Stereostructure of Pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine

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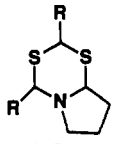
A bicyclic dithiazine with the estimated structure of pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine (I) was synthesized from ethanal, hydrogen sulfide, and 4-aminobutanal. The steric configuration of the structure was confirmed as 2*R*,4*R*,8*S* or its mirror image by an X-ray crystal structure analysis, which is the first determination of the most stable conformation of the dithiazine compounds. All of the spectral data and the KI value on a capillary column for synthesized I coincided well with those of the natural product. Almost every chemical shift in ^1H and ^{13}C NMR was also clearly assigned, in consideration of the results from the 2D-COSY method and the X-ray crystal structure analysis.

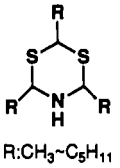
INTRODUCTION

In a preceding paper (Kubota et al., 1988), we found a new bicyclic dithiazine compound in the aroma concentrate of cooked small shrimp and estimated its structure as pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine (I). Some 5,6-dihydro-1,3,5-dithiazines (II), which have methyl,



R:CH_3

I



R:CH_3~C_5H_{11}

II

ethyl, propyl, isopropyl, butyl, or pentyl groups as the substituents on the ring structure, have been found in various cooked volatiles of food such as beef broth, krill, small shrimp, and dried squid (Kawai and Ishida, 1989). As the formation mechanism has been discussed in model reactions (Kawai et al., 1985), these monocyclic dithiazines could be prepared from mixtures of H_2S, ammonia, and the corresponding aliphatic aldehydes. However, no bicyclic dithiazine has been reported in these reaction products. This fact reveals that compound I is produced by different materials from those for compound II. In this paper, we used 4-aminobutanal instead of ammonia as the nitrogen source for compound I, which was synthesized by the condensation of H_2S and ethanal with 4-aminobutanal under the same conditions as those used for compounds II (Kawai et al., 1985). Further, since alkyl-substituted 5,6-dihydro-1,3,5-dithiazines have some asymmetric carbon atoms in the ring, several stereoisomers are possible. However, it seems that the most stable racemic isomer is generally produced as the major product, and only one of the stereoisomers has actually been reported in food volatiles. Kawai (Kawai and Ishida, 1989) also showed that minor isomers were contained at less than 10% of the corresponding major one in model reactions. Compound I found in the volatile of small shrimp also showed one major peak on the gas chromatogram and seems to have the most stable configuration for the bicyclic dithiazine structure. Although the importance of stereostructure for the quality of an aroma is now appreciated, the configuration and conformation of dithiazines have not been clearly established. Butenko only calculated the dipole moment and molar Kerr constant of 2,4,6-trimethyl-5,6-dihydro-1,3,5-dithiazine and estimated that the 2-, 4-, and 6-methyl groups were oriented in the axial, equatorial, and equatorial conformations, respectively, in the boat form (Butenko et al., 1972), although this has not been directly confirmed. This paper reports the stereostructure of synthesized compound I as confirmed by an X-ray crystal structure analysis and by NMR analyses using the 2D-COSY method (Dybowski and Lichter, 1987). A few revisions to the assignment of ^{13}C NMR spectra in the previous paper (Kubota et al., 1988) are also described.

EXPERIMENTAL PROCEDURES

Synthetic Method. *1-Pyrroline.* Commercially available 4-aminobutanal dimethyl ether (6.8 g, 0.05 mol) was hydrolyzed in 15 mL of a 12% HCl solution, before the Cl^- ion was removed by an anion-exchange resin (Amberlight 410). The aqueous solution was extracted with diethyl ether and dried over sodium sulfate, and a colorless oil was obtained by removing the diethyl ether. The main product was 1-pyrroline (0.38 g, 11.0% yield) contained as 99.6% on the gas chromatogram. Spectral data are as follows: MS *m/z* (relative intensity, %) 69 (M^+, 65), 68 (35), 42 (43), 41 (100); IR (smear, cm^{-1}) 1610–1690 (C=N).

Pyrrolidino[1,2-e]-4H-2,4-dimethyl-1,3,5-dithiazine. Ethanal (2.65 g, 0.06 mol) and 1-pyrroline (2.1 g, 0.03 mol) were dissolved in 50 mL of water at about 0 °C, and H_2S gas was bubbled moderately through the mixture with stirring until almost all the theoretical volume (0.06 mol, 1300 mL) had been consumed. After continuing to stir for 30 min, the reaction mixture was stored overnight at room temperature. The organic layer was extracted with diethyl ether, and the basic fraction was fractionated and distilled in the temperature range 81–85 °C (3.5 mmHg). The distillate of colorless oil crystallized at −15 °C, and repeated recrystallization from methanol or ethanol gave 1.09 g (0.006 mol) of colorless needles (18.6% yield): mp 40.5 °C; IR (KBr, cm^{-1}) 2960, 1460, 1380, 1185, 1020; high-resolution MS 189.0663 for $C_{18}H_{15}NS_2$, calcd as 189.0646; MS *m/z* (relative intensity, %) 189 (M^+, 12.5), 129 (12.7), 97 (19.7), 95 (26.4), 94 (26.1), 70 (40.6), 69 (53.6), 60 (100), 59 (96.1), 45 (65.3), 41 (91.7), 34 (12.8), 27 (34.7).

Crystal Structure Determination. A colorless platelike crystal with dimensions of 0.4 × 0.3 × 0.1 mm was mounted on a Rigaku AFC-5R diffractometer. The crystal was cooled to 223 K by the nitrogen gas flow method, since the melting point of the crystal was 40.5 °C. Systematic absences were $h = 2n + 1$ for $h00$, $k = 2n + 1$ for $0k0$, and $l = 2n + 1$ for $00l$. Cell parameters

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were refined by a least-squares fit for 25 reflections within the range $20 < 2\theta < 25°$. Crystal data: $M_r = 189.35$, orthorhombic, $P2_12_12_1$, $a = 9.863$ (5) Å, $b = 11.559$ (4) Å, $c = 8.794$ (3) Å, $V = 1002.5$ (7) Å^3, $Z = 4$, $d_x = 1.234$ g cm^{-3}.

Intensity data were collected up to $2\theta = 55°$, an $\omega - 2\theta$ scanning mode and scanning width of $\omega = (1.0 + 0.35 \tan\theta)°$ being applied. Background data were counted for half of the peak scanning time at both ends of the scan. Three standard reflections were recorded after every 100 reflections, and no significant intensity variations were observed. A total of 1097 reflections were collected at the rate of $32°(2\theta)$ min^{-1}, of which 448 were treated as significant ($|F_o| > 3\sigma(|F_o|)$). The data were corrected for Lorentz and polarization factors but not for absorption.

The structure was determined by using TEXSAN from the Rigaku–MSC software system (Molecular Structure Corp., 1985). Most of the hydrogen atoms were located on the difference map, and the others were treated geometrically, their isotropic temperature factors being fixed at 4.0 Å^2. The non-hydrogen atoms were refined by anisotropic temperature factors. Maximum Δ/σ and $\Delta\rho$ values in the final refinement were 0.20 and 0.2/e Å^{-3}, respectively, and final R and R_w values were 0.041 and 0.039, respectively. The computation was carried out on a MicroVaxII computer.

Instrumental Analyses. Infrared (IR) spectra were recorded on a Jasco IRA-1 instrument. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a JEOL JN-GX270 (270 MHz) instrument. Each sample was dissolved in $CDCl_3$, with tetramethylsilane as an internal standard. Low- and high-resolution mass spectra were measured by using the electron impact method (at 70 eV) with a JEOL JMS-DX 300 instrument. Kovats indices (KI) were obtained under the following conditions: gas chromatography column, 50 m × 0.25 mm (i.d.) PEG 20M chemically bonded fused silica WCOT type; carrier gas, nitrogen at 1.0 mL/min; oven temperature, 60 °C (4-min hold) programmed at 2 °C/min up to 180 °C; injection and detector temperature, 200 °C.

RESULTS AND DISCUSSION

Since it was not easy to isolate 4-aminobutanal from the reaction mixture because of its water solubility, we obtained 1-pyrroline, which is an equilibrium compound of 4-aminobutanal, and confirmed the structure by comparing the spectral data with those in the literature (Yoshikawa et al., 1965). An aqueous solution of purified 1-pyrroline, hydrogen sulfide, and ethanal produced one major compound without the formation of any monocyclic dithiazines. The spectral data of the synthesized compound coincided well with those of the natural product isolated from cooked small shrimp in a previous paper (Kubota et al., 1988), although the NMR data will be discussed further in a later section. The retention indices (KI) of the main reaction product and natural product also showed very good agreement at 1992 and 1993, respectively. From these facts, it seems that compound I was efficiently prepared by using 4-aminobutanal and, in addition, synthetic I had the same steric configuration as the natural compound. Since synthetic compound I could be recrystallized as colorless needles, an X-ray crystal structure analysis was performed to confirm the structure and to investigate the configuration.

Crystal and Molecular Structure (See Supplementary Material). The final atomic coordinates are given in Table I, and the molecular structure with the numbering of the atoms is shown in Figure 1. The configuration is 2*R*,4*R*,8*S* or its mirror image. The bond distances and angles and torsion angles are listed in Tables II and III, respectively. The six-membered ring takes a chair conformation, three hydrogen atoms being located in the axial positions and two methyl groups taking the equatorial. The crystal structure is shown in Figure 2, there being no unusually short contact between the molecules.

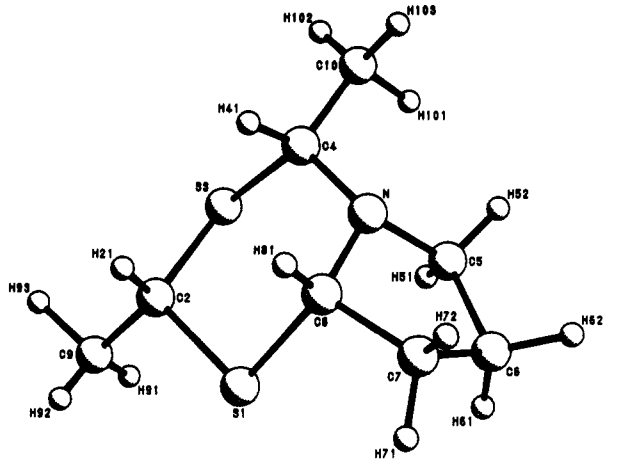


Figure 1. Molecular structure of pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine (I) with the numbering of the atoms.

Table I. Final Atomic Coordinates of Non-Hydrogen Atoms with Equivalent Temperature Factors for Compound I

atom	x	y	z	B_{eq}, Å^2
S(1)	0.8858 (3)	0.0988 (2)	0.5718 (4)	3.6
S(3)	0.8829 (3)	0.3020 (3)	0.3558 (3)	3.6
N	0.8093 (8)	0.3216 (8)	0.659 (1)	2.3
C(2)	0.843 (1)	0.149 (1)	0.381 (1)	3.1
C(4)	0.779 (1)	0.366 (1)	0.511 (1)	2.7
C(5)	0.942 (2)	0.350 (1)	0.725 (2)	5.1
C(6)	0.955 (2)	0.266 (1)	0.864 (2)	6.0
C(7)	0.828 (1)	0.187 (1)	0.851 (2)	4.6
C(8)	0.786 (1)	0.202 (1)	0.688 (1)	3.8
C(9)	0.926 (1)	0.080 (1)	0.266 (2)	4.5
C(10)	0.795 (2)	0.496 (1)	0.502 (1)	4.7

Table II. Bond Distances (Angstroms) and Angles (Degrees) for Compound I

S1–C2	1.82 (1)	N–C8	1.42 (1)
S1–C8	1.85 (1)	C2–C9	1.53 (2)
S3–C2	1.83 (1)	C4–C10	1.51 (1)
S3–C4	1.86 (1)	C5–C6	1.56 (2)
N–C4	1.43 (1)	C6–C7	1.55 (2)
N–C5	1.47 (2)	C7–C8	1.50 (2)
C2–S1–C8	100.4 (6)	S3–C4–C10	108.0 (1)
C2–S3–C4	100.1 (5)	N–C4–C10	112.0 (1)
C4–N–C5	118.0 (1)	N–C5–C6	104.0 (1)
C4–N–C8	118.2 (9)	C5–C6–C7	104.0 (1)
C5–N–C8	107.0 (1)	C6–C7–C8	103.0 (1)
S1–C2–S3	111.8 (7)	S1–C8–N	116.2 (9)
S1–C2–C9	109.0 (8)	S1–C8–C7	108.0 (1)
S3–C2–C9	108.0 (9)	N–C8–C7	103.0 (1)
S3–C4–N	114.6 (8)		

NMR of Compound I. The result of the X-ray crystal structure analysis demonstrated that the six-membered ring exists in a chair form with all of three methyne protons in the axial position, and these facts are in good agreement with the results of an NOE experiment in an NMR analysis (Kubota et al., 1988). More detailed assignments of each chemical shift in the ^{1}H and ^{13}C NMR spectra for compound I were carried out by combining the results of 2D ^{1}H–^{1}H and ^{1}H–^{13}C NMR experiments, using the torsion angles (Table III) of each proton on the five-membered ring carbons. The 2D ^{1}H–^{1}H and ^{1}H–^{13}C NMR spectra of compound I are shown in Figures 3 and 4, respectively. By ^{1}H NMR, the methyl and methylene protons were easily assigned by comparing the chemical shifts with those of compounds II (Kubota et al., 1980). Three groups of methylene protons in the five-membered ring system were clearly separated on the NMR spectra, although it was not easy to assign each signal on one-dimensional spectra. A homonuclear chemical shift correlation diagram proved

Table III. Selected Torsion Angles[a] (Degrees)

S1-C2-S3-C4	-57.7 (7)	C5-N-C4-C10	-55.0 (2)
S1-C8-N-C4	62.0 (1)	C5-N-C8-C7	44.0 (1)
S1-C8-N-C5	-74.0 (1)	C5-C6-C7-C8	19.0 (1)
S1-C8-C7-C6	85.0 (1)	C6-C5-N-C8	-31.0 (1)
S3-C2-S1-C8	56.1 (7)	C8-S1-C2-C9	175.6 (9)
S3-C4-N-C5	68.0 (1)	C8-S1-C2-H21	-61.0 (6)
S3-C4-N-C8	-63.0 (1)	C8-N-C4-C10	174.0 (1)
N-C4-S3-C2	57.2 (9)	C8-N-C4-H41	44.0 (7)
N-C5-C6-C7	6.0 (1)	H51-C5-C6-H61	-2.0 (9)
N-C8-S1-C2	-54.0 (1)	H51-C5-C6-H62	117.0 (9)
N-C8-C7-C6	-38.0 (1)	H52-C5-C6-H61	-119.0 (9)
C2-S1-C8-C7	-170.0 (9)	H52-C5-C6-H62	1.0 (9)
C2-S3-C4-C10	-177.0 (1)	H61-C6-C7-H71	27.0 (10)
C2-S3-C4-H41	-59.0 (6)	H61-C6-C7-H72	140.0 (13)
C4-S3-C2-C9	-177.7 (8)	H62-C6-C7-H71	-105.0 (8)
C4-S3-C2-H21	52.0 (8)	H62-C6-C7-H72	8.0 (11)
C4-N-C5-C6	-167.0 (1)	H71-C7-C8-H81	95.0 (10)
C4-N-C8-C7	180.0 (1)	H72-C7-C8-H81	-26.0 (12)

[a] The sign is positive if, when looking from atom 2 to atom 3, a clockwise motion of atom 1 would superimpose it on atom 4.

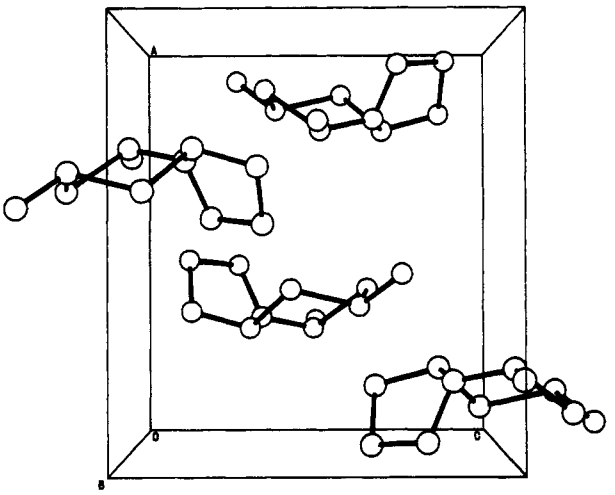


Figure 2. Crystal structure viewed along the *b* axis of compound I.

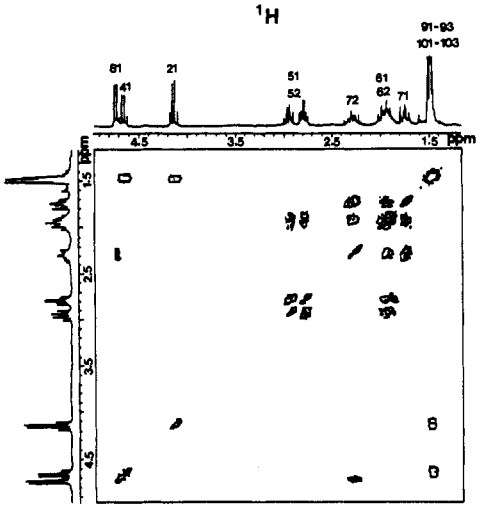


Figure 3. ^1H–^1H chemical shift correlation map (COSY) for compound I.

to be particularly helpful for the interpretation. As H61 and H62 were coupled to four adjacent protons, a complex multiplet signal at 1.84–1.97 ppm was assigned to them. Two multiplet signals of around 1.72 and 2.27 ppm and another two of around 2.76 and 2.92 ppm were divided into two methylene protons, respectively, because they had the same connectivity to each other on the diagram. H81 (4.70 ppm, d) only correlated with the signal at around

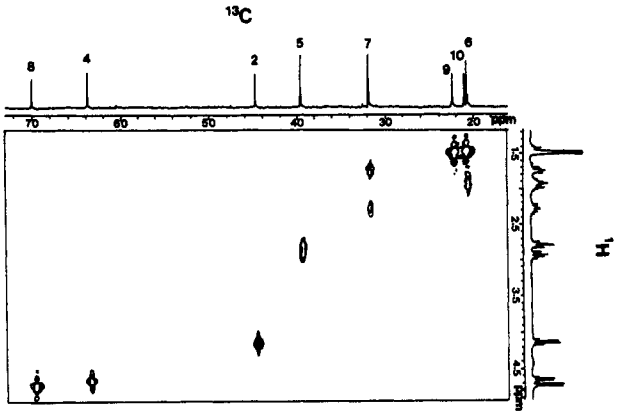
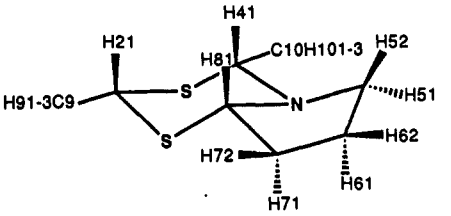


Figure 4. ^1H–^{13}C chemical shift correlated diagram for compound I.

Table IV. ^1H and ^{13}C NMR Chemical Shifts for Compound I



^{13}C			^1H	
ppm	group	assign.	ppm	assign.
70.04	CH	C8	4.70 (d)	H81
63.77	CH	C4	4.63 (q)	H41
44.87	CH	C2	4.12 (q)	H21
39.57	CH_2	C5	2.72–2.81	H51,52
			2.87–2.97	
31.84	CH_2	C7	1.67–1.76	H71
			2.20–2.34	H72
22.37	CH_3	C9	1.47 (d)	H91–93
21.00	CH_3	C10	1.45 (d)	H101–103
20.72	CH_2	C6	1.84–1.97	H61–62

2.27 ppm, which belongs to one of the adjacent protons, H71 or H72. This indicates that either H71 or H72 did not have any coupling with H81. On the other hand, the conformation angle between H81 and H71 was 95°, and that between H81 and H72 was −26° (Table III). Although the results of the crystal structure analysis could not be directly applied to the NMR solution, it is thought that H71 and H81 were almost at right angles to each other in the NMR solution because of the absence of coupling. Therefore, the chemical shifts at around 2.27 and 1.72 ppm on the ^1H NMR spectra were assigned to H72 and H71, respectively. A residual pair of chemical shifts at 2.76 and 2.92 ppm were assigned to H51 and H52. No further details to distinguish between each proton were obtained for this paper. In Figure 3, the following spin–spin interactions were observed: H101–103 (1.45 ppm, d) and H91–93 (1.47 ppm, d) coupled to the signals at 21.00 and 22.37 ppm, respectively. Therefore, these two signals by ^{13}C NMR could be assigned to the corresponding methyl carbons, whose assignments were interchanged in a previous paper (Kubota et al., 1988). The final assignments of ^1H and ^{13}C NMR data for compound I are summarized in Table IV.

As a five-membered ring structure is more unstable than a six-membered ring, it was difficult to determine the chemical shifts of each proton on the five-membered ring. In this paper, the X-ray crystal structure analysis has been shown to be very useful for a clear assignment of the NMR signals.

The most stable conformation of a novel bicyclic dithiazine, pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine, in the volatiles of cooked small shrimp was confirmed for the first time. This conformation could be applicable to some other dithiazines (II).

It seems that this bicyclic dithiazine (I) is also a secondary product during the cooking of some proteinous food and is similar to other dithiazines (II). The existence of intermediate material will be reported in another paper.

Supplementary Material Available: Final atomic parameters for hydrogen atoms and anisotropic temperature factors for non-hydrogen atoms (2 pages); F_o–F_c tables (5 pages). Ordering information is given on any current masthead page.

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