

Calcium Binding to Amide Carbonyl Groups: Structure of a Calcium Bromide Salt of D-Pantothenic Acid

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Abstract. $\text{CaBr}(\text{C}_9\text{H}_{16}\text{NO}_5)$, orthorhombic, $P2_12_12_1$, $a = 6.8210$ (2), $b = 13.6346$ (5), $c = 14.4476$ (4) Å, $V = 1343.7$ Å³, $Z = 4$, $D_c = 1.67$ Mg m⁻³, $\mu(\text{Cu } K\alpha) = 7.88$ mm⁻¹. The Ca ion is coordinated to six O atoms and to the Br ion. The shortest Ca contact, 2.291 (5) Å, is to the carbonyl O atom of the amide linkage.

Introduction. We determined the crystal structure of the calcium bromide salt of D-pantothenic acid as part of an on-going study of Ca binding to organic ligands. We were particularly interested in examining calcium interactions with the amide linkage of the pantothenate anion to obtain additional information about the factors that might be involved in calcium binding to proteins.

Clear, lath-shaped crystals of the salt were grown by evaporating an aqueous solution that contained an approximately equimolar mixture of calcium D-pantothenate and calcium bromide. Weissenberg and oscillation photographs showed that the crystals are orthorhombic; the space group is $P2_12_12_1$ as indicated by the systematic absence of reflections $h00$ with h odd, $0k0$ with k odd, and $00l$ with l odd. A crystal fragment with approximate dimensions $0.12 \times 0.18 \times 0.48$ mm was mounted on a Picker FACS-1 diffractometer with its a axis slightly inclined to the ϕ axis of the diffractometer. Intensity data were collected with the diffractometer by use of a scintillation counter, Ni-filtered Cu $K\bar{\alpha}$ ($\lambda = 1.5418$ Å) radiation, and a θ - 2θ scanning technique. The 2θ scanning speed was 1° min^{-1} and the background was counted for 20 s at each terminus of the scans. A base scan range of 0.85° was augmented to account for α_1 - α_2 splitting. Measurements were made for each of the 1323 independent reflections with $2\theta \leq 128^\circ$. The 200, 080 and 008 reflections were chosen as standards and were monitored periodically during the data-collection procedure; they did not vary significantly during the experiment. Those reflections with scan counts below background levels were given their calculated negative intensity values and were retained in all subsequent calculations. Intensities were assigned variances, $\sigma^2(I)$, according to counting statistics plus

an additional term $(0.03S)^2$, S being the scan count. The intensities and their variances were corrected for Lorentz and polarization effects, absorption corrections were applied with the program *ORABS* (Wehe, Busing & Levy, 1962), and the data were scaled by a Wilson (1942) plot. Transmission factors ranged from 0.28 to 0.52. Cell parameters were measured before and after intensity data were collected. Approximate cell parameters for use in collecting intensity data were calculated by a least-squares analysis of the angular settings for twelve Cu $K\bar{\alpha}$ reflections. More precise values for cell parameters were determined immediately after data collection by a least-squares analysis of 2θ values for 28 Cu $K\alpha_1$ reflections ($\lambda = 1.54051$ Å); these values, which are listed in the *Abstract* and were used in all subsequent calculations, are not significantly different from those obtained prior to the intensity measurements.

The trial structure was obtained by heavy-atom methods and was refined with a modified version of the full-matrix least-squares program *ORFLS* (Busing, Martin & Levy, 1962; Busing, 1971). The quantity minimized was $\sum w(F_o^2 - F_c^2/k^2)^2$, where k is a scale factor and the weights, w , are equal to $1/\sigma^2(F_o^2)$. Scattering factors for the nonhydrogen atoms were from *International Tables for X-ray Crystallography* (1974); anomalous-dispersion correction factors for these atoms were from Cromer & Liberman (1970). The atomic scattering factors of Stewart, Davidson & Simpson (1965) were used for H atoms.

Initial refinement was of a trial structure that had the incorrect enantiomer of pantothenic acid. After several cycles of refinement with anisotropic temperature factors ($R = \sum ||F_o| - |F_c|| / \sum |F_o| = 0.059$), a difference electron-density map revealed trial positions for all H atoms except the two on the hydroxyl O atoms. In subsequent refinements, limited core storage prevented simultaneous variation of all parameters; instead, subsets of parameters were refined in alternate cycles with about half the parameters varied in each cycle. Two such cycles of refinement including the positional parameters and isotropic temperature factors for 14 H atoms reduced R to 0.053.

At this stage we realized that the wrong enantiomer was being refined. A structure factor calculation with the correct enantiomer, which was obtained by inverting the asymmetric unit through the origin, produced an R of 0.042 and reduced the goodness-of-fit $\{=\sum w(F_o^2 - F_c^2)^2/(m - s)\}^{1/2}$, where m is the number of reflections and s is the number of parameters} by 20%. Trial positions for the two missing hydroxyl H atoms were deduced from the hydrogen-bonding scheme and were confirmed in a difference Fourier map. Final refinement included all positional parameters, anisotropic temperature factors for the nonhydrogen atoms, isotropic for the 16 H atoms, and an isotropic extinction parameter [after Zachariasen (1963); this is the g' parameter as formulated by Coppens & Hamilton (1970)]. Final $R = 0.032$, and the goodness-of-fit = 1.99. During the later stages of refinement, the 200 reflection was given zero weight because it seemed to suffer excessively from uncorrected extinction effects. In the last double cycle of refinement, no parameter shifted more than one-third of its standard deviation,

Table 1. Atomic coordinates and estimated standard deviations

The values for Br^- and Ca^{2+} have been multiplied by 10^5 , those for C, N and O by 10^4 , and those for H by 10^3 . The final value for the isotropic extinction parameter is $g' = 0.201(7) \text{ rad}^{-1}$.

	x	y	z
Br	89576 (8)	93214 (4)	86656 (3)
Ca	48547 (11)	68300 (6)	97118 (5)
C(1)	9967 (6)	6281 (3)	9383 (2)
C(2)	10269 (7)	5266 (3)	8989 (3)
C(3)	8441 (7)	4720 (3)	8708 (3)
N	7552 (6)	5122 (3)	7875 (2)
C(4)	5835 (6)	5569 (3)	7861 (3)
C(5)	5100 (8)	5949 (4)	6931 (3)
C(6)	5603 (8)	7040 (4)	6733 (3)
C(7)	4641 (16)	7295 (6)	5797 (4)
C(8)	7809 (11)	7210 (5)	6674 (4)
C(9)	4641 (9)	7717 (4)	7445 (3)
O(1)	11437 (4)	6831 (2)	9479 (2)
O(2)	8303 (4)	6562 (2)	9631 (2)
O(3)	4790 (4)	5677 (2)	8558 (2)
O(4)	5895 (7)	5376 (3)	6200 (2)
O(5)	5534 (5)	7726 (2)	8342 (2)
H1(C2)	1095 (8)	496 (4)	942 (4)
H2(C2)	1104 (6)	535 (3)	860 (3)
H1(C3)	901 (9)	403 (4)	858 (4)
H2(C3)	735 (8)	472 (3)	928 (3)
H(C5)	362 (8)	592 (3)	688 (3)
H1(C7)	325 (12)	722 (5)	572 (5)
H2(C7)	519 (11)	694 (5)	521 (5)
H3(C7)	462 (12)	789 (6)	565 (5)
H1(C8)	850 (11)	679 (5)	606 (5)
H2(C8)	850 (9)	712 (4)	719 (4)
H3(C8)	816 (12)	786 (5)	627 (5)
H1(C9)	483 (11)	841 (5)	720 (5)
H2(C9)	312 (10)	741 (4)	759 (4)
H(N)	817 (9)	509 (4)	736 (4)
H(O4)	484 (14)	502 (7)	590 (7)
H(O5)	631 (17)	800 (8)	876 (6)

and the average shift was less than one-tenth of the overall estimated standard deviation. A final difference Fourier map showed two peaks and two troughs ranging from 0.3 to 0.5 $e \text{ \AA}^{-3}$ in magnitude in the vicinity of the Ca and Br ions; there were no other fluctuations that exceeded 0.3 $e \text{ \AA}^{-3}$.

Discussion. Table 1 lists positional parameters and their estimated standard deviations.* The estimated errors in positional coordinates are about 0.0005 \AA for the Br ion, 0.0008 \AA for the Ca ion, 0.005 \AA for other nonhydrogen atoms, and 0.07 \AA for H atoms. The conformation of the D-pantothenate anion, together with the numbering scheme, bond lengths, bond angles and thermal ellipsoids, is shown in Fig. 1. The H atoms of the two hydroxyl groups [H(O4) and H(O5)] have refined isotropic temperature factors that, like the estimated standard deviations in positional coordinates, are about twice those values for other atoms. Bond angles and hydrogen-bonded contacts that involve atom H(O5) are distorted from the expected values; it is likely that the parameters for this atom may be affected by rippling near the Ca ion and have much larger errors than those associated with the other H atoms.

The crystal-packing and hydrogen-bonding schemes are depicted in Fig. 2. The three suitable hydrogen-bond donor sites on the pantothenate anion, the O(4)

* Lists of structure factors and thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34657 (12 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

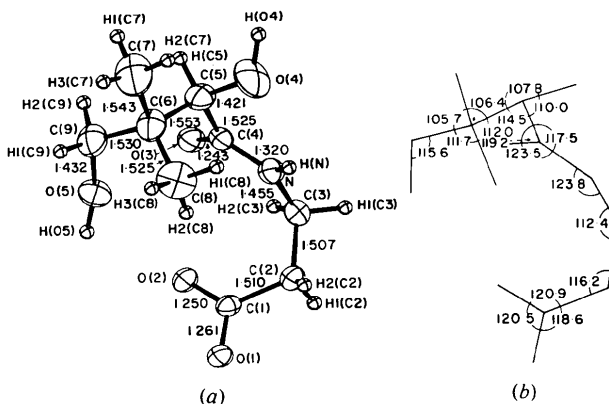


Fig. 1. Structure of the pantothenate anion, showing (a) bond lengths (\AA) and (b) bond angles ($^\circ$). In (a) nonhydrogen atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 50% probability, and H atoms are represented by spheres of 0.1 \AA radius. Angle C(5)-C(6)-C(9) is 111.1° ; angle C(7)-C(6)-C(8) is 109.6° . Estimated standard deviations are about 0.007 \AA and 0.5° for lengths and angles respectively. Bond angles involving H atoms are not shown. [This drawing, Figs. 2 and 3 were prepared with the computer program ORTEP (Johnson, 1965)].

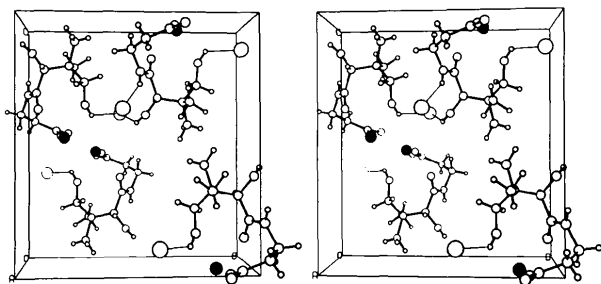


Fig. 2. Crystal packing as viewed down the a axis. The Br ions are shown as large circles and the Ca ions are depicted as solid black circles. Heavy lines represent covalent bonds and narrow lines represent hydrogen bonds.

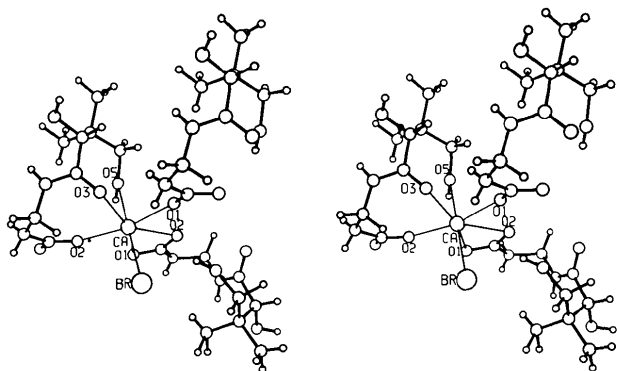


Fig. 3. Environment of the calcium ion.

and O(5) hydroxyl groups and the N–H group of the amide linkage, all form hydrogen bonds with the Br ions. The donor–acceptor distances are 3.614 (5), 3.225 (5), and 3.437 (5) Å, respectively.

The environment of the Ca ion is shown in Fig. 3. The Ca ion is coordinated to seven ligands, including six O atoms from pantothenate ions, and the Br ion. Three symmetry-related pantothenate anions are coordinated to the Ca ion. One pantothenate ion binds the Ca ion through a tridentate site that is composed of

atoms O(5) of the terminal hydroxyl group, O(3) of the carbonyl group, and O(2) from the carboxyl group; a second pantothenate ion chelates the Ca ion through the two O atoms of its carboxyl group, and the third pantothenate ion is coordinated to the Ca ion only through atom O(1) of its carboxyl group. The Ca...O distances range from 2.291 (5) to 2.613 (5) Å, with a mean value of 2.41 (11) Å; the Ca...Br distance of 2.887 (1) Å may be compared with values of 2.88 and 2.91 Å found in the crystal structure of calcium bromide (Brackett, Brackett & Sass, 1963). The shortest calcium contact, 2.291 (5) Å, is to the carbonyl O atom of the amide linkage.

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