray Diffraction Study. An X-ray structure determination was carried out in order to get the positions of the non-hydrogen atoms, which were used for the subsequent phasing of the neutron diffraction data. In addition, the presence of the gold atoms in the unit cell provided a convenient source of X-ray anomalous scatterers for the determination of the absolute configuration of the *non-hydrogen skeleton* of the carnitinium cation (specifically, of the C_3 atom). Although the absolute configuration of carnitine at C_3 has been previously determined by noncrystallographic methods,[13] it was deemed necessary to reconfirm it in the present study to allow for an unambiguous conclusion regarding the stereochemical course of the enzymatic hydration of *trans*-crotonobetaine.

$[(CH_3)_3NCH_2CH(OH)CH_2COOH]^+[AuCl_4]^-$ crystallizes in the acentric orthorhombic space group $P2_12_12_1$, with two independent molecules in the unit cell. Two octants of data were collected, and the structure was solved by standard heavy-atom methods. Refinement of the structure with an ***S*** configuration at atom C_3 resulted in a weighted agreement factor of $R_w(F_o) = 0.064$, while a similar refinement of the mirror-image structure (***R*** at C_3) yielded a much better agreement factor of $R_w(F_o) = 0.053$. Thus, the assignment of Kaneko and Yoshida[13] for L-carnitine (***R*** at C_3) is confirmed. The results of the X-ray analysis are illustrated in Figure 1.

Neutron Diffraction Study. Neutron diffraction data were collected on a crystal of dimensions 1.6 × 0.9 × 0.5 mm at low temperature (15 K) on beamline H6S at the High Flux Beam Reactor of Brookhaven National Laboratory. The positions of the non-hydrogen atoms, obtained from the X-ray analysis, were used to phase the neutron data. Difference-Fourier maps readily revealed the positions of the two deuterium atoms (positive peaks) and all 30 hydrogen atoms (negative peaks). Refinement of the structure resulted in a final agreement factor of $R(F_o) = 0.097$ for 1140 reflections with $I > 3\sigma(I)$. A plot of the carnitine portion of the structure shows the two independent carnitinium cations forming a hydrogen-bonded dimer joined via their respective COOH groups (Figure 2). Most importantly, the absolute configurations about the two chiral methylene groups [C(2)H(27)D(28) and C(12)H(25)D(26)] are both ***R***. In addition to the two intermolecular hydrogen bonds between the carboxylate groups [O(2)−H(29)···O(12) and O(11)−H(30)···O(1)], there is a third intramolecular hydrogen bond involving one of the C_3 hydroxyl oxygens [O(3)−H(31)···O(1)]. All of the distances and angles in the structure are normal and agree well with previously reported values.[16] The two carnitinium cations

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(17) For the definition of the ($a/g^+/g^-$) convention used to describe the molecular conformation in carnitine, see: Colucci, W. J.; Gandour, R. D. and Mooberry, E. A. *J. Am. Chem. Soc.* **1986**, *108*, 7141−7147. In the present study, the conformations about the C_3-C_4 bonds for both cations [i.e., the torsion angles C(2)−C(3)−C(4)−N(5) and C(12)−C(13)−C(14)−N(15)] are *trans (a)*, which is equivalent to saying that the O(3)−C(3)−C(4)−N(5) and O(13)−C(13)−C(14)−N(15) torsion angles are both *gauche (g^-)*. The *overall* conformation of the carnitine backbone C(1)−C(2)−C(3)−C(4)−N(5) is "extended", while that of C(11)−C(12)−C(13)−C(14)−N(15) is "folded" (see: Anderson, R. C.; Gullion, T.; Joers, J. M.; Shapiro, M.; Villhauer, E. B.; Weber, H. P. *J. Am. Chem. Soc.* **1995**, *117*, 10546−10550 for a definition of the extended/folded terminology).

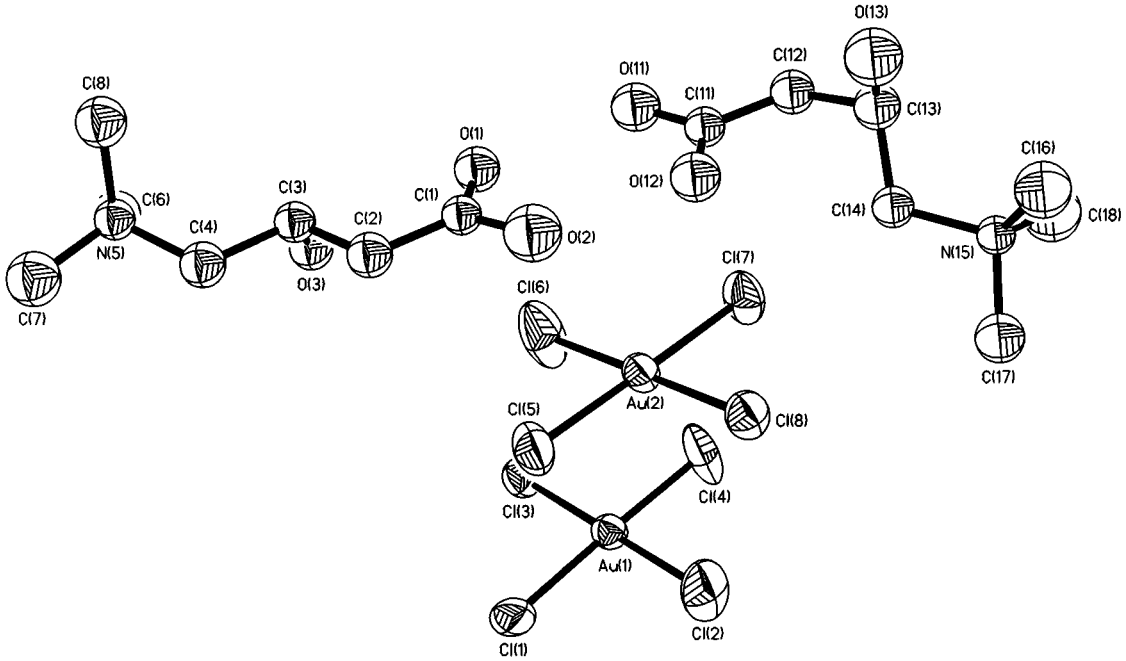

Figure 1. Structure of unlabeled carnitine tetrachloroaurate as determined by X-ray diffraction. This plot shows the two independent cations and anions in the unit cell, the latter of which are approximately parallel to each other with a Au···Au distance of 4.6 Å. By using the anomalous scattering of the gold atoms, the absolute configuration of the C(3) and C(13) atoms were determined to be **R**.

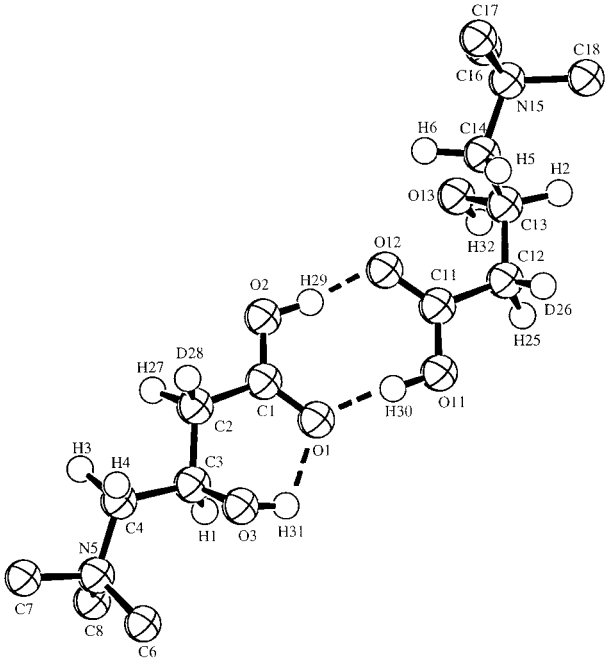

Figure 2. The structure of stereospecifically deuterated (2*R*,3*R*)-L-(−)-[2-D]carnitine tetrachloroaurate, as determined by neutron diffraction. The H atoms on the methyl groups have been omitted for clarity, and the hydrogen bonding pattern between the two carnitinium cations is illustrated. The absolute configurations of the CHD groups at the C(2) and C(12) positions were determined to be **R**. Note that the C(1)−C(2)−C(3)−C(4) backbone of the carnitinium ion on the left is in a *trans (anti)* conformation, while that on the right is *gauche* (g^+). (See footnote 17 for a more detailed discussion of the molecular conformations).

differ mainly in their backbone configuration: The orientation about the C(2)−C(3) bond [i.e., the torsion angle C(1)−C(2)−C(3)−C(4)] is *trans (anti)*, while that around the C(12)−C(13) bond [the torsion angle C(11)−C(12)−C(13)−C(14)] is *gauche* ($g+$)[17] (Figure 2). The essential conclusion from the neutron structure determination is that addition of D_2O across the double bond of crotonobetaine results in a stereospecifically-deuterated L-carnitine molecule with the absolute configuration of ***R*** at both the C_2 and C_3 positions (Scheme 1).

Discussion

L-(−)-Carnitine [*R*-(−)-3-hydroxy-4-trimethylaminobutyrate] has critical functions in the overall oxidation of fatty acids for the generation of energy in mammals. Specifically, it functions as the carrier molecule in the transport of medium and long-chain fatty acids across the inner mitochondrial membrane.[18] In peroxisomes, the site of shortening of very long-chain fatty acids, it has been suggested that L-carnitine also participates in the translocation of medium-chain fatty acyl residues into the cytosol.[19] Because of its central role in fatty acid β-oxidation and participation in the liberation of free coenzyme A with the consequent regulation of the acetyl-CoA/CoA ratio, carnitine is involved significantly in the pathophysiology of ketosis.[20]

Carnitine must be continually supplied to mammals, including humans, either through the diet or by *de novo* synthesis.[21] γ-Butyrobetaine (4-*N*-trimethylamino butyrate), the penultimate precursor derived from L-lysine, undergoes an aliphatic hydroxylation at C_3 in the terminal step of the carnitine biosynthetic pathway.[18,22,23] This hydroxylation step that involves a molecular oxygen-utilizing reaction is mediated by a ferrous ion-dependent enzyme that requires α-ketoglutarate as cosubstrate and ascorbate as reductant.[23,24] Detailed analysis of the reaction catalyzed by γ-butyrobetaine hydroxylase provided results indicating that an active site iron-oxo complex stereospe-

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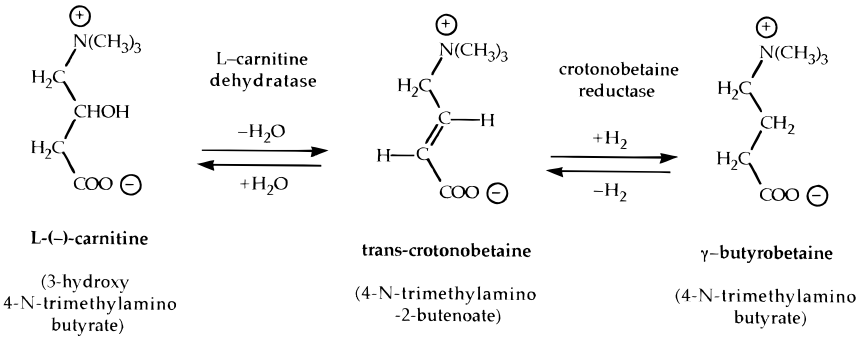
Scheme 2



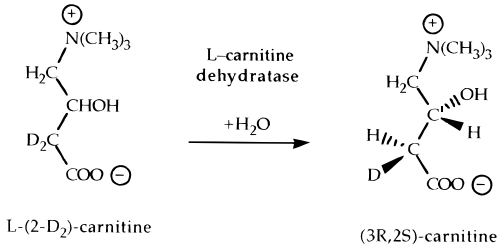
cifically abstracts, by homolytic C−H bond scission, the *pro-R* hydrogen atom at C_3 of the substrate.[25,26]

On the other hand, bacteria have evolved a diversity of catabolic pathways for the utilization of carnitine and other betaines,[27] and several species of enterobacteria, growing under partially anaerobic conditions, reductively convert L-carnitine to γ-butyrobetaine.[7,8,28] This interconversion of betaines, representing the reverse of the terminal reaction of the carnitine biosynthetic pathway, has been shown to proceed through crotonobetaine (4-*N*-trimethylamino-2-butenoate) as an intermediate[7,8,28] (Scheme 2). The dehydration of L-carnitine to crotonobetaine is catalyzed by an induced, reversible carnitine dehydratase (L-carnitine dehydratase, EC.4.2.1.89) that has been purified from *Escherichia coli* O44 K74 grown anaerobically in the presence of L-(−)-carnitine or crotonobetaine, and its kinetic and molecular properties have been characterized.[11,29] The *caiB* gene from *E. coli* O44 K74 that encodes L-carnitine dehydratase has been cloned, sequenced, and overexpressed.[12] Subsequently, molecular characterization of the *cai* operon in *E. coli* revealed the presence of five additional genes necessary for carnitine metabolism in that organism.[30]

In an earlier study, we demonstrated that resting cell suspensions of *E. coli* O44 K74 derived from cultures grown in complex medium supplemented with either DL-carnitine or crotonobetaine, when incubated with crotonobetaine in the presence of ^3H_2O, yielded monotritiated L-carnitine (L-[2-^{3}H]-carnitine); no further incorporation of ^{3}H occurred after chemical equilibrium had been reached.[31] We further established that the reisolated "biologically" synthesized L-[2-^{3}H]carnitine, on reincubation with similarly induced resting cell suspensions of *E. coli* O44 K74, rapidly lost its ^{3}H atom to the aqueous medium and that (a) the accumulated product(s) contained no detectable radioactivity and (b) the rate of ^{3}H release far exceeded the rate of formation of crotonobetaine. These results were interpreted as being compatible with a stereospecific mechanism of hydration and dehydration involving a carbanion intermediate in the reversible interconversion of crotonobetaine and L-carnitine.

The stereospecific course of the L-carnitine dehydratase reaction enabled us, in a subsequent study, to synthesize the two C_2 monodeuterated isomers of L-carnitine. One isomer (the title compound) was prepared by incubation of suitably induced resting cell suspensions of *E. coli* O44 K74 with crotonobetaine in the presence of D_2O (>99.5%) (Scheme 1) and the "opposite" isomer by the reverse carnitine dehydratase-catalyzed proton exchange of L-[2-$^2H_2$]carnitine (Scheme 3). These two species of L-[2-^{2}H]carnitine were then examined by ^{1}H NMR, allowing us to independently monitor the pD dependence and coupling constants of the individual C_2 protons of L-carnitine and of *O*-acetyl-L-carnitine. The results obtained indicated that there was little effect of the carboxyl charge on the conformational states(s) of L-carnitine about the C_2-C_3 bond and pointed to the conformational similarities in aqueous solution of L-carnitine and its *O*-acyl esters.[14] These NMR studies, however, did not allow an unambiguous absolute configurational assignment at the C_2 position of either of the two enantiomorphs of L-[2-^{2}H]-carnitine.

Scheme 3



Establishment of the stereochemical course of the L-carnitine dehydratase-catalyzed addition of D_2O across the *trans* C=C double bond of crotonobetaine is predicated on the determined absolute configuration of the C_2 position of the product L-[2-^{2}H]carnitine. Although comparative NMR and optical rotatory data have been used extensively in the assignment of absolute configurations of deuterated products of enzymatic reactions,[32] the unavailability of related molecules of known stereochemistry precluded the application of a similar approach to the stereospecifically synthesized monodeuterated L-carnitine. As outlined in the introduction, in more recent years application of single-crystal neutron diffraction techniques has allowed the unambiguous determination of the absolute configuration of several enzymatically synthesized molecules that possess chiral methylene groups.[3−6] In the present study the product of the L-carnitine dehydratase-catalyzed hydration of crotonobetaine in D_2O was crystallized as the $[AuCl_4]^-$ salt and subjected to single-crystal neutron diffraction analysis. The results establish the product of the enzymatic synthesis to be (3*R*,2*R*)-carnitine-2-^{2}H, a finding that is consistent with an L-carnitine dehydratase-catalyzed stereospecific *syn* addition to the *trans* C=C double bond of crotonobetaine (Scheme 1).

Of the 15 enzymatically catalyzed hydratase−dehydatase reactions examined with respect to their stereochemistry, seven

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(29) During the purification procedure a low-molecular-weight effector essential for enzymatic activity was separated from the enzyme. The addition of this still unknown effector resulted in the reactivation of the apoenzyme.[11] To date, it has not been possible to replace this component with any of the known enzyme cofactors.

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examples are known to proceed with *anti* stereochemistry, and eight have been shown to produce the *syn* elimination of water.[33,34] The enzymatic dehydrations proceeding with *anti* stereochemistry have been categorized as those in which "the abstracted proton of the substrate is α to a carboxylate group", whereas for the dehydratases that produce the *syn* elimination of water, "the proton abstracted is α to a carbonyl group of a thioester or a ketone".[34] The present demonstration of the L-carnitine dehydratase-catalyzed *cis* addition to the *trans* C═C double bond of crotonobetaine is a clear exception to this proposed duality in classification based on the environment of the reactive substrate α carbon atom and of the consequent postulated biological significance of this dichotomy.

Experimental Section

1. Materials. L-(−)-Carnitine (*R*-(−)-3-hydroxy-4-trimethylaminobutyric acid) and crotonobetaine (4-trimethylamino-2-butenoic acid) were gifts from Lonza (Basel, Switzerland) or from Sigma-Tau (Rome, Italy). The *trans*-configuration of crotonobetaine was confirmed by ^{1}H-NMR spectroscopy in D_2O at room temperature (Varian UNITY400 spectrometer). The coupling constant with 15.6 Hz and the coupling pattern between the C_3 and C_4 protons with 6.2 ppm were the same as those reported by Yokozeki *et al.*,[35] indicating that the structure around the double bond was of a *trans*-configuration. L-Carnitine acetyl transferase (EC 2.3.1.7; 80 U/mg) was purchased from Boehringer (Mannheim, Germany). L-(−)-[*methyl*-^{14}C] carnitine hydrochloride, sterilized aqueous solution (50 mCi/mmol), and [1-^{14}C]acetyl coenzyme A, solid (60 mCi/mmol), were products from Amersham Buchler (Braunschweig, Germany). Deuterium oxide (^2H_2O, > 99.95 atom % D) was obtained from Fluka Chemie (Buchs, Switzerland). Carnitine dehydratase (EC 4.2.1.89) was isolated from *Escherichia coli* O44K74 grown under anaerobic conditions at 37°C in a complete medium supplemented with L-(−)-carnitine. The enzyme was purified by chromatography on phenyl-sepharose, hydroxyapatite, and DEAE-sepharose.[11] During the purification procedure of carnitine dehydratase a low molecular-weight effector essential for enzymatic activity was separated from the enzyme. To date, the structural identity of this effector has not been determined. The effector was enriched by $(NH_4)_2SO_4$ fractionation, phenol extraction, and chromatography on DEAE-sephadex A25 and sephadex G25.[36] The incubation mixture for the enzymatic synthesis of stereospecifically labelled [2-^{2}H]carnitine contained a predetermined optimized amount of enriched effector.

2. Synthesis of L-(−)-[2-^{2}H]Carnitine from *trans*-Crotonobetaine. Purified L-(−)-carnitine dehydratase (free of carnitine racemase[30,37]) in phosphate buffer was lyophilized and then redissolved in D_2O. This step was repeated once more. The same procedure was performed with the effector preparation. *trans*-Crotonobetaine (1.8 g) was dissolved in 100 mL of 0.05 M potassium phosphate buffer with pH 7.8 (0.126 mol/L) and lyophilized twice (each time following redissolving the dried material in 25 mL of D_2O). Finally, the three lyophilized lots were combined and dissolved in 100 mL of D_2O. The L-carnitine dehydratase (specific activitiy 2.1 U/mg) incubation mixture was allowed to react for 8 h in a thermoshaker maintained at 37°C. The reaction mixture was then stored at −80 °C, and an aliquot was taken for carnitine determination. With the modifications that involved substitution of Hepes for Tris and of *N*-ethylmaleimide for tetrathionate, the specific radiochemical enzymatic assay of McGarry and Foster was used to quantitate the amount of L-(−)-carnitine produced.[38] The L-carnitine concentration in the reaction mixture was determined to be 0.028 mol/L; this is equivalent to 454 mg of L-(−)-[2-^{2}H] carnitine (M_r 162.2), representing a yield of 22.3%.

3. Purification of the L-(−)-[2-^{2}H]Carnitine. L-(−)-[*methyl*-^{14}C] carnitine, 2.5 μCi, was added to the reaction mixture for assessing the recovery of carnitine following elution from the chromatographic columns. The anions from the reaction mixture were initially removed by treatment with Dowex 2 (AG 2 × 8, 50−100 mesh, OH^--form). The chromatographic separation of L-carnitine from the excess residual crotonobetaine was performed on a cation exchange column (2.5 × 50 cm) of Dowex 50 (AG WX8, 200−400 mesh, H^+-form). Following the application of the reaction mixture, the column was washed with water (45 fractions, 10 mL each) and then with 0.1 N hydrochloric acid. The column was subsequently eluted at a flow rate of 40 mL/h with a linear gradient reaching 3.0 N hydrochloric acid that was formed with 600 mL each of 0.1 and 3.0 N HCl.[15] A 100 μL aliquot of each 10 mL fraction was checked for radioactivity content by liquid scintillation counting (Tri-carb 2500, Packard). L-Carnitine was eluted from the column in fractions 140−156. These fractions were examined by thin layer chromatography for the amount of remaining crotonobetaine (adsorbent: silica gel, solvent system: phenol/butanol/ammonia, 50/50/20, v/v) according to Seim *et al*.[8] Fractions 140−145 were completely free of crotonobetaine. Fraction 146 already contained 5% crotonobetaine and 95% carnitine, with significant increases in the crotonobetaine content of the subsequent fractions. Accordingly, only fractions 140−145 representing 60.1% of the enzymatically synthesized deuterated carnitine were combined and lyophilized. Only this lot (mp 153−154 °C)[39] was used for the crystallization of L-(−)-[2-^{2}H] carnitine tetrachloroaurate.

4. Preparation of Tetrachloroaurate(III) Salt and X-ray Diffraction Study. The highly hygroscopic carnitine can be crystallized as a stable compound as its tetrachloroaurate(III) salt, which can be prepared by treating the inner salt (zwitterionic form) of carnitine or its hydrochloride species with an equivalent amount of $HAuCl_4$ in aqueous solution. The resulting yellow solution was filtered and allowed to evaporate slowly until the first crystals appeared. At this

Table 1. Summary of Experimental Conditions and Refinement Results for the Neutron Diffraction Study of $[Carnitinium]^+[AuCl_4]^-$

chemical formula	$C_7H_{15}DAuCl_4NO_3$
formula weight (M_r, daltons)	502.033
crystal class	orthorhombic
space group	$P2_12_12_1$
crystal dimensions (mm)	1.6 × 0.9 × 0.5
crystal color	yellow−orange
a (Å)	10.855 (2)
b (Å)	11.678 (3)
c (Å)	22.776 (6)
V (Å^3)	2887(2)
Z	8
density (g cm^{-3})	2.303
wavelength (Å)	1.16395(10)
abs. coeff (μ_N, cm^{-1})	2.209
trans. factor range	0.816−0.907
temp (K)	15.0 (±0.5)
diffractometer	port H6S, Brookhaven HFBR
scan mode	$\omega/2\theta$, with $\Delta 2\theta = 3.0°$ for $6° < 2\theta < 60°$, and $\Delta 2\theta = (-2.35° + 8.06° \tan\theta)$ for $60° < 2\theta < 106°$
index limits	$0 \le h \le 14$; $0 \le k \le 16$; $0 \le l \le 31$
no. of independent reflcns	4360
no. of reflcns used in refinement (N_o)	2961
no. of varied parmtrs (N_v)	257
$R(F_o)^a$	0.097
$R_w(F_o)^a$	0.116
S^b	1.14

[a] Calculated for 1140 reflections with $I > 3\sigma(I)$. For all 2961 reflections, $R(F_o^2) = 0.329$, $R_w(F_o^2) = 0.240$. [b] $S = [\sum w(F_o^2 - k^2F_c^2)^2/(N_o - N_v)]^{1/2}$.

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Table 2. Selected Distances in Carnitine Tetrachloroaurate (Neutron Results)

O(1)−C(1)	1.263(18)	O(11)−C(11)	1.331(20)
O(2)−C(1)	1.319(19)	O(12)−C(11)	1.250(18)
O(2)−H(29)	0.990(39)	O(11)−H(30)	1.010(30)
O(3)−H(31)	0.955(39)	O(13)−H(32)	0.965(30)
O(3)−C(3)	1.471(19)	O(13)−C(13)	1.518(19)
N(5)−C(4)	1.516(14)	N(15)−C(14)	1.519(14)
N(5)−C(6)	1.535(15)	N(15)−C(16)	1.553(14)
N(5)−C(7)	1.532(15)	N(15)−C(17)	1.543(16)
N(5)−C(8)	1.573(15)	N(15)−C(18)	1.525(16)
C(1)−C(2)	1.521(16)	C(11)−C(12)	1.508(17)
C(2)−H(27)	1.133(29)	C(12)−H(25)	1.109(28)
C(2)−D(26)	1.130(29)	C(12)−D(28)	1.110(28)
C(2)−C(3)	1.533(16)	C(12)−C(13)	1.541(17)
C(3)−C(4)	1.558(16)	C(13)−C(14)	1.541(17)

hydrogen bonds	O−H (Å)	H···O (Å)	O−H···O (°)
O(2)−H(29)···O(12)	0.990(39)	1.624(32)	174.4(29)
O(11)−H(30)···O(1)	1.010(30)	1.697(29)	176.1(26)
O(3)−H(31)···O(1)[a]	0.955(39)	2.303(39)	119.4(29)

[a] Intramolecular hydrogen bond.

point, the solution was filtered again and stored in a closed vial. After several days, bright yellow-orange needles of a suitable size were obtained.

For the X-ray structure determination, a crystal of the size 0.3 × 0.3 × 0.5 mm was mounted in a glass capillary for data collection, which was carried out at room temperature with Mo Kα radiation on a Siemens P4 diffractometer. The structure was solved with standard heavy-atom techniques and refined to give final agreement factors of $R(F_o) = 0.047$, $R_w(F_o) = 0.053$ for 3123 nonzero reflections [$F > 4\sigma(F)$].[40] The crystal structure of carnitinium-tetrachloroaurate(III) is shown in Figure 1. The two independent molecules form a dimeric unit with their carboxyl groups facing each other, leading to intermolecular hydrogen-bonds (*ca.* 2.65 Å) between the carbonyl and hydroxyl groups of two adjacent molecules. The side chains of the molecules are oriented in a head-to-tail fashion with the hydroxy groups at C(3) and C(13) pointing in opposite directions. The chiral centers at C(3) and C(13), respectively, are in an ***R***−configuration. If an ***S***-configuration is assumed instead, $R_w(F_o) = 0.064$, a significantly poorer agreement with the data. Details of the X-ray structural analysis are available as Supporting Information (see paragraph at the end of this paper).

(40) For the X-ray structure analysis, the programs used were SHELX-76 (Sheldrick, G. M. Cambridge University, U.K., 1976) and SHELX-86 (Sheldrick, G. M. University of Göttingen, Germany, 1986).

(41) DISPLEX Model CS−202; APD Cryogenics, Inc.

5. Neutron Diffraction Study. A specimen of volume 0.7 mm^3 was mounted on an aluminum pin with halocarbon grease and sealed under a helium atmosphere inside an aluminum container. This container was placed in a closed-cycle helium refrigerator,[41] cooled to 15.0 ± 0.5 K, and mounted on a four-circle diffractometer at port H6S of the Brookhaven High Flux Beam Reactor. Data (4360 reflections) were collected at a wavelength 1.16395(10) Å. Initial coordinates were obtained from the X-ray structure determination. All hydrogen and deuterium atoms were found from a difference Fourier map, and the structure refined to agreement factors of $R(F_o) = 0.097$ and $R_w(F_o) = 0.116$, based on 1140 reflections with $I > 3\sigma(I)$. A summary of the parameters used in the neutron diffraction analysis is given in Table 1, and selected distances are listed in Table 2. Full details of the neutron structure determination, together with a complete set of atomic coordinates, distances and angles, have been deposited as Supporting Information (see paragraph at the end of this paper).

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Supporting Information Available: Full descriptions of the X-ray and neutron structural analyses of carnitine tetrachloroaurate, tabulations of the experimental conditions and refinement results, and complete listings of the atomic coordinates and molecular parameters (distances and angles) for both the X-ray structure determination (Tables S-1−S-4) and the neutron diffraction analysis (Tables S-5−S-8) (14 pages). See any current masthead page for ordering and Internet access instructions.

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