organic compounds

A commonly used spin label: S-(2,2,5,5-tetramethyl-1-oxyl-Δ³-pyrrolin-3-ylmethyl) methanethio-sulfonate

Vitali Zielke,[a] Henning Eickmeier,[b] Kalman Hideg,[c] Hans Reuter[b]* and Heinz-Jürgen Steinhoff[a]

[a]Makromolekülstrukturen, Fachbereich Physik, Universität Osnabrück, Barbarastrasse 7, D-49076 Osnabrück, Germany, [b]Anorganische Chemie II, Institut für Chemie, Universität Osnabrück, Barbarastrasse 7, D-49069 Osnabrück, Germany, and [c]Institute of Organic and Medicinal Chemistry, University of Pécs, PO Box 99, 7602 Pécs, Hungary

Correspondence e-mail: hreuter@uos.de

The title compound, $C_{10}H_{18}NO_3S_2$, which finds application as a spin label, has triclinic ($P\bar{1}$) symmetry at 100 (2) K with two independent molecules in the asymmetric unit. Both molecules are very similar with respect to bond lengths and angles, but molecule 2 shows disordering of its side chain. The pyrroline rings differ slightly with respect to the position of the NO group, which in both cases are sterically shielded by the surrounding methyl groups. The crystal structure of the title compound represents the first example of a 2,2,5,5-tetramethyl-1-oxyl-Δ^3-pyrroline derivative with a side chain at the double bond which is linked to it through an sp^3-hybridized C atom. In the solid state, the side chain adopts a conformation with the methyl group above/below the pyrroline ring and a H atom directed towards a C atom of the double bond. The disordered side chain of molecule 2 represents a second conformation with low potential energy. Both molecules exhibit planar chirality, but in the solid state both pairs of stereoisomers are present. These four stereoisomers are stacked one behind the other in four different columns, denoted A, A', B and B', the angle between the vectors of the N—O bonds in columns A and B being 80.38 (8)°.

Comment

Nitroxide radicals are gaining increasing importance since they exhibit magnetic properties similar to nitronyl nitroxides (Osiecki & Ullman, 1968), which can be used as building blocks for purely organic molecule-based magnets (Fegy et al., 1998; Hirel et al., 2001). Nitroxides are used as a tool for spin labelling in EPR (electron paramagnetic resonance) spectroscopy (see, for example, Altenbach et al., 1990; Steinhoff et al., 1994; Hubbell et al., 1996; Bordignon & Steinhoff, 2007). Via the technique of site-directed spin labelling, information about the structure and conformational dynamics of macromolecules such as water-soluble or membrane proteins of arbitrary molecular weight can be obtained. The title compound (Berliner et al. 1982), abbreviated to methanethiosulfonate spin label (MTSSL, also known as HO-225), has emerged in recent years as one of the most frequently used spin labels. In addition, various of its derivatives, such as pyrroline ring-based artificial amino acids or lipids, have been synthesized (Balog et al., 2003, 2004, 2007). Increasing interest in molecular dynamics simulations of spin-labelled proteins requires high-resolution information on the spin label structure (Beier & Steinhoff, 2006; Budil et al., 2006; DeSensi et al., 2008; Sezer et al., 2008a,b). Additionally, EPR spectra of crystalline MTSSL have been used as a reference for spectra of adsorbed radicals on surfaces (Niermann et al., 2006; Niermann et al., unpublished). For quantitative analyses of these EPR data, the inter-spin distances and relative orientations of the pyrroline rings in the MTSSL crystal must be known.
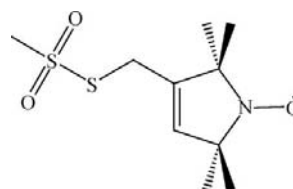
MTSSL crystallizes with two independent molecules in the asymmetric unit (molecule 1 is shown in Fig. 1). The bond lengths and angles within these molecules differ only marginally. The main difference between the two molecules results from a disorder of the side chain in molecule 2.

The bond lengths for $Cn2—Cn3$ [1.5025 (18) Å for molecule 1 ($n = 1$) and 1.5008 (18) Å for molecule 2 ($n = 2$)] and $Cn4—$
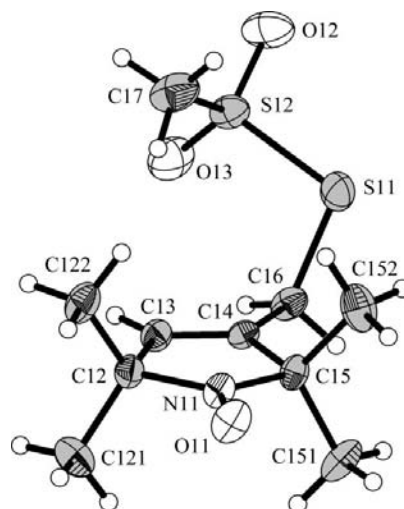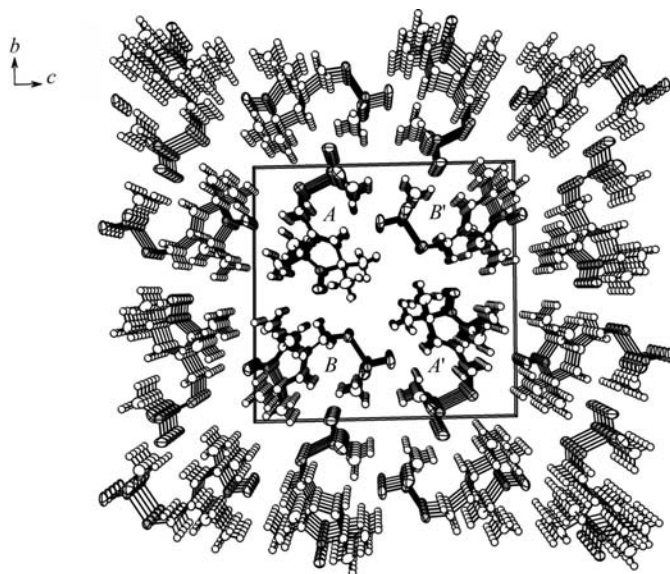

Figure 1
A view of molecule 1 in the asymmetric unit of MTSSL, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level (key: O white, C grey with octant shading, N white with octant shading and S grey) and H atoms are shown as small spheres of arbitrary radii.
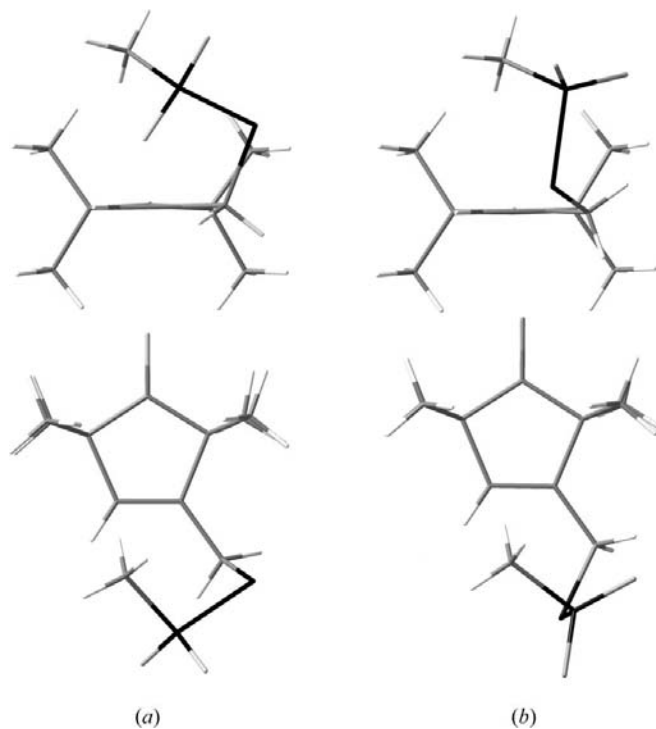
Figure 2
A perspective view of the crystal structure of MTSSL, showing columns of molecules along [100]. For the sake of clarity, H atoms have been omitted and only the major part of the side chain of molecule 2 is shown. The columns of different stereoisomers are marked A/A' and B/B'.

$Cn5$ [1.5173 (17) Å for $n = 1$ and 1.5146 (17) Å for $n = 2$] conform to single bonds (Allen *et al.*, 1987), while those of $Cn3—Cn4$ [1.3301 (19) Å for $n = 1$ and 1.3358 (17) Å for $n = 2$] correspond to double bonds, in agreement with the scheme. The corresponding N—O distances are 1.2767 (14) ($n = 1$) and 1.2740 (15) Å ($n = 2$), which are in good agreement with the values found in comparable nitroxides (Turley & Boer, 1972; Wiley *et al.*, 1991; Duskova *et al.*, 2001). All other bond lengths and angles, especially those involving S atoms, are in good agreement with calculated data.

Within the pyrroline rings, the carbon skeletons are nearly planar [maximum deviations from the least-squares planes through the four C atoms are 0.0084 (8) Å for atom C13 in molecule 1 and 0.0040 (8) Å for atom C23 in molecule 2], with the N atoms slightly further from these planes [0.066 (2) Å for atom N11 and 0.031 (2) Å for atom N21]. Because the vectors of the nitroxide groups form angles of 2.93 (7) (molecule 1) and -1.86 (7)$°$ (molecule 2) with the least-squares planes through the ring C atoms, the nitroxide O atoms lie on the same side [0.131 (3) Å for O11 in molecule 1] of the pyrroline ring as the corresponding N atom, or on the opposite side [-0.011 (3) Å for O21 in molecule 2]. In both cases, the NO radical is perfectly shielded by the surrounding four methyl groups.

Numerous crystal structures of nitroxide radicals are described in the literature, and those of 3-pyrrolinyl-1-oxy radicals are widespread. In the latter case, all known examples are 2,2,5,5-tetramethyl derivatives, most of them with one additional side chain at the double bond. All of these side chains are connected to the pyrroline ring *via* an sp^2- or sp-hybridized C atom, which allows the multiple bonds [–CN (Dickman & Doedens, 1983; Porter & Doedens, 1984),

Figure 3
Wire models showing the different conformations of the side chains from two perspectives. At the top are views parallel to the pyrroline ring, and below are views perpendicular to the pyrroline ring. (*a*) The conformation of molecule 1 and the major part of molecule 2. (*b*) The second conformation (minor part) of molecule 2.

–COOR (Wiley *et al.* 1991), –CONH$_2$ (Turley & Boer, 1972) or –COO$^-$ (Papoutsakis *et al.*, 1999; Boeyens & Kruger, 1970)] in the side chain to hyperconjugate with the double bond of the pyrroline ring. As a result of this hyperconjugation, these side chains are almost coplanar with the pyrroline ring.

MTSSL represents the first example with a side chain that is connected to the pyrroline ring *via* an sp^3-hybridized C atom. Moreover, the second atom of the side chain, an S atom, is also sp^3-hybridized, giving the side chain a high conformational flexibility. From all conformers possible as a result of rotation around the C—C single bond, those realized in the solid state are those where the side chain is nearly perpendicular [the C—C—C—S torsion angle is 99.30 (15)$°$ for molecule 1 and 99.65 (14)$°$ for the major part of molecule 2] to the pyrroline ring. In addition, the side chains are folded back at the S atoms in the direction of the pyrroline ring, giving the molecules a 'scorpion-like' habit. As a result of this folding back, the methyl group of the methanethiosulfonate residue is positioned above/below the pyrroline ring with a H atom pointing in the direction of the C atom of the double bond. Because this effect is observed in both independent molecules, this conformation must be accompanied by a very low potential energy. Moreover, this conformation of the molecules produces planar chirality. However, because of crystal symmetry, both stereoisomers of the two independent molecules are present, so that the solid state represents a racemic mixture. Whether the complete rotation around the C—C

single bond is prevented in solution so that both conformations can exist side by side is difficult to determine. The orientation, folding and intramolecular interactions of the side chain, however, suggest that the energy barrier can be high enough, especially at low temperatures.

The solid-state structure of MTSSL also reveals that there is a second minimum in the potential energy curve of the molecule. This local minimum is represented by the disordered side chain of molecule 2, with a low site-occupancy factor of 0.0821 (14). This conformation is characterized by a C23—C24—C26B—S21B torsion angle of 22.6 (2)°. Although this value is very different from that of the previous conformation, the remaining part of the methanethiosulfonate residue is folded in a similar way, so that the methyl group is also positioned above/below the pyrroline ring with one H atom directed towards a C atom of the double bond.

In the crystal structure, the molecules are stacked one behind the other in two different columns; one column (A) is formed from molecule 1 and the second one (B) from molecule 2. Both columns are parallel to the a axis. The distance between the individual molecules within each column, as well as between their nitroxide groups, therefore corresponds to the length of the a axis [5.7386 (3) Å]. At the same time, this is the shortest distance between nitroxide groups in the crystal structure of MTSSL. Due to space group symmetry, the NO groups of column A are consequently antiparallel to those of column A' and those of column B are antiparallel to those of column B'. The vectors of the N—O bonds in adjacent columns are nearly perpendicular to one another [80.38 (8)°]. It is interesting to observe that the unit cell metric is not far from tetragonal and that the molecules in related columns seem to be, in projection along the a axis, roughly related by a fourfold axis at, for example, $(x, \frac{1}{2}, 0)$ and $(x, 0, \frac{1}{2})$. The near perpendicularity of the N—O bonds in adjacent molecules is consistent with this idea of very approximate C_4 symmetry.

Intermolecular interactions are restricted mainly to van der Waals forces. No π–π interactions or classical hydrogen bonds are found, although there are some short intermolecular C—H···O contacts between O atoms and adjacent H atoms. The two shortest C—H···O contacts are O22A^{i}···H13 = 2.29 Å, O22A^{i}···C13 = 3.1683 (19) Å and O22A^{i}···H13—C13 = 153°, and O21$^{\mathrm{ii}}$···H162 = 2.25 Å, O21$^{\mathrm{ii}}$···C16 = 3.2385 (18) Å and O21$^{\mathrm{ii}}$···H162—C16 = 172° [symmetry codes: (i) $-x$, $1 - y$, $1 - z$; (ii) $-x$, $1 - y$, $-z$].

Experimental

The synthesis of MTSSL used for the crystallization was as described by its discoverer (Berliner *et al.*, 1982). For crystal growth, a saturated solution was prepared by diluting MTSSL (6.4 mg) in acetonitrile (Sigma–Aldrich; 12–13 μl). The solvent was allowed to evaporate overnight in a closed tube with a small opening at a temperature of 277 K. Large pale-yellow needles of MTSSL were obtained. A suitable single crystal was selected under a polarization microscope, cut to an appropriate size and mounted on a 400/25 μm Micro Mesh MiTeGen Micromount using Fromblin Y perfluoropolyether (LVAC 16/6, Aldrich).

Crystal data

$C_{10}H_{18}NO_3S_2$	$\gamma = 83.009$ (2)°
$M_r = 264.37$	$V = 1329.28$ (11) Å^3
Triclinic, $P\bar{1}$	$Z = 4$
$a = 5.7386$ (3) Å	Mo $K\alpha$ radiation
$b = 15.0677$ (6) Å	$\mu = 0.39$ mm^{-1}
$c = 15.4981$ (7) Å	$T = 100$ (2) K
$\alpha = 88.411$ (2)°	$0.32 \times 0.29 \times 0.25$ mm
$\beta = 88.550$ (2)°	

Data collection

Bruker APEXII diffractometer with a CCD area-detector	85640 measured reflections
Absorption correction: multi-scan (*SADABS*; Bruker, 2004)	7469 independent reflections
$T_{\min} = 0.884$, $T_{\max} = 0.909$	6384 reflections with $I > 2\sigma(I)$
	$R_{\mathrm{int}} = 0.035$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.035$	16 restraints
$wR(F^2) = 0.091$	H-atom parameters constrained
$S = 1.04$	$\Delta\rho_{\max} = 0.45$ e Å^{-3}
7469 reflections	$\Delta\rho_{\min} = -0.41$ e Å^{-3}
313 parameters	

In order to refine the disordered side chain of molecule 2, a common site-occupancy factor was refined for all atoms of each conformation, while the total site occupancy of the two components was constrained to unity. The anisotropic atomic displacement parameters of the corresponding atoms within the two different conformations were constrained to be equal. In addition, all chemically equivalent bond lengths (C—C, C—S, S—S and S—O) in the disordered region were restrained to common values. With the exception of the H atoms in the disordered part of molecule 2, all H atoms could be located in a difference Fourier map. In order to minimize the number of refined parameters, the positions of all H atoms were subsequently calculated using geometric criteria and constrained to ride on their parent atoms, with C—H = 0.95 (pyrroline), 0.99 (methylene) or 0.98 Å (methyl). For each independent molecule, a separate common isotropic displacement parameter was refined for the H atoms.

Data collection: *SMART* (Bruker, 2004); cell refinement: *SAINT* (Bruker, 2007); data reduction: *SAINT*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *DIAMOND* (Brandenburg, 1999); software used to prepare material for publication: *SHELXTL* (Sheldrick, 2008).

References

Allen, F. H., Kennard, O., Watson, D. G., Brammer, L., Orpen, A. G. & Taylor, R. (1987). *J. Chem. Soc. Perkin Trans 2*, pp. S1–19.

Altenbach, C., Marti, T., Khorana, H. G. & Hubbell, W. L. (1990). *Science*, **248**, 1088–1092.

Balog, M., Abé, C., Kalai, T., Steinhoff, H.-J., Jeko, J. & Hideg, K. (2007). *Synthesis*, pp. 1663–1670.

Balog, M., Kalai, T., Jekö, J., Berente, Z., Steinhoff, H.-J., Engelhard, M. & Hideg, K. (2003). *Tetrahedron Lett.* **44**, 9213–9217.

Balog, M., Kalai, T., Jekö, J., Steinhoff, H.-J., Engelhard, M. & Hideg, K. (2004). *Synlett*, **14**, 2591–2593.

Beier, C. & Steinhoff, H. J. (2006). *Biophys. J.* **91**, 2647–2664.

Berliner, L. J., Grunwald, J., Hankovszky, H. O. & Hideg, K. (1982). *Anal. Biochem.* **119**, 450–455.

Boeyens, J. C. A. & Kruger, G. J. (1970). *Acta Cryst.* B**26**, 668–672.

Bordignon, E. & Steinhoff, H. J. (2007). *Biological Magnetic Resonance*, Vol. 27, *ESR Spectroscopy in Membrane Biophysics*, edited by M. A. Hemminga & L. J. Berliner, pp. 129–164. Berlin: Springer.

Brandenburg, K. (1999). *DIAMOND*. Version 2.1c. Crystal Impact GbR, Bonn, Germany.

Bruker (2004). *SADABS* and *SMART*. Bruker AXS Inc., Madison, Wisconsin, USA.

Bruker (2007). *SAINT*. Version 7.34A. Bruker AXS Inc., Madison, Wisconsin, USA.

Budil, D. E., Sale, K. L., Khairy, K. A. & Fajer, P. G. (2006). *J. Phys. Chem. A*, **110**, 3703–3713.

DeSensi, S. C., Rangel, D. P., Beth, A. H., Lybrand, T. P. & Hustedt, E. J. (2008). *Biophys. J.* **94**, 3798–3809.

Dickman, M. H. & Doedens, R. J. (1983). *Inorg. Chem.* **22**, 1591–1594.

Duskova, J., Labsky, J., Hasek, J. & Cisarova, I. (2001). *Acta Cryst.* E**57**, o85–o86.

Fegy, K., Sanz, N., Luneau, D., Belorizky, E. & Rey, P. (1998). *Inorg. Chem.* **37**, 4518–4523.

Hirel, C., Vostrikova, K. E., Pécaut, J., Ovcharenko, V. I. & Rey, P. (2001). *Chem. Eur. J.* **7**, 2007–2014.

Hubbell, W. L., Mchaourab, H. S., Altenbach, C. & Lietzow, M. A. (1996). *Structure*, **4**, 779–783.

Niermann, N., Degefa, T. H., Walder, L., Zielke, V., Steinhoff, H. J., Onsgaard, J. & Speller, S. (2006). *Phys. Rev. B*, **74**, 235424.

Osiecki, J. H. & Ullman, E. F. (1968). *J. Am. Chem. Soc.* **90**, 1078–1079.

Papoutsakis, D., Kirby, J. P., Jackson, J. E. & Nocera, D. G. (1999). *Chem. Eur. J.* **5**, 1474–1480.

Porter, L. C. & Doedens, R. J. (1984). *Acta Cryst.* C**40**, 1814–1817.

Sezer, D., Freed, J. H. & Roux, B. (2008a). *J. Phys. Chem. B*, **112**, 5755–5767.

Sezer, D., Freed, J. H. & Roux, B. (2008b). *J. Chem. Phys.* **128**, 165106.

Sheldrick, G. M. (2008). *Acta Cryst.* A**64**, 112–122.

Steinhoff, H.-J., Mollaaghababa, R., Altenbach, C., Hideg, K., Krebs, M., Khorana, H. G. & Hubbell, W. L. (1994). *Science*, **266**, 105–122.

Turley, J. W. & Boer, F. P. (1972). *Acta Cryst.* B**28**, 1641–1644.

Wiley, D. W., Calabrese, J. C., Harlow, R. L. & Miller, J. S. (1991). *Angew. Chem. Int. Ed.* **30**, 450–452.