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Trihydrate 1/1 Salt Between (*R*)-Carnitine Amide and (1*R*,3*S*)-Camphoric Acid

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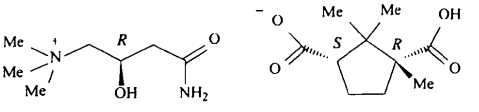
Abstract

The crystal structure of the trihydrate 1/1 salt between (*R*)-carnitine amide and (1*R*,3*S*)-camphoric acid, (*R*)-3-hydroxy-4-(trimethylammonio)butanamide (1*R*,3*S*)-1-carboxy-1,2,2-trimethyl-3-cyclopentanecarboxylate trihydrate, $C_7H_{17}N_2O_2^+.C_{10}H_{15}O_4^-.3H_2O$, is characterized by alternating layers of camphoric acid and carnitine amide molecules, both connected by a network of hydrogen bonds involving water molecules. Molecular-mechanics calculations using periodic boundary conditions, with two different force fields (*CVFF* and *AMBER*) and a dielectric constant ranging between 1 and 10, indicate that such commercial packages are not fully suitable when different kinds of forces are involved.

Comment

Carnitine and its derivatives are found to occur naturally in both plant and animal tissues, and in relatively high concentrations in the hearts and muscles of vertebrates (Fraenkel & Friedman, 1957; Hosein, Booth, Gasoi & Kato, 1967). The acetyl derivative is involved in carbohydrate metabolism (Childress, Sacktor & Traynor, 1966), as well as in the reversible transfer of the acetyl group between acetyl carnitine and acetyl coenzyme A. The acyl-carnitines play an important role in fatty acid oxidation by providing the transport of fatty acyl groups across mitochondria membranes (Colucci & Gandour, 1988). Acyl transfer is catalyzed by carnitine acyltransferase enzymes that comprise a family of proteins with different sub-cellular localization, substrate specificity and sensitivity to inhibitors (Bieber, 1988). In view of such properties, carnitine is useful in the therapeutic treatment of myocardial ischemia (Visioli, Pasini & de Giuli, 1992), acetylcarnitine is useful in cerebral aging and peripheral neuropathies (Calvani, 1993), while carnitine palmitoyl transferase inhibitors have potential therapeutic applications in the treatment of diabetes (McGarry, Woeltje, Kuwajima & Foster, 1989).

The study of the salts between (*R,S*)-carnitine derivatives and molecules or macromolecules capable of discriminating the two enantiomers can be useful to identify the topographical arrangement of the key recognition sites on carnitine binding enzymes, as well as to suggest alternative methods for stereoselective separation in analytical applications. Therefore, we have undertaken solid-state structural studies involving carnitine derivatives such as the title compound, (I), and protein moieties, in order to investigate the origin of the chiral discrimination of carnitine derivatives, as well as to provide a test of force-field models with respect to their adoption in theoretical calculations.



(I)

The crystal structure is characterized by alternate layers, nearly perpendicular to the *b* axis, of camphoric acid molecules and carnitine amide molecules connected by a network of hydrogen bonds involving the water molecules. Such a structure appears to be particularly stable as the same crystal cell parameters and X-ray diffraction data are obtained when the compound is recrystallized from isobutanol. The structure of the independent salt unit is shown in Fig. 1. Bond lengths and angles are as expected except for those involved in the camphoric rings that are reported in Table 1. Furthermore, consideration of the C—O bond lengths in the carboxyl groups allows assignment of the anion site to C17, in agreement with chemical evidence. Carnitine amide conformational parameters are very similar to those found in related carnitine derivatives (Gandour, Colucci & Fronczek, 1985; Colucci, Gandour & Mooberry, 1986). The molecule assumes an extended conformation even if an amide group replaces the carboxylate, or the counterion is different.

The packing in the *bc* plane, showing the hydrogen-bonding network, is illustrated in Fig. 2. The crystal structure is characterized by a three-dimensional

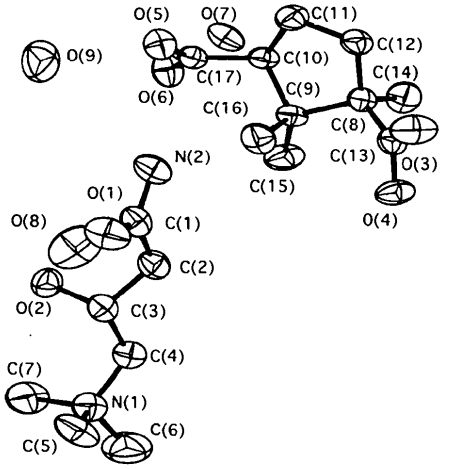


Fig. 1. Molecular drawing showing the numbering of the non-H atoms. Displacement ellipsoids are plotted at the 50% probability level.

network of hydrogen bonds involving the water molecules (see Table 2). The layers of camphoric acid molecules are connected along the screw-axis direction by a chain of hydrogen bonds bridging pairs of different carboxylic groups through the water molecules [O(7)]. The carboxylate atoms O(5) and O(6) provide the binding of carnitine amide through a chain of hydrogen bonds involving the amide NH_2 H atoms and extending along the *a* axis. The carnitine amide hydroxy groups interconnect, face-to-face, the bilayer of carnitine moieties *via* the water molecules O(8) and O(9), which also bridge the camphoric acid layers. The H atoms of the water molecules could not be localized, so a full description of the geometry of the hydrogen bonding in which these molecules act as donors is not possible. Nevertheless, the contact distances (Table 2) are indicative of these interactions.

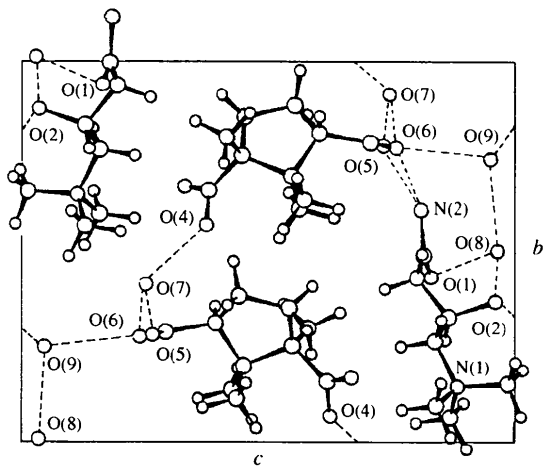


Fig. 2. The *bc* projection of the crystal structure showing the hydrogen-bonding network.

The crystal structure is stabilized by different kinds of interactions that usually characterize the force field in protein systems. Hydrogen bonds involving water molecules, coulombic interactions between ions, and van der Waals interactions cooperate in the stabilization of the crystal structure. Theoretical investigations of such intermolecular forces using standard molecular-mechanics program packages can be useful in selecting the most suitable package for further investigation of the binding of carnitine derivatives to relevant enzymes. The *DISCOVER* program (Biosym Technologies, 1994) was adopted using *CVFF* and *AMBER* force fields; minimization procedures were carried out by conjugated gradients (convergence set at 0.04 kJ $Å^{-1}$ for the first derivative). The coordinates of the crystal structure were used as a starting point and experimental cell parameters were imposed as periodical constraints to the crystal lattice. Packing and conformational energies were minimized together. The explicit-image model, with a non-bond cutoff of 23 Å as the periodic boundary condition, was used. Furthermore, the dielectric constant was changed from 1 to 10 to test the role of electrostatic forces and hydrogen bonds in crystal-structure stabilization.

In all cases, average deviations of 0.5 Å were observed for both the camphoric acid and carnitine amide molecules; the carnitine amide moiety appears to be less sensitive to force-field variations due to the more effective hydrogen-bond network in which it is involved. Such deviations appear to decrease on increasing the dielectric constant, suggesting that electrostatic forces play a minor role in stabilizing the crystal structure. Structure variations related to a dielectric constant > 5 are insignificant.

With respect to the force fields, the *CVFF* structure appears to be closer to the crystal structure than the *AMBER* structure, possibly because the electrostatic contribution to the energy is very different in the two cases: in *AMBER*, a separate term takes into account hydrogen-bonding interactions and the electrostatic term is predominant over non-bonded interactions, whereas in *CVFF*, the balance between electrostatic and non-bond forces is more even. Particularly interesting are the shifts of the water molecules as a consequence of perturbations of the force field, due to changes in the dielectric constant. These shifts seem to mirror the corresponding anisotropic displacement parameters of the crystal structure in magnitude and direction. Atoms O(8) and O(9) show displacements of 1.0 Å, twice as large as that of O(7).

The comparison between the crystal structure and the minimum-energy structures, as obtained by adopting two different force fields, indicates that commercial molecular-mechanics packages present a rather approximate model of the real force fields when different kinds of energy contributions to crystal packing and conformational energies are involved, as in the present case.

Therefore, we are currently attempting to obtain a better partition of the different energy contributions to reproduce the crystal structure and then extend this force field to carnitine molecular recognition by proteins.

In conclusion, the typical bilayer crystal structure in which the hydrophobic moiety of the camphoric acid is sandwiched by the polar water–carnitine amide layers suggests that the diastereomeric resolution as obtained by crystallization from the racemic solution, in the presence of camphoric acid, appears to be essentially a surface process of chiral discrimination. At present, we are attempting a chiral discrimination of carnitine amide raceme by using as a template a disymmetric distribution of carboxylic groups on a molecular surface. Preliminary results obtained using a solution of poly-L-glutamic acid in an α-helical conformation as a chiral template seem to confirm this hypothesis.

Experimental

Crystals of the title compound suitable for X-ray diffraction analysis were obtained by fractional crystallization of the carnitine raceme mixture in an aqueous solution, as in the industrial procedure (Cavazza, 1981).

Crystal data

$C_7H_{17}N_2O_2^+.C_{10}H_{15}O_4^-.3H_2O$
M_r = 414.49
Monoclinic
$P2_1$
a = 6.610 (2) Å
b = 11.371 (1) Å
c = 14.683 (1) Å
β = 91.73 (1)°
V = 1103.1 (4) Å^3
Z = 2
D_x = 1.248 Mg m^{-3}
D_m not measured

Cu $K\alpha$ radiation
λ = 1.54178 Å
Cell parameters from 23 reflections
θ = 30–65°
μ = 0.84 mm^{-1}
T = 293 K
Prism
0.25 × 0.20 × 0.05 mm
Colourless

Data collection

Rigaku AFC-5 diffractometer
$\theta/2\theta$ scans
Absorption correction: none
2114 measured reflections
1982 independent reflections
R_{int} = 0.05

θ_{max} = 65°
$h = 0 \rightarrow 7$
$k = 0 \rightarrow 13$
$l = -17 \rightarrow 17$
3 standard reflections every 97 reflections
intensity decay: none

Refinement

Refinement on F
R = 0.059
wR = 0.081
S = 0.995
1982 reflections
252 parameters
H atoms rigidly bound to parent C atom

$w = 1/[0.00001 + 0.04069F + 0.00109F^2]$
$(\Delta/\sigma)_{max}$ = 0.01
$\Delta\rho_{max}$ = 0.4 e Å^{-3}
$\Delta\rho_{min}$ = −0.2 e Å^{-3}
Extinction correction: none
Scattering factors from *International Tables for Crystallography* (Vol. C)

Table 1. *Selected geometric parameters (Å, °)*

O(3)—C(13)	1.211 (4)	C(9)—C(10)	1.566 (6)
O(4)—C(13)	1.303 (6)	C(9)—C(15)	1.529 (6)
O(5)—C(17)	1.243 (3)	C(9)—C(16)	1.530 (5)
O(6)—C(17)	1.279 (3)	C(10)—C(17)	1.511 (4)
C(12)—C(8)—C(9)	104.2 (2)	O(4)—C(13)—O(3)	121.8 (4)
C(14)—C(8)—C(13)	107.1 (3)	C(8)—C(13)—O(3)	123.9 (4)
C(10)—C(9)—C(8)	101.1 (3)	C(8)—C(13)—O(4)	114.2 (3)
C(16)—C(9)—C(8)	114.8 (3)	O(6)—C(17)—O(5)	121.3 (3)
C(16)—C(9)—C(15)	108.8 (4)	C(10)—C(17)—O(5)	121.0 (2)
C(11)—C(10)—C(9)	104.5 (3)	C(10)—C(17)—O(6)	117.7 (2)
C(17)—C(10)—C(9)	113.8 (3)	C(5)—N(1)—C(4)	110.2 (3)
C(17)—C(10)—C(11)	115.1 (3)	C(6)—N(1)—C(4)	107.2 (4)
C(12)—C(11)—C(10)	105.5 (3)	C(7)—N(1)—C(4)	113.6 (4)
C(11)—C(12)—C(8)	107.9 (3)		
N(2)—C(1)—C(2)—C(3)	−141.3 (3)		
C(1)—C(2)—C(3)—C(4)	−171.5 (3)		
C(2)—C(3)—C(4)—N(1)	170.6 (3)		

Table 2. *Contact distances (Å)*

O(1)···O(8^{i})	2.806 (6)	O(5)···N(2^{i})	2.934 (8)
O(2)···O(8)	2.694 (8)	O(6)···O(9)	2.701 (5)
O(2)···O(9^{ii})	2.802 (8)	O(6)···O(7)	2.718 (6)
O(4)···O(7^{iii})	2.601 (7)	O(6)···N(2)	3.058 (7)
O(5)···O(7^{i})	2.736 (6)	O(8)···O(9^{iv})	2.746 (13)

Symmetry codes: (i) $x-1, y, z$; (ii) $1-x, y-\frac{1}{2}, -z$; (iii) $1-x, y-\frac{1}{2}, 1-z$; (iv) $1+x, y, z$.

I and $\sigma(I)$ values were corrected for Lorentz, polarization and shape-anisotropy effects (North, Phillips & Mathews, 1968). A total of 1982 independent reflections were processed by the direct-methods program package *SIR*92 (Altomare *et al.*, 1994), which provided the complete structure. All non-H atoms were refined by full-matrix least squares with anisotropic displacement parameters. H atoms were idealized (C—H = 1.05 Å; Allen *et al.*, 1987). Each H atom was assigned the equivalent isotropic displacement parameter of the parent atom and allowed to ride on it. The final difference Fourier map, with a root-mean-square deviation on electron density of 0.06 e Å^{-3}, showed no significant features.

Data collection: *MSC/AFC Diffractometer Control Software* (Molecular Structure Corporation, 1988). Cell refinement: *MSC/AFC Diffractometer Control Software*. Data reduction: *TEXSAN PROCESS* (Molecular Structure Corporation, 1985). Program(s) used to refine structure: *CAOS* (Camalli *et al.*, 1986). Molecular graphics: *CAOS*. Software used to prepare material for publication: *CAOS*. Computer: DEC3500 AXP (Istituto di Strutturistica Chimica CNR).

Supplementary data for this paper are available from the IUCr electronic archives (Reference: NA1264). Services for accessing these data are described at the back of the journal.

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trans-Myristic Acid 3-*tert*-Butoxycarbonylamino-2-oxopiperidin-5-yl Ester,† a New Anthelmintic Compound

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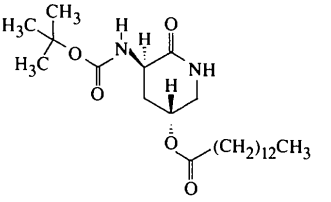
Abstract

The title compound, $C_{24}H_{44}N_2O_5$, is a 3-amino-5-hydroxy-piperidin-2-one derivative. Single-crystal X-ray diffraction was performed to determine the relative stereochemistry at the chiral centres. The substituents of the 2-piperidone ring have a *trans* configuration. The molecule crystallized in a monoclinic cell with a long *c* axis [47.742 (5) Å]; the space group is $P2_1/c$. The crystal structure is stabilized by hydrogen bonds along the *b*-axis direction.

Comment

As part of a general program of synthesis of new anthelmintic compounds, a series of 3-amino-5-hydroxy-piperidin-2-one derivatives has been synthesized. These compounds are structural analogues of secondary marine metabolites, which exhibit promising *in vitro* and *in vivo* anthelmintic activity (Crews & Hunter, 1993). Synthesis of the title compound, (I), involves a spontaneous lactone–lactam interconversion (Bols & Lundt, 1991; Chida, Tobe, Murai, Yamazaki & Ogawa, 1994), giving the racemic product. The ^{1}H NMR data were not sufficient to give the relative stereochemistry of the substituents at C2 and C4; thus, X-ray diffraction was used to determine the molecular structure and obtain the relative configurations.



(I)

The molecule contains a 2-piperidone ring substituted at C2 and C4. The substituents are a *tert*-butoxycarbonylamino group at C2 and a myristate group at C4. These substituents are *trans* with respect to each other, as is shown in Fig. 1; this configuration was expected, based on the synthesis. The 2-piperidone ring has a distorted half-chair conformation as in the 2-piperidone ring of (3*S*)-3-*tert*-butoxycarbonylamino-2-piperidone, (II) (Valle, Crisma, Toniolo, Yu & Johnson, 1989). The puckering parameters q_1, φ_2 and q_2 (Cremer & Pople, 1975) have values of 0.277 (2) Å, 282.7 (5)° and −0.341 (2) Å, respectively, for (I), and 0.331 (8) Å, −86.9 (15)° and −0.381 Å for (II) (Valle *et al.*, 1989). The presence of the substituent at C4 increases distortion from the symmetrical conformation of the ring, but comparison of torsion angles of the rings in (I) and (II) shows that they have similar conformations (see Table 3).

The N2 atom is equatorial to the ring, while O4 is axial, and in this conformation, the two substituents avoid overcrowding on one side of the ring. The hydrocarbon chain of myristic acid is planar, the maximum deviation from the least-squares plane being 0.04 (2) Å for C24. The *tert*-butoxycarbonylamino group is in its usual extended (*trans-trans*) arrangement, as in (II)

† Alternative name: 5-(*tert*-butoxycarbonylamino)-6-oxopiperidin-3-yl tetradecanoate.