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Syntheses of Althiomycin Analogs in Relation to Antibacterial Activities[1)]

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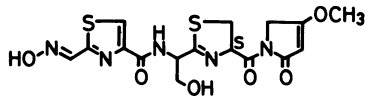
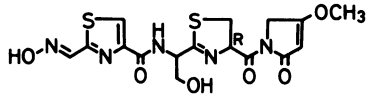
Four analogs of an antibiotic althiomycin were synthesized in order to clarify the structure-activity relationships, especially with regard to two asymmetric centers and the aldoxime configuration. The antibiotic activity of de(hydroxymethyl) analog is comparable to that of the original antibiotic for Gram-positive bacteria.

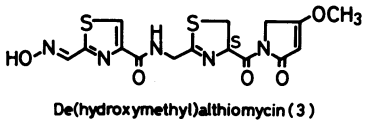
Althiomycin (**1**), isolated from *Streptomyces althioticus*,[2)] inhibits the protein synthesis in both Gram-positive and negative bacteria. Recently, we achieved the total synthesis of this antibiotic.[3)] For investigation of the structure-reactivity relationships of althiomycin, we synthesized the following analogs (**2**—**5**) (Fig. 1).

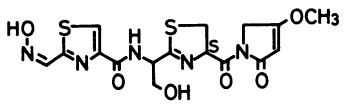
First of all, the enantiomer of althiomycin, i.e., epialthiomycin (**2**), was synthesized according to the synthetic pathway of an original antibiotic (Fig. 2).

Secondly, de(hydroxymethyl) analog (**3**) was prepared from 1-[(*S*)-2-(benzyloxycarbonylaminomethyl)-2-thiazolin-4-yl-carbonyl]-4-methoxy-3-pyrrolin-2-one (**10**) which was the key intermediate of our synthetic study on althiomycin[3)] (Fig. 3). The


Althiomycin (1)


Epialthiomycin (2)


De(hydroxymethyl)althiomycin (3)


(Z)-Althiomycin (4)

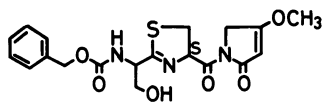

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Fig. 1.

benzyloxycarbonyl (*Z*) group of this compound was removed with anhydrous hydrogen fluoride, followed by the coupling with the thiazole part by the azide method using potassium carbonate as the base.

Finally, we attempted to achieve the total synthesis of (*Z*)-althiomycin, which is the isomer of the natural (*E*)-althiomycin with respect to the aldoxime. (Fig. 4) The thiazole part of this compound was prepared from ethyl (*Z*)-2-(hydroxyiminomethyl)-4-thiazolecarboxylate (**11**) via successive procedures of conversion to the hydrazide with hydrazine and azidation with nitrite ester. The compound thus obtained was coupled with the thiazoline-pyrrolinone part (**5**)[3)] to give no desired product, but its bisanhydro derivative in very low yield. Therefore, we then tried to convert the natural althiomycin into its stereoisomer with anhydrous hydrogen fluoride at room temperature. Since this product was a mixture of (*E*)- and (*Z*)-althiomycins (about 1:1), the separation was performed with high-performance liquid chromatography (HPLC). The desired compound thus obtained gives two singlets at δ 8.59 and 8.09 which can be assigned as signals of the proton on C-5 in the thiazole ring and the methine proton of the aldoxime, while the original (*E*)-althiomycin gives the corresponding singlets at δ 8.37 and 8.35 in NMR respectively.[4)]

The antibacterial activities of these analogs, including the synthetic intermediate **5** of althiomycin, were shown in Table 1. This result suggests the following conclusions. The absence of the hydroxymethyl group attached to the adjacent carbon of C-2 in the thiazoline ring has no influence on the antibacterial activity of the parent antibiotic, while the inversion of the configuration of C-4 in the thiazoline ring or the aldoxime results in a significant decrease of the biological activity. The replacement of the thiazole part by benzyloxycarbonyl group gives the total loss of the antibiotic activity.

Experimental

All melting points are uncorrected. The ^{1}H NMR spectra were obtained with a Varian XL-100-15 NMR spectrometer, a JEOL FX-90Q NMR spectrometer, and a JEOL JNM-PMX 60 NMR spectrometer. The chemical shifts are given in δ, with tetramethylsilane (TMS) as the internal standard.

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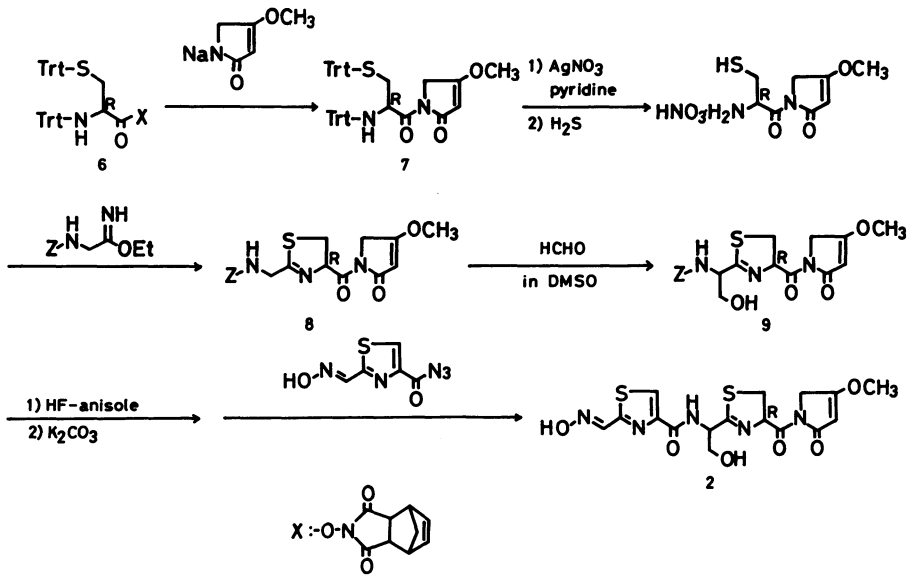

Fig. 2.

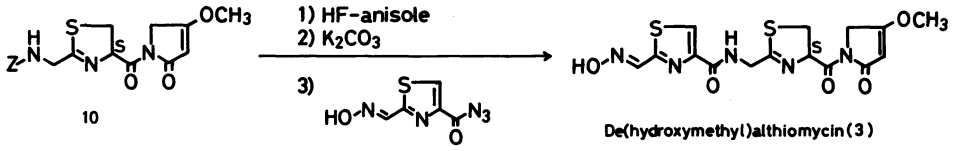

Fig. 3.

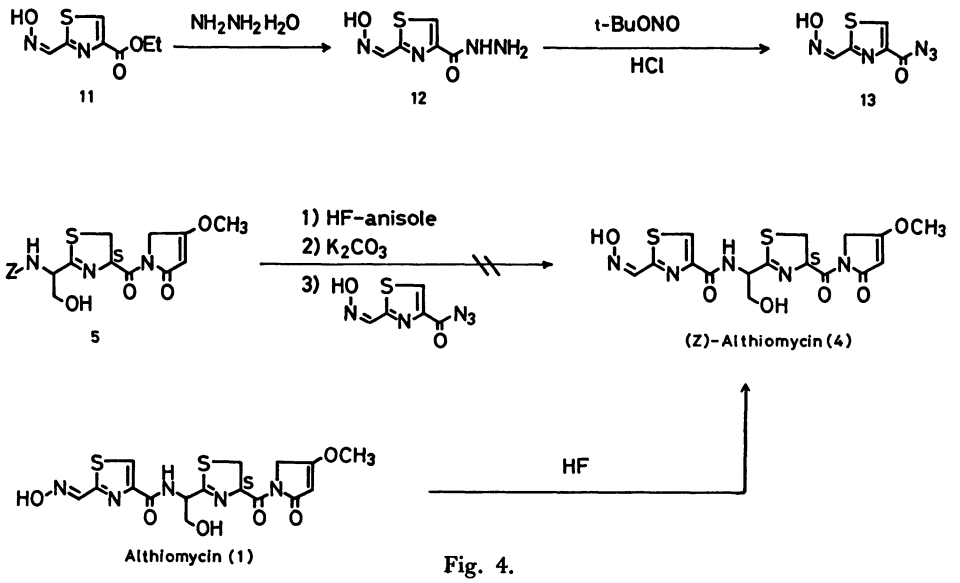

Fig. 4.

The UV spectra were obtained with a Hitachi 124 spectrophotometer. The specific rotations were obtained with a Perkin-Elmer 141 polarimeter. HPLC was performed with a Shimadzu LC-5A liquid chromatograph. TLC was carried out by the ascending method on a Merck Kieselgel 60 F_{254}.

***N,S*-Dititryl-L-cysteine 5-Norbornene-*endo*-2,3-dicarboximidyl Ester (6).** To a suspension of *N,S*-dititryl-L-cysteine diethylamine salt (10.0 g, 14.7 mmol) in 700 ml of ether, 1 M(1 M=1 mol dm^{-3}) aqueous citric acid (5 ml) was added and vigorously stirred for 30 min at 0 °C. The ethereal layer was washed with water and concentrated in vacuo. To the solution of the residual oil in 40 ml of THF, *N*-hydroxy-5-norbornene-*endo*-2,3-dicarboximide (3.95 g,

Table 1. Minimum Inhibitory Concentrations (mcg/ml) of Althiomycin Analogs

Test organism	5	Epialthiomycin	(Z)-Althiomycin	De(hydroxymethyl)-althiomycin	Natural althiomycin
Staph. aureus ATCC 6538P	>100	>100	>100	25	25
Staph. aureus MS353	>100	>100	>100	25	25
Staph. aureus MS353 C36	>100	>100	>100	25	25
Staph. aureus MS353 AO	>100	>100	>100	25	25
Staph. aureus 0116	>100	>100	>100	25	25
Staph. aureus 0119	>100	>100	>100	25	25
Staph. aureus 0126	>100	>100	>100	6.3	25
Staph. aureus 0127	>100	>100	>100	6.3	25
Staph. epidermidis sp-al-1	>100	>100	>100	25	25
Strept. pyogenes N.Y.5	>100	25	12.5	3.1	3.1
Strept. pyogenes 1022	>100	25	25	3.1	3.1
Strept. faecalis 1501	>100	>100	>100	50	100
Strept. agalactiae 1020	>100	50	25	12.5	6.3
Sarcina lutea ATCC 9341	>100	25	25	6.3	1.6
M. flavus ATCC 10240	>100	25	25	12.5	3.1
C. diphtheriae P.W.8	>100	12.5	12.5	3.1	0.8
Bac. subtilis ATCC 6633	>100	>100	>100	25	25
E. coli NIHJ-JC2	>100	>100	>100	>100	>100
E. coli B	>100	>100	>100	>100	50
K. pneumoniae ATCC 10031	>100	>100	50	25	6.3
Salm. typhosa H 901	>100	50	>100	>100	>100
Salm. entritidis Gaertner	>100	>100	>100	>100	>100
Shigella flexineri type 3a	>100	>100	>100	>100	25
Shigella sonnei E33	>100	>100	>100	>100	>100
Proteus vulgaris OX19	>100	>100	>100	>100	50
Serratia marcescens	>100	>100	>100	>100	>100
Ps. aeruginosa LAM1095	>100	>100	>100	>100	>100

22.1 mmol) and dicyclohexylcarbodiimide (3.63 g, 17.6 mmol) were added and stirred at 0 °C for 2 h. After additional stirring at room temperature for 10 h, the reaction mixture was concentrated in vacuo. The residual oil was dissolved in ethyl acetate, and the insoluble material was filtered off. The filtrate was washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was recrystallized from ethyl acetate and hexane; yield 9.43 g (83.5%). Mp 195—196 °C (decomp). $[\alpha]_D^{20}$ +74.0° (*c* 0.978, chloroform). Found: C, 77.88; H, 5.71; N, 3.56; S, 4.10%. Calcd for $C_{50}H_{42}N_2O_4S \cdot 0.3(C_2H_5)_2O$: C, 77.92; H, 5.75; N, 3.55; S, 4.06%.

1-(*N,S*-Dityrityl-L-cysteinyl)-4-methoxy-3-pyrrolin-2-one (7). To a solution of 4-methoxy-3-pyrrolin-2-one (3.63 g, 32.1 mmol) in 100 ml of anhydrous THF, 60% sodium hydride (1.03 g, 25.7 mmol) was added and heated under reflux for 10 min. To the solution, *N,S*-dityrityl-L-cysteine 5-norbornene-*endo*-2,3-dicarboximidyl ester (**6**) (15.6 g, 21.4 mmol) was added and vigorously stirred for 50 min at room temperature. Ethyl acetate was added to the reaction mixture, and the solution was washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was recrystallized from ethyl acetate and hexane; yield 14.1 g (94%). Mp 206—207 °C (decomp). $[\alpha]_D^{27}$ +23.1° (*c* 1.72, chloroform). 100 MHz ^{1}H NMR ($CDCl_3$) δ=7.0—7.6 (30H, m), 4.86(1H, s), 4.84(1H, br., s), 3.78(3H, s), 3.77(1H, d, *J*=18 Hz), 3.43(1H, br., t), 3.34(1H, d, *J*=18 Hz), 2.80(1H, dd, *J*=12 Hz, 6 Hz), 2.58(1H, dd, *J*=12 Hz, 4 Hz). Found: C, 78.56; H, 5.85; N, 3.97; S, 4.55%. Calcd for $C_{46}H_{40}N_2O_3S$: C, 78.83; H, 5.75; N, 4.00; S, 4.57%.

1-[(*R*)-2-(Benzyloxycarbonylaminomethyl)-2-thiazolin-4-yl-carbonyl]-4-methoxy-3-pyrrolin-2-one (8). To a suspension of 1-(*N,S*-dityrityl-L-cysteinyl)-4-methoxy-3-pyrrolin-2-one (**7**) (2.50 g, 3.57 mmol) in a mixture of 12.5 ml of methanol and 8.77 ml of benzene, pyridine (0.320 ml, 3.93 mmol) and silver nitrate (670 mg, 3.93 mmol) were added and stirred for 4 h at room temperature. Hydrogen sulfide was bubbled through the reaction mixture for 30 min at room temperature. Silver sulfide was filtered off and the solution was concentrated in vacuo. The residue was dissolved in 50 ml of anhydrous ethanol and ethyl benzyloxycarbonylaminoacetimidate (842 mg, 3.57 mmol) was added to the solution. After stirring for 20 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with saturated aqueous sodium hydrogencarbonate and then brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was recrystallized from ethyl acetate and hexane; yield 660 mg (47.5%). Mp 178—179 °C (decomp). $[\alpha]_D^{27}$ −16.4° (*c* 0.335, chloroform). 100 MHz ^{1}H NMR ($CDCl_3$) δ=7.34(5H, s), 6.24(1H, mt), 5.54(1H, br., s),

5.26(1H, s), 5.25(2H, s), 4.30(2H, s), 4.26(2H, dd, J=6 Hz, 2 Hz), 3.90(3H, s), 3.62(1H, dd, J=9 Hz, 6 Hz), 3.50(1H, dd, J=9 Hz, 6 Hz). Found: C, 55.12; H, 5.06; N, 10.41; S, 8.11%. Calcd for $C_{18}H_{19}N_3O_5S \cdot 0.25H_2O$: C, 54.88; H, 4.99; N, 10.67; S, 8.14%.

1-[(4*R*)-2-[1-(Benzyloxycarbonylamino)-2-hydroxyethyl]-2-thiazolin-4-ylcarbonyl]-4-methoxy-3-pyrrolin-2-one (9). A mixture of **8** (100 mg, 0.257 mmol) and pulverized paraformaldehyde (154 mg, 5.14 mmol) in 1 ml of degassed dimethyl sulfoxide was stirred at room temperature for 7 d. After the insoluble material was filtered off, the filtrate was diluted with ethyl acetate, and washed with water. The organic layer was dried over anhydrous magnesium sulfate. The solution was concentrated in vacuo and the residue was lyophilized; yield 108 mg (100%). Mp 72—73 °C (decomp). 100 MHz ^{1}H NMR ($CDCl_3$) δ=7.33 (5H, s), 6.30 (1H, m), 5.88 (1H, m), 5.16 (1H, s), 5.13 (2H, s), 4.28 (2H, s), 4.02 (1H, s), 3.91(2H, m), 3.90(3H, s), 3.47 (1H, d, J=6 Hz), 3.34 (1H, d, J=6 Hz).

Epialthiomycin (2). To a solution of **9** (83.3 mg, 0.199 mmol) in 215 μl of anisole, anhydrous hydrogen fluoride (ca. 1 ml) was introduced within 10 min at −78 °C and stirred at 0 °C for 20 min. Hydrogen fluoride was evaporated off at 0 °C under reduced pressure for 20 min. The residual oil was dissolved in 1 ml of DMF at −78 °C, and potassium carbonate (824 mg, 5.97 mmol) was added to the solution, and then the suspension was vigorously stirred for 5 min at room temperature. Under cooling with Dry Ice–methanol bath, (*E*)-2-(hydroxyiminomethyl)-4-thiazolecarbonyl azide[3] (39.2 mg, 0.199 mmol) was added and the suspension was stirred at 0 °C for 30 min. After additional stirring at room temperature for 2.5 h, the reaction mixture was diluted with ethyl acetate, and washed with 0.1 M potassium phosphate buffer (pH 7.15) and water. The organic layer was dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by silica-gel preparative TLC using a developing solvent chloroform–methanol 7:1 gave colorless powder; yield 19.9 mg (22.7%). Mp 174—176 °C (decomp). $[\alpha]_D^{14}$ −20.5° (c 0.073, Methyl Cellosolve). UV_{max}(methanol): 223 nm (ε 34700), 240 nm (ε 26000), and 286 nm (ε 8590). 90 MHz ^{1}H NMR(DMSO-d_6) δ=8.40 (1H, br., s, NH), 8.38 (1H, s, CH of oxime), 8.35 (1H, s, 5-H of thiazole), 6.08 (1H, mt, 4-H of thiazoline), 5.43 (1H, s, 3-H of pyrrolinone), 4.93 (1H, br.,-C<u>H</u>-CH_2OH), 4.33(2H, s, 5-H of pyrrolinone), 3.89 (3H, s, OCH_3), 3.83 (2H, m, -CH-C<u>H</u>$_2$OH), 3.50 (2H, t, 5-H of thiazoline). Found: C, 44.42; H, 4.48; N, 13.84; S, 12.87%. Calcd for $C_{16}H_{17}N_5O_6S_2 \cdot 0.3H_2O \cdot 0.6C_4H_8O_2$: C, 44.40; H, 4.54; N, 14.07; S, 12.88%.

De(hydroxymethyl)althiomycin (3). To a solution of 1-[(*S*)-2-(benzyloxycarbonylaminomethyl)-2-thiazolin-4-yl-carbonyl]-4-methoxy-3-pyrrolin-2-one (**10**)[3] (100 mg, 0.257 mmol) in 278 μl of anisole, anhydrous hydrogen fluoride (ca. 1 ml) was introduced within 10 min at −78 °C. After stirring at 0 °C for 20 min, hydrogen fluoride was evaporated off under reduced pressure at 0 °C for 20 min. Under cooling with Dry Ice–methanol bath, the residual oil was dissolved in 1 ml of DMF, and the solution was stirred vigorously for 5 min at room temperature. To the suspension, (*E*)-2-(hydroxyiminomethyl)-4-thiazolecarbonyl azide (50.6 mg, 0.257 mmol) was added and stirred at room temperature for 2.5 h after stirring at 0 °C for 30 min. The reaction mixture was diluted with ethyl acetate, and washed with 0.1 M potassium phosphate buffer (pH 7.15) and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by silica-gel preparative TLC (chloroform–methanol 9:1) gave colorless solid; yield 30.7 mg (29.2%). Mp 116—117 °C (decomp). $[\alpha]_D^{14}$ +11.6° (c 0.147, Methyl Cellosolve). 90 MHz ^{1}H NMR(DMSO-d_6) δ=9.00(1H, br., d, NH), 8.33 (2×1H, s, CH of oxime and 5-H of thiazole), 6.14 (1H, mt, 4-H of thiazoline), 5.42 (1H, s, 3-H of pyrrolinone), 4.33 (2H, s, 5-H of pyrrolinone), 4.25 (2H, m, $-CH_2-\overset{N^-}{\overset{\|}{C}}-S-$), 3.88 (3H, s, OCH_3), 3.40 (2H, m, 5-H of thiazoline). Found: C, 43.97; H, 4.09; N, 15.24; S, 14.01%. Calcd for $C_{15}H_{15}N_5O_5S_2 \cdot 0.5H_2O \cdot 0.4C_4H_8O_2$: C, 43.96; H, 4.34; N, 15.22; S, 14.13%.

(*Z*)-2-(Hydroxyiminomethyl)-4-thiazolecarbohydrazide (12). Ethyl (*Z*)-2-(hydroxyiminomethyl)-4-thiazolecarboxylate (**11**)[3] (1.50 g 7.50 mmol) was dissolved in 13 ml of DMF. To the solution, 100% hydrazine hydrate (8.02 ml, 0.165 mol) was added and allowed to stand at 4 °C for 20 h. To the reaction mixture, 100 ml of methanol was added and the precipitates were collected; yield 1.17 g (83.6%). Mp >220 °C. Found: C, 32.45; H, 3.30; N, 29.93; S, 17.35%. Calcd for $C_5H_6N_4O_2S$: C, 32.35; H, 3.25; N, 30.09; S, 17.22%.

(*Z*)-2-(Hydroxyiminomethyl)-4-thiazolecarbonyl Azide (13). To a suspension of the hydrazide **12** (155 mg, 0.834 mmol) in 1.25 ml of DMF, *t*-butyl nitrite (109 μl, 0.918 mmol) and 4.35 M hydrogen chloride in THF (0.420 ml, 1.80 mmol) were added at −50 °C and stirred at −30 °C for 40 min. The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to give colorless needles; yield 116 mg (70.7%). Mp 118—119 °C (decomp).

(*Z*)-Althiomycin (4). Althiomycin (**1**)(148 mg, 0.337 mmol) was dissolved in ca. 5 ml of anhydrous hydrogen fluoride and stirred at room temperature for 40 min. Hydrogen fluoride was evaporated off under reduced pressure and the residue was dissolved in 2 ml of DMF. Potassium carbonate (1.40 g, 10.1 mmol) was added to the solution and the suspension was vigorously stirred for 10 min at room temperature. The reaction mixture was diluted with ethyl acetate and washed with 0.1 M potassium phosphate buffer (pH 7.15) and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to give colorless solid. Separation and purification by HPLC (Nagel Nucleosil 7C_{18} (8 mm×250 mm), 31% aqueous acetonitrile) gave colorless solid (retention time: 5.6 min); yield 13.1 mg (8.9%). Mp 170—172 °C (decomp). $[\alpha]_D^{14}$ +10.7° (c 0.197, Methyl Cellosolve). 90 MHz ^{1}H NMR (DMSO-d_6) δ=8.59 (1H, s, CH of oxime), 8.54 (1H, br., NH), 8.09 (1H, s, 5-H of thiazole), 6.27 (1H, mt, 4-H of thiazoline), 5.43 (1H, s, 3-H of pyrrolinone), 5.14 (1H, mt, OH), 4.90 (1H, m, -C<u>H</u>-CH_2OH), 4.33(2H, s, 5-H of pyrrolinone), 3.89 (3H, s, OCH_3), 3.85 (2H, m, -CH-C<u>H</u>$_2$OH), 3.59 (2H, t, 5-H of thiazoline). Found: C, 43.97; H, 4.52; N, 13.43; S, 12.27%. Calcd for $C_{16}H_{17}N_5O_6S_2 \cdot 0.75H_2O \cdot 0.75C_4H_8O_2$: C, 43.96; H, 4.76; N, 13.49; S, 12.36%.

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References

1) This work was presented at the 9th International Congress of Heterocyclic Chemistry, Tokyo, August 1983.

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3) K. Inami and T. Shiba, *Bull. Chem. Soc. Jpn.*, **58**, 352 (1985).

4) The model compound of (*Z*)-aldoxime, ethyl (*Z*)-2-(hydroxyiminomethyl)thiazole-4-carboxylate, gives two doublets (J=1 Hz) at δ 8.67 and 8.10, see T. Shiba, K. Inami, K. Sawada, and Y. Hirotsu, *Heterocycles*, **13**, 175 (1979).