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# Structure of Bufothionin

BY KAZUMASA HONDA,* MIDORI GOTO AND MASAYASU KURAHASHI

*National Chemical Laboratory for Industry, Tsukuba, Ibaraki* 305, *Japan*

TOSHIFUMI AKIZAWA AND MASANORI YOSHIOKA

*Department of Pharmaceutical Sciences, Setsunan University*, 45-1 *Nagaotoge-cho, Hirakata, Osaka* 573-01, *Japan*

AND VINCENT P. BUTLER JR

*Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY* 10032, *USA*

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**Abstract.** (1,3,4,5-Tetrahydro-5,5-dimethylpyrrolo-[4,3,2-*de*]quinolinio)-6-sulfate (bufothionin), $C_{12}H_{14}N_2O_4S$, $M_r = 282{\cdot}32$, monoclinic, $P2_1/c$, $a = 7{\cdot}000\ (1)$, $b = 9{\cdot}709\ (1)$, $c = 18{\cdot}238\ (5)$ Å, $\beta = 95{\cdot}51\ (4)°$, $V = 1233{\cdot}7\ (4)$ Å$^3$, $Z = 4$, $D_m = 1{\cdot}52$, $D_x = 1{\cdot}520$ Mg m$^{-3}$, Cu $K\alpha_1$, $\lambda = 1{\cdot}5406$ Å, $\mu = 2{\cdot}415$ mm$^{-1}$, $F(000) = 592$, $T = 295$ K, final $R = 0{\cdot}047$ for 2267 unique reflections. The molecule has a positive and a negative charge in a single structure. Intermolecular hydrogen bonds link the centrosymmetrical molecules to form a dimeric structure.

**Introduction.** The title compound is one of the major components of the extract of the skin of *Bufo marinus* (L.). The molecular structure has not been determined by means of nuclear magnetic resonance (NMR) or mass spectrometry (MS) because of the difficulty of interpreting the spectra. This study was therefore performed in order to clarify the structure.

**Experimental.** Bufothionin was purified from an extract of the toad skins (purchased from National Reagents, Bridgeport, Connecticut, USA) by using high-performance liquid chromatography (Akizawa, Ohtani, Kasai, Goto & Yoshioka, 1990). Colorless needle crystals were grown from the eluate (water–acetonitrile, 9:1 *v/v*) by slow evaporation at room temperature. The proposed chemical formula is consistent with that estimated by NMR, MS and X-ray fluorescence spectrometry.

Crystal size $0{\cdot}65 \times 0{\cdot}18 \times 0{\cdot}15$ mm. $D_m$ by flotation in $CHCl_3$–$CCl_4$. Data collection on an Enraf–Nonius CAD-4 diffractometer, graphite-monochromated Cu $K\alpha_1$ radiation, cell dimensions refined using 20 reflections having $25 < \theta < 30°$, intensity data $1{\cdot}5 \le \theta \le 75°$; $\omega$–$2\theta$ scans; $\Delta\omega = (0{\cdot}5 + 0{\cdot}15\tan\theta)°$; horizontal detector aperture $(1{\cdot}0 + 0{\cdot}5\tan\theta)$ mm; $-8 \le h \le 8$, $0 \le k \le 12$, $0 \le l \le 22$. Three standard reflections ($0\bar{1}\bar{2}$, 111, $2\bar{1}0$) showed no significant intensity variation during X-ray exposure time. Corrections for Lorentz and polarization, and an empirical absorption correction using $\psi$ scans (transmission factors 0·892–0·997). 2581 reflections collected, 2349 were above the significance level of $3\sigma(F_o)$ and 2267 were unique ($R_{int} = 0{\cdot}016$). Structure solved by direct methods using *MULTAN*11/82 (Main, Fiske, Hull, Lessinger, Germain, Declercq & Woolfson, 1982). Ten H atoms introduced from difference Fourier maps. Refinement on $F$ by full-matrix least squares with anisotropic temperature factors for non-H atoms and isotropic ones for H atoms. Among the remaining four H atoms, three [H(C9), H′(C9) and H″(C12)] were placed at the calculated positions. With H(N1) attached in a correct position, N(1) and O(3)$(1-x, -y, -z)$ are linked *via* a hydrogen bond. All H atoms fixed with

* To whom correspondence should be addressed.

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Table 1. *Fractional atomic coordinates and equivalent isotropic thermal parameters for non-H atoms, with e.s.d.'s in parentheses*

$B_{eq} = (4/3)\sum_i\sum_j \beta_{ij}\mathbf{a}_i.\mathbf{a}_j$.

| | $x$ | $y$ | $z$ | $B_{eq}$(Å$^2$) |
|---|---|---|---|---|
| S(1) | 0·91603 (9) | 0·07275 (6) | 0·20119 (3) | 2·60 (1) |
| O(1) | 0·8795 (2) | 0·2146 (2) | 0·1521 (1) | 2·74 (3) |
| O(2) | 1·0483 (3) | −0·0073 (2) | 0·1636 (1) | 4·14 (4) |
| O(3) | 0·7272 (3) | 0·0109 (2) | 0·2009 (1) | 3·27 (4) |
| O(4) | 0·9890 (3) | 0·1284 (2) | 0·2707 (1) | 4·44 (5) |
| N(1) | 0·3459 (4) | 0·2036 (3) | −0·0907 (1) | 3·58 (5) |
| N(2) | 0·4985 (3) | 0·3580 (2) | 0·1467 (1) | 2·09 (3) |
| C(1) | 0·2099 (4) | 0·2868 (3) | −0·0642 (2) | 3·67 (6) |
| C(2) | 0·2738 (4) | 0·3344 (3) | 0·0045 (1) | 2·92 (5) |
| C(3) | 0·4577 (4) | 0·2741 (3) | 0·0210 (1) | 2·39 (4) |
| C(4) | 0·5803 (3) | 0·2821 (2) | 0·0857 (1) | 2·08 (4) |
| C(5) | 0·7503 (4) | 0·2101 (2) | 0·0892 (1) | 2·40 (4) |
| C(6) | 0·7981 (4) | 0·1351 (3) | 0·0274 (1) | 3·25 (5) |
| C(7) | 0·6759 (5) | 0·1236 (3) | −0·0365 (1) | 3·49 (6) |
| C(8) | 0·5019 (4) | 0·1931 (3) | −0·0386 (1) | 2·89 (5) |
| C(9) | 0·2006 (4) | 0·4282 (3) | 0·0602 (2) | 3·55 (6) |
| C(10) | 0·3679 (4) | 0·4753 (3) | 0·1144 (1) | 2·97 (5) |
| C(11) | 0·3827 (4) | 0·2575 (3) | 0·1873 (1) | 3·27 (5) |
| C(12) | 0·6465 (4) | 0·4250 (3) | 0·2007 (2) | 3·60 (6) |

Table 2. *Bond distances* (Å) *and angles* (°)

| | | | |
|---|---|---|---|
| S(1)—O(1) | 1·649 (2) | C(1)—C(2) | 1·369 (4) |
| S(1)—O(2) | 1·432 (2) | C(2)—C(3) | 1·419 (4) |
| S(1)—O(3) | 1·452 (2) | C(2)—C(9) | 1·493 (4) |
| S(1)—O(4) | 1·427 (2) | C(3)—C(4) | 1·393 (3) |
| O(1)—C(5) | 1·392 (3) | C(3)—C(8) | 1·402 (3) |
| N(1)—C(1) | 1·372 (4) | C(4)—C(5) | 1·377 (3) |
| N(1)—C(8) | 1·380 (3) | C(5)—C(6) | 1·408 (4) |
| N(2)—C(4) | 1·493 (3) | C(6)—C(7) | 1·382 (4) |
| N(2)—C(10) | 1·542 (3) | C(7)—C(8) | 1·389 (4) |
| N(2)—C(11) | 1·508 (3) | C(9)—C(10) | 1·527 (4) |
| N(2)—C(12) | 1·507 (3) | | |

| | | | |
|---|---|---|---|
| O(1)—S(1)—O(2) | 105·5 (1) | C(3)—C(2)—C(9) | 118·0 (2) |
| O(1)—S(1)—O(3) | 104·5 (1) | C(2)—C(3)—C(4) | 129·0 (2) |
| O(1)—S(1)—O(4) | 100·9 (1) | C(2)—C(3)—C(8) | 109·2 (2) |
| O(2)—S(1)—O(3) | 113·8 (1) | C(4)—C(3)—C(8) | 121·7 (2) |
| O(2)—S(1)—O(4) | 115·9 (1) | N(2)—C(4)—C(3) | 114·1 (2) |
| O(3)—S(1)—O(4) | 114·1 (1) | N(2)—C(4)—C(5) | 127·5 (2) |
| S(1)—O(1)—C(5) | 118·4 (1) | C(3)—C(4)—C(5) | 118·0 (2) |
| C(1)—N(1)—C(8) | 109·3 (2) | O(1)—C(5)—C(4) | 120·8 (2) |
| C(4)—N(2)—C(10) | 109·6 (2) | O(1)—C(5)—C(6) | 119·4 (2) |
| C(4)—N(2)—C(11) | 108·0 (2) | C(4)—C(5)—C(6) | 119·8 (2) |
| C(4)—N(2)—C(12) | 114·3 (2) | C(5)—C(6)—C(7) | 122·7 (3) |
| C(10)—N(2)—C(11) | 109·8 (2) | C(6)—C(7)—C(8) | 117·1 (2) |
| C(10)—N(2)—C(12) | 106·0 (2) | N(1)—C(8)—C(3) | 105·9 (2) |
| C(11)—N(2)—C(12) | 109·1 (2) | N(1)—C(8)—C(7) | 133·5 (2) |
| N(1)—C(1)—C(2) | 110·2 (2) | C(3)—C(8)—C(7) | 120·6 (2) |
| C(1)—C(2)—C(3) | 105·4 (2) | C(2)—C(9)—C(10) | 109·3 (2) |
| C(1)—C(2)—C(9) | 136·5 (3) | N(2)—C(10)—C(9) | 114·6 (2) |

thermal parameters of parent atoms in the final refinement, $R = 0{\cdot}047$, $wR = 0{\cdot}065$ $[w^{-1} = \sigma^2(F_o)]$, max. $\Delta/\sigma = 0{\cdot}01$. Max. and min. residual densities 0·36 and −0·57 e Å$^{-3}$. Scattering factors for neutral atoms taken from *International Tables for X-ray Crystallography* (1974, Vol. IV). All the calculations performed with the Enraf–Nonius *SDP* (B. A. Frenz & Associates Inc., 1982) package.

**Discussion.** Tables 1 and 2 summarize the final atomic parameters and the bond distances and angles, respectively.* The molecular structure is shown in Fig. 1(*a*) with the numbering system.

The molecular structure is identical to that postulated by Robinson, Smith, Jackson, Shaw, Frydman & Deulofeu (1961). The molecule has positive and negative charged groups [N(2)$^+$ and $SO_3^-$] in a single structure. This may be one of the reasons why the interpretation of the NMR and MS spectra is difficult. It is considered that this compound arises from bufotenine 3-[2-(dimethylamino)ethyl]-5-hydroxyindole (Fig. 1*b*) by a ring closure of the —$CH_2$—$CH_2$—$N(CH_3)_2$ chain onto the C(4) position, accompanied with the sulfonation on the OH group at the C(5) position. The bond angle of 129·0 (2)° for C(2)—C(3)—C(4) is smaller than the corresponding angle of 133·3 (7)° of bufotenine (Falkenberg, 1972), whereas 121·7 (2)° for C(4)—C(3)—C(8) is larger than the 117·2 (7)° of bufotenine. This distortion is presumably due to a tension caused by the additional ring closure. The indole nucleus [N(1)—C(1)—C(2)—C(3)—C(4)—

* Lists of structure factors, anisotropic thermal parameters, H-atom parameters and selected least-squares-planes data have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53789 (18 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

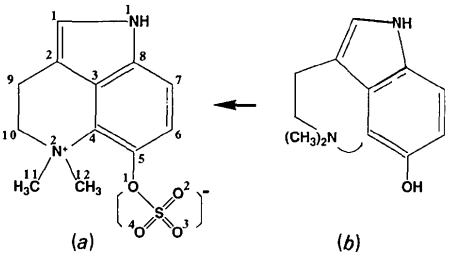


Fig. 1. Molecular structure of bufothionin (*a*) with the numbering system for the non-H atoms. A possible precursor bufotenine (*b*) is also depicted.

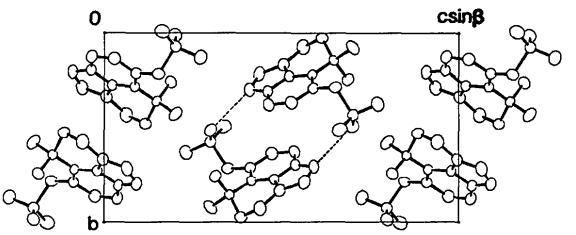


Fig. 2. Perspective view of the crystal structure along the *a* axis (Johnson, 1976). Thermal ellipsoids are drawn at 40% probability. Intermolecular hydrogen bonding between N(1) and O(3) is also indicated.

C(5)—C(6)—C(7)—C(8)] of bufothionin is almost planar within the maximum deviations of 0·04 Å [C(5)].

Fig. 2 depicts the crystal structure projected along the *b* axis. The shortest distance between the positive charge center $N(2)^+$ and the negative charge center $SO_3^-$ is 3·64 Å [N(2)···O(3)($1-x$, $\frac{1}{2}+y$, $\frac{1}{2}-z$)]; two methyl groups, C(11) and C(12), block intermolecular interaction between the positive and negative charge centers. A short intermolecular distance of 2·905 (1) Å between the N(1) and O(3) atoms is observed around the center of inversion, which suggests that the two centrosymmetrically related molecules form a dimer linked by hydrogen bonds. Slight enlargement of the double-bond length to 1·452 (2) Å for S(1)—O(3), compared with those for S(1)—O(2) [1·432 (2) Å] and S(1)—O(4) [1·427 (2) Å] is in good agreement with the formation of the hydrogen bond.

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# Proline Conformations in Linear Peptides. Structure Determination of the Methyl Ester of *N*-Benzyloxycarbonyl-L-prolyl-D-alanine (*N*-Z-L-Pro-D-Ala-OMe)

BY JEANETTE A. KRAUSE AND DRAKE S. EGGLESTON

*Department of Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, PA* 19406, *USA*

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**Abstract.** *N*-Z-L-Pro-D-Ala-OMe, $C_{17}H_{22}N_2O_5$, $M_r$ = 334·38, crystallizes in the orthorhombic space group $P2_12_12_1$, with the cell dimensions $a$ = 5·005 (5), $b$ = 17·690 (9) and $c$ = 18·70 (1) Å$^3$, $V$ = 1656·3 Å$^3$, $Z$ = 4, $D_x$ = 1·341 g cm$^{-3}$, Cu $K\alpha$, $\lambda$ = 1·54184 Å, $\mu$ = 7·830 cm$^{-1}$, $F(000)$ = 712, $T$ = 198 K, final $R$ (on $F$) = 0·036 for 1575 observed reflections with $I \geq 3\sigma(I)$. The pyrrolidine ring takes on the $C_2$-C$^\gamma$-*endo* conformation. The urethane bond is in the *cis* conformation [$\omega_0$ = 6·0 (3)°] while the peptide bond is in the *trans* conformation [$\omega_1$ = 170·8 (2)°]; $\varphi_1/\psi_1$ values are −88·0 (3)° and 151·3 (2)°. Intermolecular hydrogen bonding occurs between the C-terminus and the symmetry-related amide. Systematic examination of the pyrrolidine ring in linear peptides reveals no correlation exists between the *cis–trans* orientation of the proline and the conformation of the pyrrolidine ring.

**Introduction.** Proline is an important constituent of many proteins. Its presence in proteins imposes certain conformational restrictions, particularly as a helix breaker. Peptides containing proline residues have been extensively studied because of the possibility of *cis–trans* isomerization about the *X*-Pro bond (Carver & Blout, 1976; Grathwohl & Wuthrich, 1976; Nair & Vijayan, 1981) and the different modes of puckering that the pyrrolidine ring can undergo (Balasubramanian, Lakshminarayanan, Sabesan, Tegoni, Venkatesan & Ramachandran, 1971; Ashida & Kakudo, 1974).

Recently it was proposed (Trikha, Patel & Singh, 1990) that for proline in the *cis* conformation the pyrrolidine ring adopts only the $C_2$-C$^\gamma$-*endo* conformation. The structure of *N*-Z-L-Pro-D-Ala-OMe displays an *X*-Pro bond which is *cis* and in which the pyrrolidine ring geometry is $C_2$-C$^\gamma$-*endo*. Observation of this ring conformation in conjuction with a *cis* proline appears to further confirm the previously drawn correlations. However, closer examination of a larger database of linear proline-containing peptides reveals the previous assertion to be erroneous.

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