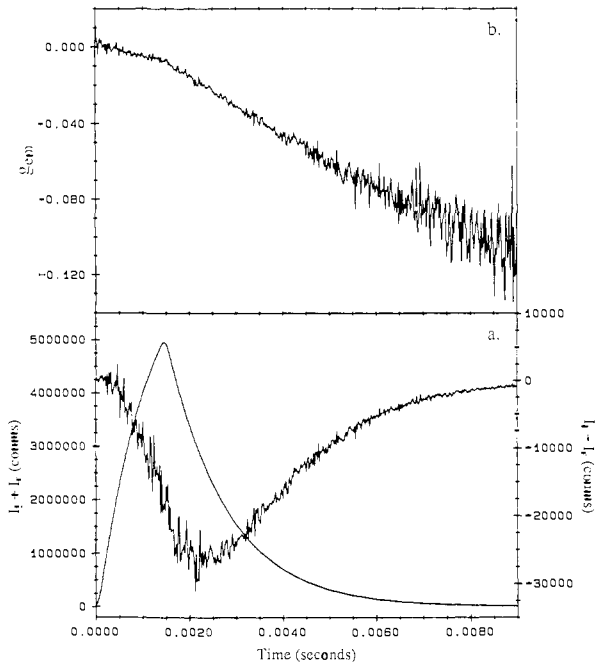


Figure 3. (a) Time-resolved circularly polarized luminescence (noisy trace, right scale) and total luminescence (smooth trace, left scale) plotted vs time for a solution 10 mM in $Tb(dpa)_3^{3-}$ and 5 μM in Λ-(+)-$Ru(phen)_3^{2+}$. Excitation wavelength 325 nm (He–Cd laser, chopped at 53 Hz), emission at 543.5 nm. The laser first excites the sample at time 0.0 and is chopped off at time 0.0016 s. The initial rise in the TL intensity reflects the kinetics of the emission. (b) Dissymmetry factor calculated from data in (a) plotted vs time.

Kagan et al.,[7] and Rau.[8] Kagan developed an equation expressing the enantiomeric excess as a function of time:

$$y = \frac{[S] - [R]}{[S] + [R]} = \tanh\,[\tfrac{1}{2}(k_S - k_R)t] \qquad (1)$$

Here, y is the enantiomeric excess, and k_S and k_R are the first-order rate constants for the photodestruction of the S and R enantiomers by the circularly polarized light. For our system, an analogous equation can be derived:

$$g_{em}(t) = g_{em}(\lim)\,\tanh\,[\tfrac{1}{2}(k_{\Lambda\Lambda} - k_{\Delta\Lambda})[Q]t] \qquad (2)$$

where $g_{em}(t)$ is the dissymmetry at time t, $g_{em}(\lim)$ is the limiting dissymmetry for fully resolved (Λ or Δ) $Tb(dpa)_3^{3-}$, [Q] represents the concentration of the resolved $Ru(phen)_3^{2+}$ quencher, and $k_{\Lambda\Lambda}$ and $k_{\Delta\Lambda}$ are the rate constants for quenching of the Λ-$Tb(dpa)_3^{3-}$ isomer by Λ-$Ru(phen)_3^{2+}$ and of the Δ-$Tb(dpa)_3^{3-}$ by Λ-$Ru(phen)_3^{2+}$, respectively. By fitting of the dissymmetry data in Figure 3b to eq 2, we derived approximate values for the rate constants of 1×10^8 and 2×10^8 M^{-1} s^{-1} and a limiting dissymmetry factor magnitude for the $Tb(dpa)_3^{3-}$ complex of 1.2×10^{-1}. We are currently uncertain as to which interaction (Λ–Λ or Δ–Λ) produces the larger quenching rate. However, the difference between the two rates demonstrates that the enantioselectivity in this quenching process is very large.

This induction of optical activity into a large population of (excited) racemic terbium complexes by a small, resolved population of ruthenium complexes results in a large amplification of the optical activity of the system. This, then, is a very sensitive probe of transition metal complex enantiomeric resolution. We are continuing to develop this probe and are using it to study the enantioselective binding of transition metal complexes to DNA.

Acknowledgment. This work was supported by the National Science Foundation (Grants CHE-8215815 and CHE-8600012), a NIH Biomedical Research Support Subgrant, and a post-doctoral fellowship to G.L.H. from Monsanto.

(7) Balavoine, G.; Moradpour, A.; Kagan, H. B. *J. Am. Chem. Soc.* **1974**, *96*, 5152.
(8) Rau, H. *Chem. Rev.* **1983**, *83*, 535.

Carbon-13 Spin System Directed Strategy for Assigning Cross Peaks in the COSY Fingerprint Region of a Protein

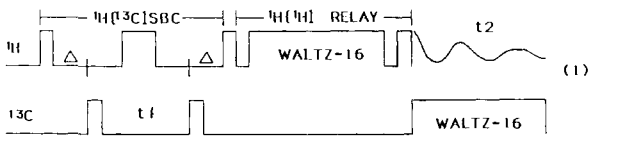
Byung-Ha Oh, William M. Westler, and John L. Markley*

Department of Biochemistry
College of Agricultural and Life Sciences
University of Wisconsin—Madison, 420 Henry Hall
Madison, Wisconsin 53706

Received November 14, 1988

We previously demonstrated that the ^{13}C spin systems of amino acids in proteins (uniformly labeled with ^{13}C to a level of about 30%) can be traced out and classified according to 18 different amino acid types by a single $^{13}C\{^{13}C\}$ double quantum correlation ($^{13}C\{^{13}C\}$DQC) experiment.[1-3] The remaining ambiguities of Glu = Gln and Asp = Asn can be resolved by means of a $^{13}C\{^{15}N\}$ single-bond correlation ($^{13}C\{^{15}N\}$SBC) experiment.[4] ^1H spin systems then can be elucidated by using $^1H\{^{13}C\}$ single-bond correlation ($^1H\{^{13}C\}$SBC) data to translate carbon assignments into assignments of directly bonded hydrogens.[5] In principle, data from these three experiments are sufficient for extensive identification of cross peaks in the ^1H COSY fingerprint region (recorded in ^1H_2O). In practice, however, overlaps of C^α or H^α resonances from different residues lead to ambiguities in such cross assignments. These ambiguities, which appear in $^1H\{^{13}C\}$SBC or ^1H COSY spectra, can be resolved by additional information that links the ^{13}C and ^1H spin systems through other scalar coupling pathways. We show here that $^1H\{^{13}C\}$ single-bond correlation with ^1H relay ($^1H\{^{13}C\}$SBC–^{1}HR) data, along with ^1H correlated relay (^1H RELAY)[6] data, provides such pathways to extensive residue-type identifications of [H^α,H^N] cross peaks in the COSY[7] fingerprint region. Such identifications are a prerequisite for sequential resonance assignments based on interresidue NOESY (nuclear Overhauser effect spectroscopy)[8] connectivities.[9] The protein sample studied was the oxidized form of ferredoxin (M_r = 11 000) from *Anabaena* 7120 (a photosynthetic cyanobacterium).

In this work, homonuclear Hartmann–Hahn mixing[10] was used to provide the ^1H relay in the $^1H\{^{13}C\}$SBC experiment. This approach differs from that designed by Brühwiler and Wagner, which incorporates an additional coherence transfer step.[11] The pulse sequence (1) used is[10]



The [H^α,(C^α,C^β)] connectivities from the $^1H\{^{13}C\}$SBC–^{1}HR spectrum (Figure 1B) can be correlated directly with [(C^α,C^β),$C^{\alpha+\beta}$] connectivities from the $^{13}C\{^{13}C\}$DQC spectrum (Figure 1A). Similarly, the [(H^α,H^β),C^α] connectivities from the $^1H\{^{13}C\}$SBC-^{1}HR spectrum (Figure 2A) can be related directly

(1) Westler, W. M.; Kainosho, M.; Nagao, H.; Tomonaga, N.; Markley, J. L. *J. Am. Chem. Soc.* **1988**, *110*, 4093–4095.
(2) Stockman, B. J.; Westler, W. M.; Darba, P.; Markley, J. L. *J. Am. Chem. Soc.* **1988**, *110*, 4095–4096.
(3) Oh, B.-H.; Westler, W. M.; Darba, P.; Markley, J. L. *Science* **1988**, *240*, 908–911.
(4) Westler, W. M.; Stockman, B. J.; Hosoya, Y.; Miyake, Y.; Kainosho, M.; Markley, J. L. *J. Am. Chem. Soc.* **1988**, *110*, 6256–6258.
(5) For a review, see: Griffey, R. H.; Redfield, A. G. *Q. Rev. Biophys.* **1987**, *19*, 51–82.
(6) Wagner, G. *J. Magn. Reson.* **1983**, *55*, 151–156.
(7) Nagayama, K.; Kumar, A.; Wüthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1980**, *40*, 321–334.
(8) Kumar, A.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1–6.
(9) Wüthrich, K. *NMR of Protein and Nucleic Acids*; Wiley: New York, 1986; pp 130–161.
(10) Rance, M. *J. Magn. Reson.* **1987**, *74*, 557–564.
(11) Brühwiler, D.; Wagner, G. *J. Magn. Reson.* **1986**, *69*, 546–551.

0002-7863/89/1511-3083$01.50/0

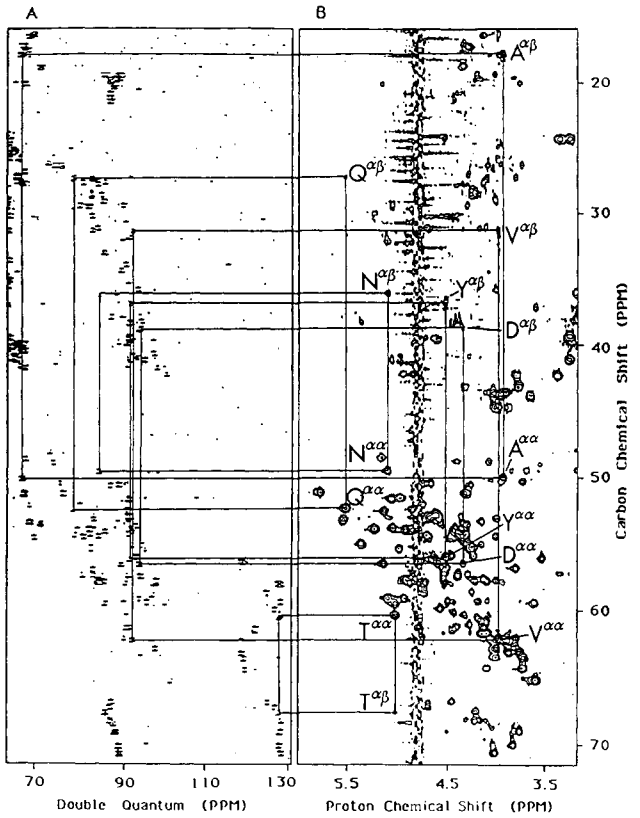


Figure 1. Selected regions of (A) the $^{13}C\{^{13}C\}$DQC spectrum and (B) the $^{1}H\{^{13}C\}$SBC–^{1}HR spectrum of oxidized [26% U-^{13}C]ferredoxin[13] from *Anabaena* 7120. The sample was 0.4 mL of 9.0 mM ferredoxin in $^{2}H_2O$ containing 50 mM phosphate buffer. The uncorrected pH meter reading was 7.5. [H^{α},(C^{α},C^{β})] connectivities for seven different amino acids in the $^{1}H\{^{13}C\}$SBC–^{1}HR spectrum are matched to [$C^{\alpha+\beta}$,(C^{α},C^{β})] connectivities in the $^{13}C\{^{13}C\}$DQC spectrum. Spectrum A was collected by using a Bruker 5-mm broad-band probe. 508 blocks of free induction decays (FIDs) were collected as 8192 data points; each represented the average of 512 transients. The experiment time was 92 h. Spectrum B was collected with a 5-mm inverse broad-band probe by using Bruker reverse electronics. WALTZ-16 ^{13}C decoupling[14] was used during acquisition to collapse ^{13}C–^{1}H splittings. 512 blocks of FIDs were collected as 4096 data points (each represented the average of 72 transients). The experiment time was 13 h.

to [(H^{α},H^{β}),H^{N})] connectivities from the ^{1}H RELAY spectrum (Figure 2B). For clarity, only selected connectivities are drawn in the figures. The $^{1}H\{^{13}C\}$SBC–^{1}HR spectrum also showed [(H^{α},H^{N}),C^{α}] connectivities from 11 slowly exchanging amide protons (data not shown) that can be readily correlated with [H^{α},H^{N}] cross peaks in the ^{1}H COSY spectrum (recorded in $^{2}H_2O$). At the Hartmann–Hahn mixing time used (15 ms), we did not observe cross peaks from ^{13}C and ^{1}H nuclei separated by more than two bonds. Some of the expected two-bond connectivities were not observed in the $^{1}H\{^{13}C\}$SBC–^{1}HR spectrum probably because of either small coupling constants or paramagnetic broadening of signals caused by the 2Fe·2S* center.[17]

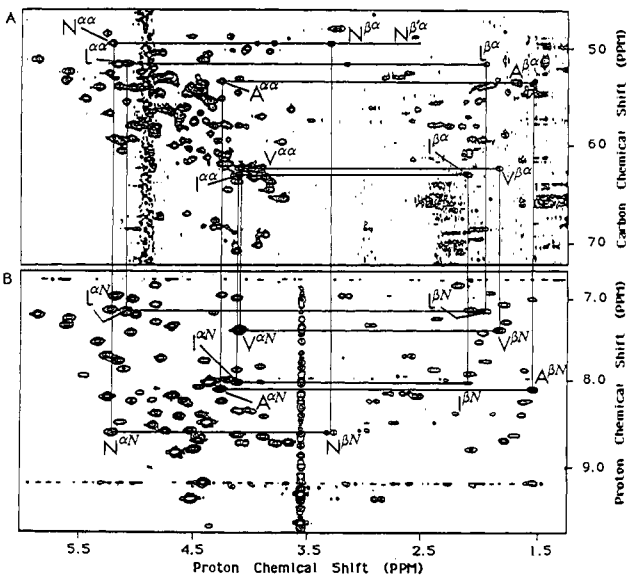


Figure 2. (A) Another region of the $^{1}H\{^{13}C\}$SBC–^{1}HR spectrum described in Figure 1. (B) The ^{1}H RELAY spectrum of unlabeled ferredoxin. [(H^{α},H^{β}),C^{α}] connectivities for different amino acids in the $^{1}H\{^{13}C\}$SBC–^{1}HR spectrum are matched to [(H^{α},H^{β}),H^{N})] connectivities in this spectrum. The sample was 0.5 mL of 9 mM ferredoxin in 90% $^{1}H_2O$/10% $^{2}H_2O$ containing 50 mM phosphate buffer at pH 7.1. The absolute value mode RELAY[6] spectrum was obtained with 8-step phase cycling.[15] 512 blocks of FIDs were collected as 2048 data points; each represented the average of 96 transients. The experiment time was 17 h.

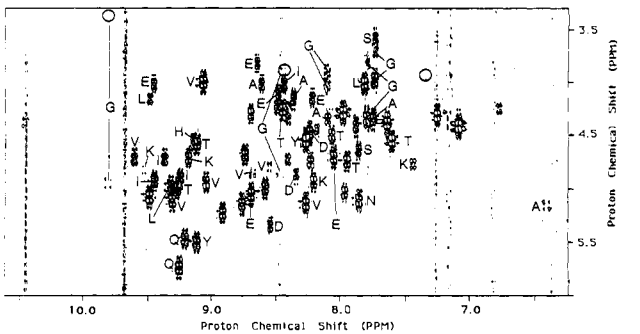


Figure 3. Fingerprint region of the double-quantum filtered COSY spectrum of the same sample described in Figure 2B. Solvent suppression was achieved by irradiation at the solvent frequency during the relaxation delay (1.2 s). Phase cycling for this experiment was as described in ref 16. 512 blocks of FIDs were collected as 2048 data points; each represented the average of 160 transients. The experiment time was 28 h. Classifications are designated by the one-letter code for amino acids. The circles indicate cross peaks visible at lower contour levels.

However, most of the ambiguities in [H^{α},C^{α}] or [H^{α},H^{N}] cross peak assignments were removed by using the $^{1}H\{^{13}C\}$SBC–^{1}HR data to eliminate all other assignment possibilities.

The data permitted first-order assignments of 56 of the 76 observed COSY fingerprint peaks (Figure 3) to 51 different residues (two peaks were identified for each of five glycines). The strategy presented here provides a more complete classification than could be achieved on the basis of ^{1}H spin systems alone. Only 33 of the 51 identified spin systems (Figure 3) would be distinguishable on the basis of the "8 ^{1}H spin system" classification, and only 37 of the 51 would be distinguishable with the "15 ^{1}H spin system" classification[9] that sometimes is difficult to obtain with larger or paramagnetic proteins.

Acknowledgment. We thank Dr. E. S. Mooberry for assistance with NMR instrumentation and Mr. B. J. Stockman for help in

(12) Similar information is provided by the $^{1}H\{^{13}C\}$ multiple-bond correlation ($^{1}H\{^{13}C\}$MBC) experiment.[18] In our experience, $^{1}H\{^{13}C\}$SBC–^{1}HR data provide much stronger cross peaks except for those arising from methyl groups.

(13) The method for ^{13}C enrichment of the protein was published in ref 3. All NMR experiments presented in this paper were carried out at 25 °C on a Bruker AM-500 spectrometer (500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C). Chemical shifts were referenced to internal (trimethylsilyl)propionate for ^{1}H and external tetramethylsilane for ^{13}C, where the resonance of external dioxane was taken to be at 67.8 ppm.

(14) Shaka, A. J.; Keeler, J.; Frenkiel, T.; Freeman, R. *J. Magn. Reson.* **1983**, *52*, 335–338.

(15) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *65*, 355–360.

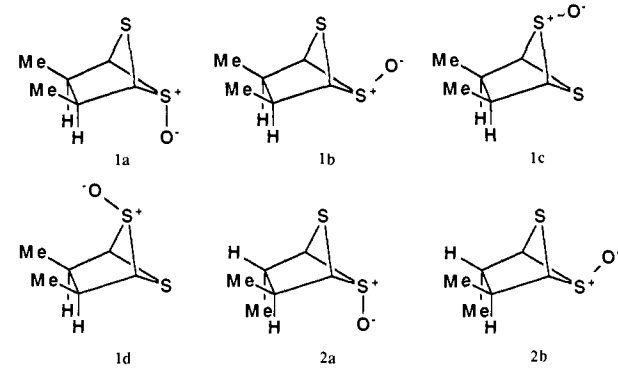
(16) Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479–485.

(17) Tsukihara, T.; Fukuyama, K.; Katsube, Y. In *Iron–Sulfur Protein Research*: Matsubara, H., Katsube, Y., Wada, K., Eds.; Japan Scientific Society Press: Tokyo, 1986; pp 59–68.

(18) Stockman, B. J.; Reily, M. D.; Westler, W. M.; Ulrich, E. L.; Markley, J. L. *Biochemistry* **1989**, *28*, 230–236.

preparing the protein samples. This work was supported by USDA Competitive Grant 88-37262-3406 and National Institutes of Health Grant RR02301 from the Biomedical Research Technology Program, Division of Research Resources. This study made use of the National Magnetic Resonance Facility at Madison, which is supported in part by Grant RR023021. Additional equipment in the facility was purchased with funds from the University of Wisconsin, the NSF Biological Biomedical Research Technology Program (Grant DMB-8415048), NIH Shared Instrumentation Program (Grant RR02781), and the U.S. Department of Agriculture. B.H.O. is supported by a Peterson Fellowship from the University of Wisconsin—Madison.

Scheme I



Zwiebelanes: Novel Biologically Active 2,3-Dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-Oxides from Onion

Thomas Bayer[1a] and Hildebert Wagner*

Institute of Pharmaceutical Biology, University of Munich 8000 Munich 2, Federal Republic of Germany

Eric Block,* Serge Grisoni, and Shu Hai Zhao

Department of Chemistry State University of New York at Albany Albany, New York 12222

Andras Neszmelyi

Central Research Institute for Chemistry of the Hungarian Academy of Sciences H-1020 Budapest, Hungary

Received November 21, 1988

A variety of remarkable low molecular weight cyclic and acyclic organosulfur compounds has been isolated from extracts and essential oils of onion (*Allium cepa*) and garlic (*Allium sativum*) and have been shown to contain C_3, C_6, or C_9 units derived from the stable precursors *trans*-(+)-*S*-1- or (+)-*S*-2-propenyl L-cysteine sulfoxide, respectively.[1b-g] In connection with the search for antiasthmatic agents from onion[2] we have discovered two isomeric biologically active compounds of formula $C_6H_{10}OS_2$ which we name zwiebelane A and B (**1** and **2**, respectively).[3] We present evidence that **1** and **2** are, respectively, *cis*- and *trans*-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxides and that they originate from 1-propenesulfenic acid (**3**). We also report a mechanistically based, stereospecific one-step synthesis of **1** and **2**.

Allium cepa bulbs were peeled and chopped and, after ca. 30 min, squeezed to give onion juice, which was extracted with chloroform. The concentrated extract was then subjected (sequentially) to flash chromatography (C-18 silica gel, methanol; to remove triterpenes), chromatography on a Chromatotron (silica gel, chloroform), column chromatography (silica gel, 5:1 toluene–ethyl acetate), and finally HPLC (silica gel, 100:1 methylene chloride:acetone) affording **1**, **2**, and thiosulfinates (*E*,*Z*)-RS(O)SCH=CHCH$_3$ and RS(O)SR′ (R and R′ = Me or *n*-Pr), among other compounds.[2c,d] Compound **1** is a colorless oil of formula $C_6H_{10}OS_2$ (elemental analysis[4a] and CI- and EI-MS; prominent EI-MS fragment ions at *m*/*e* 99 and 113[4b]) with intense IR bands at 1065 and 1085 cm^{-1} (S=O) [UV λ_{max} 250 nm; ^{1}H NMR ($CDCl_3$)[4c] δ 4.12 (H_A, $J_{AA'}$ = 6.7, J_{AB} = 0.9 Hz, 2 H, CHS_2), 2.92 (H_B, J_{BC} = 6.8, $J_{BC'}$ = 0.3, $J_{BB'}$ = 5.8 Hz, 2 H, $CHCH_3$), 1.17 (H_C, 6 H, CH_3); ^{13}C NMR δ 79.5 (CH), 33.3 (CH), 12.6 (CH_3)]. Compound **2**, present in smaller amounts, also has formula $C_6H_{10}OS_2$ by MS [^{1}H NMR ($CDCl_3$)[4c] δ 4.25 (H_A, $J_{AA'}$ = 6.65, J_{AB} = 0.9 Hz, 1 H, CHS_2), 4.21 ($H_{A'}$, $J_{A'B'}$ = 1.1 Hz, 1 H, CHS_2), 2.85 (H_B, $J_{BB'}$ = 4.0, J_{BC} = 6.7 Hz, 1 H, $CHCH_3$), 2.33 ($H_{B'}$, $J_{B'C'}$ = 7.3 Hz, 1 H, $CHCH_3$), 1.45 ($H_{C'}$, d, 3 H, CH_3), 1.37 (H_C, d, 3 H, CH_3); ^{13}C NMR δ 79.4, 77.7, 48.0, 39.4 (CH), and 15.7, 14.2 (CH_3)]. On the basis of the above spectroscopic data we propose that **1** and **2** are, respectively, *cis*- and *trans*-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide. The mixture of **1** and **2** showed a 65–90% inhibition of thrombin-induced TXB_2 biosynthesis in human platelet rich plasma at a concentration of 0.1–1.0 mg/mL.[4d]

Four distinct isomers of **1** and *two* isomers of **2** are possible, namely **1a–d** and **2a,b** (see Scheme I)[5] although only one isomer each of **1** and **2** is observed in this work. On the basis of Eu(fod)$_3$ shift reagent and aromatic solvent induced shift studies[7] we propose that **1** and **2** have the respective structures (1α, 2α, 3α, 4α, 5β)- and ($\pm$)-(1α, 2α, 3β, 4α, 5β)-2,3-dimethyl-5,6-dithiabicyclo-[2.1.1]hexane 5-oxide (structures **1a** and **2a**, respectively). The 5,6-dithiabicyclo[2.1.1]hexane ring system, a bicyclic derivative of the well-studied 1,3-dithietane ring system,[6] has not been previously reported although the related, strained[8a] 5-thiabicyclo[2.1.1]hexane system[8b] is known.

(1) (a) Present address SUNY-Albany. (b) Block, E.; Penn, R. E.; Revelle, L. K. *J. Am. Chem. Soc.* **1979**, *101*, 2200. (c) Block, E.; Revelle, L. K.; Bazzi, A. A. *Tetrahedron Lett.* **1980**, *21*, 1277. (d) Block, E.; Bazzi, A. A.; Revelle, L. K. *J. Am. Chem. Soc.* **1980**, *102*, 2490. (e) Block, E.; Ahmad, S.; Jain, M. K.; Crecely, R. W.; Apitz-Castro, R.; Cruz, M. R. *J. Am. Chem. Soc.* **1984**, *106*, 8295. (f) Block, E. *Sci. Am.* **1985**, *252*, 114. (g) Block, E.; Ahmad, S.; Catalfamo, J.; Jain, M. K.; Apitz-Castro, R. *J. Am. Chem. Soc.* **1986**, *108*, 7045. (h) Block, E.; Iyer, R.; Grisoni, S.; Saha, C.; Belman, S.; Lossing, F. P. *J. Am. Chem. Soc.* **1988**, *110*, 7813 and references therein.

(2) (a) Dorsch, W.; Ettl, M.; Hein, G.; Scheftner, P.; Weber, J.; Bayer, T.; Wagner, H. *Int. Arch. Allergy Appl. Immun.* **1987**, *82*, 535. (b) Dorsch, W.; Adelmann-Grill, B.; Bayer, T.; Ettl, M.; Hein, G.; Jaggy, H.; Ring, J.; Scheftner, P.; Wagner, H. *Allergologie* **1987**, *10*, 316. (c) Dorsch, W.; Wagner, H.; Bayer, T.; Fessler, B.; Hein, G.; Ring, J.; Scheftner, P.; Sieber, W.; Strasser, T.; Weiss, E. *Biochem. Pharmacol.*, in press. (d) Bayer, T. Ph.D. Dissertation, University of Munich, 1988. (e) Bayer, T.; Wagner, H.; Wray, V.; Dorsch, W. *Lancet* **1988**, 906. (f) Wagner, H.; Dorsch, W.; Bayer, T.; Breu, W.; Willer, F., submitted for publication. (g) Kawakishi, S.; Morimitsu, Y. *Lancet* **1988**, 330.

(3) "Zwiebel" is German for onion.

(4) (a) Anal. Calcd for $C_6H_{10}OS_2$: C, 44.4; H, 6.2; O, 9.9; S, 39.5. Found: C, 44.5; H, 6.1; O, 9.3; S, 38.2. (b) High resolution EI-MS: 113.0429 corresponding to C_6H_9S. (c) The ^{1}H NMR spectra of **1a** and **2a** are not first order and were therefore interpreted through LAOCOON III analysis of the 10 spin systems; full details will be given elsewhere. Coupling constants are in excellent agreement with those determined for isomers of 2-bromo-5-thiabicyclo[2.1.1]hexane and its 5-oxide: Naganathan, S.; Block, E., unpublished results. (d) Dorsch, W., Wagner, H., private communication.

(5) (a) According to the Cahn–Ingold–Prelog convention **1a–d**, **2a**, and **2b** are named (1α, 2α, 3α, 4α, 5β)-, (1α, 2α, 3α, 4α, 5α)-, (1α, 2β, 3β, 4α, 5α)-, (1α, 2β, 3β, 4α, 5β)-, ($\pm$)-(1α, 2α, 3β, 4α, 5α), and ($\pm$)-(1α, 2α, 3β, 4α, 5β)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide, respectively. (b) Oxygen assumes an equatorial position in 1,3-dithietane 1-oxide itself[6] which would correspond to the oxygen orientation in **2a**.

(6) Block, E.; Corey, E. R.; Penn, R. E.; Renken, T. L.; Sherwin, P. F.; Bock, H.; Hirabayshi, T.; Mohmand, S.; Solouki, B. *J. Am. Chem. Soc.* **1982**, *104*, 3119.

0002-7863/89/1511-3085$01.50/0