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## $\beta$ -Methylanthionine: a Sulfur Amino Acid in Subtilin and Nisin Antibiotics

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**Abstract.**  $\beta$ (S)-Methylanthionine,  $C_7H_{14}SN_2O_4 \cdot 2H_2O$ , F. W. 258, monoclinic,  $P2_1$ ,  $a=11.118$  (5),  $b=16.371$  (5),  $c=6.783$  (2) Å,  $\beta=91.09$  (4)°,  $D_x=1.39$  g cm<sup>-3</sup> for  $Z=4$ ,  $D_m=1.39 \pm 0.02$  g cm<sup>-3</sup> by flotation,  $V=1234$  Å<sup>3</sup>. The structure was solved by direct methods and refined to an  $R$  value of 10.2%. Both D and L  $\alpha$ -carbon atoms were found in this bacterial amino acid. The average C-S bond distance is 1.83 (2) Å and the C-S-C angle is 103°.

**Introduction.**  $\beta$ -Methylanthionine is a rare non-protein amino acid which occurs in the bacterial antibiotics nisin and subtilin (Gross & Morell, 1970, 1971). In addition to early chemical work by Alderton (1953), who showed the compound contains three asymmetric C atoms, recent hypotheses by Gross & Morell (1970, 1971) on the conformation of this amino acid in antibiotics led us to undertake this study.

Prismatic crystals of the amino acid from subtilin were grown overnight by slow evaporation from water at room temperature. Space group and lattice parameters were determined from precession photographs. The systematic extinctions are  $0k0$  for  $k$  odd and indicate space group  $P2_1(C_2^2, \text{No. } 4)$ . Unit cell constants were refined by a least-squares fitting of diffractometer settings for 12 centered reflections.

A crystal measuring  $0.2 \times 0.2 \times 0.4$  mm was sealed in a glass capillary. Intensity data were collected with Mo  $K\alpha$  radiation on a Picker Corporation FACS-I four-circle diffractometer. A  $1^\circ \text{ min}^{-1} 2\theta$  scan was used with stationary 20 s background counts on either side of the nominal  $2^\circ$  scan. Three standard reflections were monitored every 100 reflections. Of the 1647 unique reflections measured to  $50^\circ 2\theta$ , 438 reflections had background-corrected intensities  $I_o$  less than  $\sigma(I_o)$  and were assigned an intensity of  $I_o + \sigma(I_o)$ . The standard error  $\sigma$  is estimated as  $[\text{CN} + \text{BG} + p^2 I_o^2]^{1/2}$  where CN is the peak count, BG is the total background count, and  $p$  is an arbitrary stability factor taken as 5%. Corrections were made for Lorentz-polarization effects, but not for absorption [ $\mu(\text{Mo } K\alpha) = 2.78 \text{ cm}^{-1}$ ]. Fourier and least-squares calculations were done with the X-RAY System (1972) on an IBM 360 computer. Scattering curves were taken from *International Tables for X-ray Crystallography* (1968).

Observed reflections were converted to normalized  $|E|$  values by means of a  $K$  curve, and the symbolic addition procedure for non-centrosymmetric crystals was applied (Karle & Karle, 1966). Phases used for origin and enantiomorph assignment were  $70\bar{6}$ ,  $30\bar{2}$ ,

$\bar{2}14$ , and  $\bar{2}\bar{6}3$ . To implement the phasing procedure, three symbolic phases were systematically assigned numeric values in increments of  $\pi/4$  radians. The multiple-solution tangent formula procedure developed by Drew, Templeton & Zalkin (1969) was used. After 30 cycles of tangent formula refinement, including in three stages the 225 reflections with  $|E| > 1.5$ , the phase set with an  $R$  (Karle) index equal 0.23 was used to calculate an  $E$  map which revealed all of the structure except methyl groups and water molecules which were found by difference Fourier syntheses.

In the full-matrix least-squares refinement the quantity minimized was  $\sum w(|F_o| - |F_c|)^2$ , where  $w = \sigma^{-2}(F_o)$ . After six cycles of refinement with isotropic temperature factors, parameter shifts became less than their standard deviations and the  $R$  value  $\sum ||F_o| - |F_c|| / \sum |F_o|$  was 11.8% for non-hydrogen atoms. At this stage H atoms were seen in difference Fourier maps. Water H atoms could not be located, and methyl H atoms had to be placed in most likely positions. Two additional cycles of refinement using anisotropic temperature factors only for S atoms (see *Discussion*) gave weighted and unweighted  $R$  values of 8.5% and 10.2% for 1207 observed reflections, at which point all parameter shifts were less than  $0.4\sigma$ . The goodness-of-fit parameter was 1.3. Positional and isotropic thermal factors (of parent atom) for H atoms were not varied. Unobserved reflections were omitted from the refinement. The final difference map contained only random background. The final atomic coordinates [L configuration at C(2) and C(12)] and temperature factors are given in Table 1.

**Discussion.** This amino acid is unusual in that the two  $\alpha$ -C atoms have opposite configurations. Earlier chemical studies (Gross & Morell, 1971) assigned the D configuration to the  $\alpha$ -C atom in the methylated portion of the molecule, and the L configuration to the second  $\alpha$ -C atom. The configuration of the methylated  $\beta$ -C atom had not been established. By imposing these chemically determined  $\alpha$  configurations on our model, we find directly that the configuration of the methylated  $\beta$ -C atom is (S). An effort was made to confirm the overall absolute configuration. A refinement with a full data set collected with copper radiation\* indi-

\* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31200 (22 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

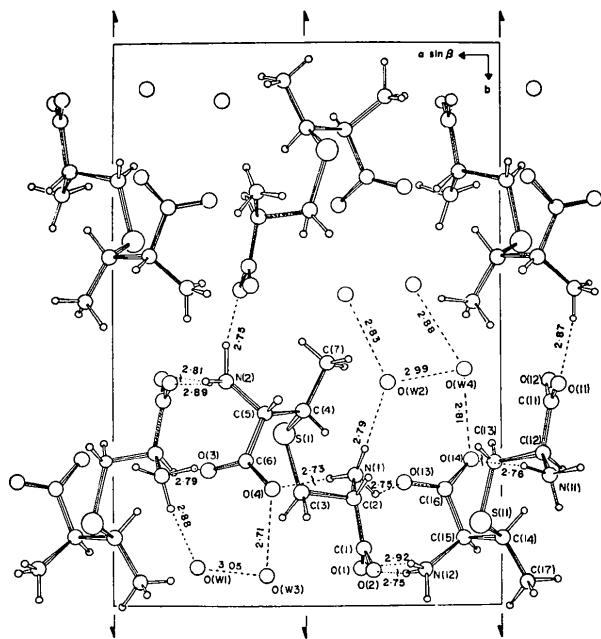


Fig. 1. View of the crystal structure along the  $c$  axis.

cated the  $\alpha$  configurations were correct, in that reversing the sign of the  $\Delta f''$  component for sulfur produced an increase in the  $R$  value from 11.3% to 12.0%. A test of the  $R$  factor ratio (Hamilton, 1965) showed the increase in  $R$  (and weighted  $R$ ) is significant at the 99% confidence level. However, for this structure the absolute configuration determination is somewhat less than certain because of difficulties encountered when a full anisotropic refinement was attempted, as discussed below.

The two molecules in the asymmetric unit were compared with the molecular fit program of Nyburg (1974). Least-squares matching of non-hydrogen atomic coordinates showed the deviation in position between chemically equivalent atoms ranged from 0.04 to 0.34 Å with an r.m.s. value of 0.15 Å. A constrained refinement with S atoms exactly superimposed gave values from 0.06 to 0.33 Å and an r.m.s. value of 0.20 Å. For comparison, the approximate positional error estimated from the structure refinement is only 0.015 Å. Nevertheless, the overall conformations of the two molecules are very similar. In each molecule, the amino groups are on opposite sides of the C-S-C plane. In the crystal (Fig. 1) the molecules are closely related by

Table 1. Fractional coordinates  $\times 10^4$  ( $\times 10^3$  for H) and temperature factors ( $\text{Å}^2 \times 10^3$ )

Standard deviations are given in parentheses.  $T = \exp(-8\pi^2 U \sin^2 \theta / \lambda^2)$  except where noted.

	$x$	$y$	$z$	$U$		$x$	$y$	$z$	$U$
C(1)	3540 (13)	18964 (11)	2280 (23)	20 (4)	H1(N1)	325	800	-25	27
C(2)	3784 (14)	8073 (12)	2512 (23)	26 (4)	H2(N1)	440	774	-20	27
C(3)	5107 (14)	7971 (12)	3412 (22)	25 (4)	H3(N1)	350	720	100	27
S(1)	5588 (4)	6917 (6)	3423 (6)	†	H(C2)	345	770	325	29
O(1)	3583 (10)	9294 (8)	621 (16)	32 (3)	H1(C3)	563	840	255	31
O(2)	3296 (10)	9344 (8)	3836 (15)	36 (3)	H2(C3)	500	840	450	31
N(1)	3745 (12)	7649 (9)	522 (19)	29 (4)	H(C4)	450	690	625	23
C(4)	5056 (14)	6514 (11)	5775 (22)	24 (4)	H(C5)	575	632	880	24
C(5)	6062 (14)	6555 (11)	7391 (22)	19 (4)	H1(N2)	750	600	555	16
C(6)	6567 (14)	7382 (11)	7753 (22)	23 (4)	H2(N2)	705	536	700	16
C(7)	4443 (15)	5676 (13)	5488 (25)	41 (5)	H3(N2)	757	598	830	16
O(3)	7547 (10)	7565 (9)	7069 (15)	35 (3)	H1(C7)	405	573	412	45
O(4)	5869 (10)	7879 (9)	8652 (16)	44 (4)	H2(C7)	389	560	650	45
N(2)	7065 (10)	5981 (9)	6839 (15)	17 (3)	H3(C7)	510	529	542	45
C(11)	-1338 (14)	6357 (11)	1652 (24)	25 (4)	H1(N11)	-210	754	430	22
C(12)	-1108 (12)	7268 (10)	1507 (19)	14 (4)	H2(N11)	-60	752	420	22
C(13)	172 (15)	7424 (12)	846 (22)	34 (5)	H3(N11)	-155	826	325	22
S(11)	555 (4)	8509 (6)	679 (6)	†	H(C12)	-162	760	45	16
O(11)	-1501 (11)	6043 (9)	3333 (17)	37 (3)	H1(C13)	57	716	180	29
O(12)	-1309 (10)	5987 (9)	54 (16)	44 (3)	H2(C13)	22	710	-70	29
N(11)	-1299 (11)	7670 (9)	3466 (18)	22 (4)	H(C14)	-63	830	-200	25
C(14)	-51 (14)	8771 (11)	-1814 (22)	24 (4)	H(C15)	63	900	-475	15
C(15)	983 (13)	8763 (11)	-3322 (22)	20 (4)	H1(N12)	232	924	-160	26
C(16)	1474 (15)	7906 (11)	-3613 (22)	22 (4)	H2(N12)	247	940	-375	26
C(17)	-705 (16)	9569 (13)	-1738 (25)	44 (5)	H3(N12)	192	988	-250	26
O(13)	2511 (10)	7777 (8)	-3020 (15)	35 (3)	H1(C17)	-86	979	-37	44
O(14)	783 (10)	7379 (9)	-4375 (15)	39 (3)	H2(C17)	-150	952	-247	44
N(12)	1950 (12)	9330 (9)	-2798 (18)	29 (4)	H3(C17)	-22	1001	-245	44
O(W1)	7804 (11)	9310 (10)	3066 (18)	58 (4)					
O(W2)	2870 (12)	6052 (10)	415 (19)	68 (4)					
O(W3)	6049 (12)	9485 (10)	-415 (19)	69 (4)					
O(W4)	914 (12)	5824 (10)	-2628 (19)	67 (4)					

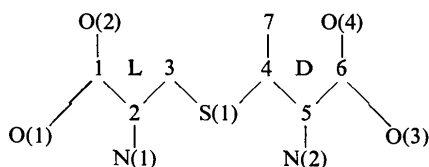
$$\dagger T = \exp[-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*hkU_{12} + 2a^*c^*hlU_{13} + 2b^*c^*klU_{23})]$$

	$U_{11}$	$U_{22}$	$U_{33}$	$U_{12}$	$U_{13}$	$U_{23}$
S(1)	35 (3)	32 (3)	32 (3)	12 (3)	-2 (2)	-2 (2)
S(11)	38 (3)	40 (3)	26 (3)	-21 (3)	10 (2)	-9 (3)

pseudo twofold screw axes parallel to the **a** and **c** directions. This higher pseudosymmetry and the nearly 90°  $\beta$  angle approximate space group  $P2_12_1$ , with one molecule per asymmetric unit. Herein may lie the cause of our failure to complete a full non-hydrogen anisotropic refinement without having six atoms assume nonpositive-definite temperature factors. The copper data set, collected from a second crystal, showed similar

Table 2. Bond distances (Å) and angles (°) in  $\beta$ -methyl-lanthionine

Standard deviations are approximately 0.2 Å and 1°.



(Numbering for molecule 2 is  $i + 10$ .)

S(1)–C(3)	1.81	S(11)–C(13)	1.83
S(1)–C(4)	1.83	S(11)–C(14)	1.86
C(2)–C(3)	1.59	C(12)–C(13)	1.52
C(4)–C(5)	1.55	C(14)–C(15)	1.55
C(4)–C(7)	1.54	C(14)–C(17)	1.50
C(1)–C(2)	1.49	C(11)–C(12)	1.52
C(5)–C(6)	1.48	C(15)–C(16)	1.52
C(2)–N(1)	1.52	C(12)–N(11)	1.50
C(5)–N(2)	1.51	C(15)–N(12)	1.46
C(2)–O(1)	1.25	C(12)–O(11)	1.27
C(2)–O(2)	1.26	C(12)–O(12)	1.24
C(5)–O(3)	1.23	C(15)–O(13)	1.23
C(5)–O(4)	1.29	C(15)–O(14)	1.26
C(3)–S(1)–C(4)	104	C(13)–S(11)–C(14)	101
S(1)–C(3)–C(2)	112	S(11)–C(13)–C(12)	114
S(1)–C(4)–C(5)	111	S(11)–C(14)–C(15)	110
C(7)–C(4)–S(1)	111	C(17)–C(14)–S(11)	110
C(7)–C(4)–C(5)	116	C(17)–C(14)–C(15)	113
C(3)–C(2)–C(1)	108	C(13)–C(12)–C(11)	110
C(3)–C(2)–N(1)	108	C(13)–C(12)–N(11)	110
N(1)–C(2)–C(1)	111	N(11)–C(12)–C(11)	110
C(4)–C(5)–C(6)	115	C(14)–C(15)–C(16)	111
C(4)–C(5)–N(2)	109	C(14)–C(15)–N(12)	113
N(2)–C(5)–C(6)	109	N(12)–C(15)–C(16)	111
C(2)–C(1)–O(1)	120	C(12)–C(11)–O(11)	119
C(2)–C(1)–O(2)	116	C(12)–C(11)–O(12)	114
O(2)–C(1)–O(1)	124	O(12)–C(11)–O(11)	126
C(5)–C(6)–O(3)	120	C(15)–C(16)–O(13)	117
C(5)–C(6)–O(4)	115	C(15)–C(16)–O(14)	118
O(3)–C(6)–O(4)	125	O(13)–C(16)–O(14)	125

Selected torsion angles

C(4)–S(1)C(3)–C(2)	–88
C(3)–S(1)C(4)–C(5)	–95
S(1)–C(3)C(2)–C(1)	–171
S(1)–C(4)C(5)–C(6)	57
C(7)–C(4)C(5)–C(6)	–175
N(1)–C(2)C(1)–O(1)	–15
N(2)–C(5)C(6)–O(3)	20
C(14)–S(11)C(13)–C(12)	–84
C(13)–S(11)C(14)–C(15)	–99
S(11)–C(13)C(12)–C(11)	–179
S(11)–C(14)C(15)–C(16)	67
C(17)–C(14)C(15)–C(16)	–170
N(11)–C(12)C(11)–O(11)	–8
N(12)–C(15)C(16)–O(13)	14

refinement behavior. Further, a higher data-to-parameter ratio may be necessary for full refinement.

The two independent molecules have normal bond distances and angles (Table 2), and corresponding bonds and angles in each molecule are equal to their mean within two standard deviations. The mean of the four S–C distances is 1.83 (2) Å, the S–CH(CH<sub>3</sub>) bond being slightly longer (0.03 Å) in each case than the S–CH<sub>2</sub> bond. The two C–S–C angles average 102.5°. Comparable S–C bond and angle values in the unmetallated *meso*-lanthionine.HCl are 1.81 Å and 103.7° (Rosenfield & Parthasarathy, 1972). As expected for a zwitterionic molecule, the C–O bonds in each carboxyl group are equal to the mean (1.25 Å) within 1.5 $\sigma$ , and three of the four C–C–O angles *cis* to the amino group are larger, by 4.5°, than the *trans* angles, a difference seen in other zwitterions (Marsh & Donohue, 1967). While in *meso*-lanthionine both carboxyl groups are *trans* to the S atom across each  $\alpha$ C– $\beta$ C bond, in  $\beta$ -methylanthionine this is true only for the carboxyl group at the L end. Presumably the bulky methyl group initiates the 180° rotation of the D carboxyl group about the  $\alpha$ – $\beta$  bond. We have discussed elsewhere the conformation this amino acid might assume in the peptide antibiotics nisin and subtilin (Knox & Keck, 1973).

No intramolecular hydrogen bonds are found, although in models it is possible for the D carboxyl group to rotate to form a hydrogen bond with the amino group on the L end of the molecule. Instead, the molecule participates in a full complement of hydrogen bonds to neighboring molecules and to water molecules. Each protonated amino group is involved in three NH $\cdots$ O hydrogen bonds 2.73 to 2.92 Å long.

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