SYNTHESIS AND GEOMETRIC CONFIGURATION OF 2-(HYDROXYIMINOMETHYL)THIAZOLE-4-CARBOXYLIC ACID IN ANTIBIOTIC ALTHIOMYCIN

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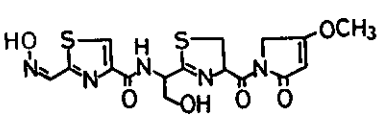
2-(Hydroxyiminomethyl)thiazole-4-carboxylic acid, a component of an antibiotic althiomycin, was synthesized. A configuration of the aldoxime group in the natural althiomycin was unambiguously determined to be *syn*-form by means of nuclear Overhauser enhancement and the behavior of methylation by reference to the synthetic compound.

A sulfur-containing antibiotic, althiomycin, isolated first from *Streptomyces althioticus* by H.Umezawa *et al.*,[1] was found to inhibit the protein synthesis in both Gram-positive and negative bacteria.[2] Although D.J.Cram *et al.* investigated the structure of althiomycin, they did not reach to a final proposal of the correct structure.[3] In 1974, Umezawa and his collaboraters succeeded to assign the total structure (1) of the antibiotic from results of the chemical degradation of the original antibiotic and X-ray analysis of its bisanhydro derivative.[4,5] At the time, they proposed an *anti*-form for the configuration of the aldoxime group in the moiety of 2-(hydroxyiminomethyl)thiazole-4-carboxylic acid as shown in 1 based on the following two experiments.[4]



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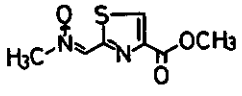

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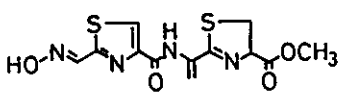

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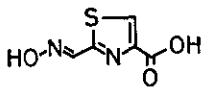
First, its aldoxime group was readily dehydrated to a cyano group by treatment with *N*-(bromoacetoxy)succinimide, in accordance with the general character of an *anti*-aldoxime. Secondly, they obtained a nitrone derivative (2) in the reaction of one of the products of the alkaline hydrolyzate with diazomethane in methanol. It has been accepted that an *anti*-aldoxime is mainly converted to a nitrone, whereas *syn*-one affords an *O*-methyl derivative.[6] Meanwhile, H.A.Kirst *et al*. suggested that the configuration of the aldoxime should be *syn*-form by means of X-ray crystallographic study for the methanolysis product (3) of althiomycin.[7]



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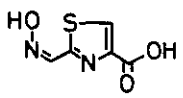

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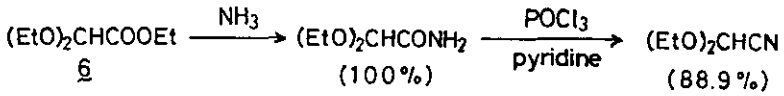
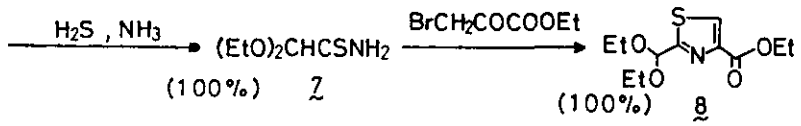
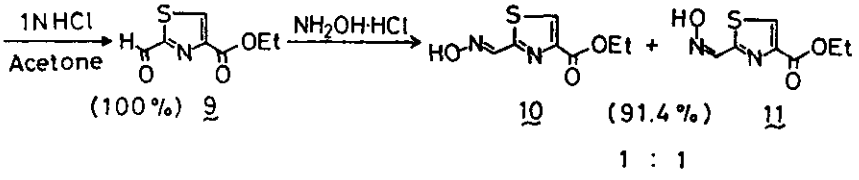






In order to solve the discrepancy in these different conclusions, we investigated thoroughly an isomerism of 2-(hydroxyiminomethyl)thiazole-4-carboxylic acid in this study. Both *syn*(4)- and *anti*(5)-aldoxime of this acid were synthesized from ethyl diethoxyacetate (6)[8] for the first time as shown in the scheme. Thus, successive procedures of amidation, dehydration, and addition of hydrogen sulfide for ethyl diethoxyacetate (6), afforded an intermediate of thioamide acetal (7), which was then coupled with ethyl bromopyruvate to yield thiazole ester (8). After removal of acetal from 8 by refluxing in acetone-1N HCl (10:1) for 1 h, an aldehyde (9) formed was converted to a mixture of the aldoximes (10,11) with hydroxylamine

Table 1. Nuclear Overhauser enhancement of 10 and 11.

irradiated signal	10 OH (aldoxime) 2	10 CH (aromatic) 4	10 CH (aldoxime) 5	11 OH (aldoxime) 1	11 CH (aromatic) 3	11 CH (aldoxime) 6
2	*	-	30±13%	-	-	-
1	-	-	-	*	-	-

sample: 10:11=1:2 in DMSO-d_6

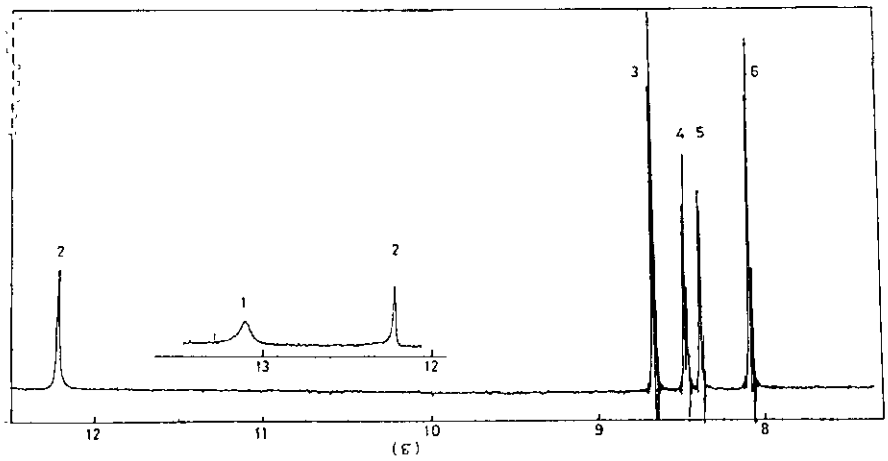
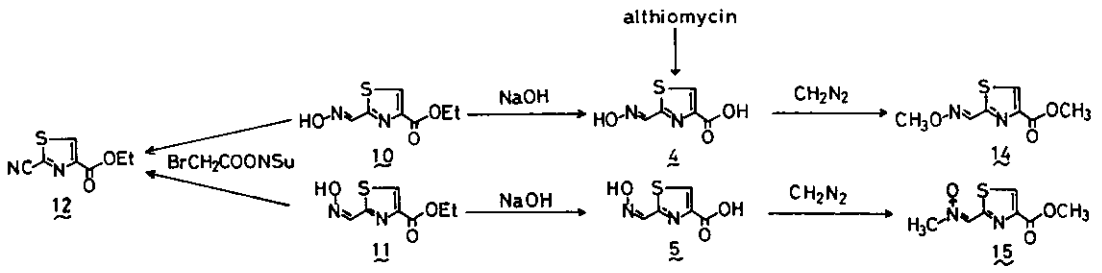


Fig. 1. NMR of ethyl 2-(hydroxyiminomethyl)thiazole-4-carboxylate (10:11=1:2). (100 MHz, Varian XL-100-15)



hydrochloride in pyridine. The ratio of *syn*- and *anti*-form in the aldoximes (1:1) was estimated from the integrations of nmr signals of the product before purification. Each aldoxime was obtained by silica gel column chromatography, preparative thin-layer chromatography, and fractional recrystallization successively.

For assignment of the configuration of each aldoxime, nuclear Overhauser enhancement was observed for a mixture of the isomers in dimethyl sulfoxide-d_6, as demonstrated in Table 1. This clearly shows that the one isomer 10 must be assigned

to *syn*-aldoxime, while 11 to *anti*-one.

The dehydration reaction of the aldoxime with *N*-(bromoacetoxy)succinimide was carried out according to the literature.[4] Actually both aldoximes (10,11) gave the nitrile (12) in the same way. The fact suggests that a liability to the dehydration can not be considered as a reliable index for the configurational assignment of the aldoxime in this case.

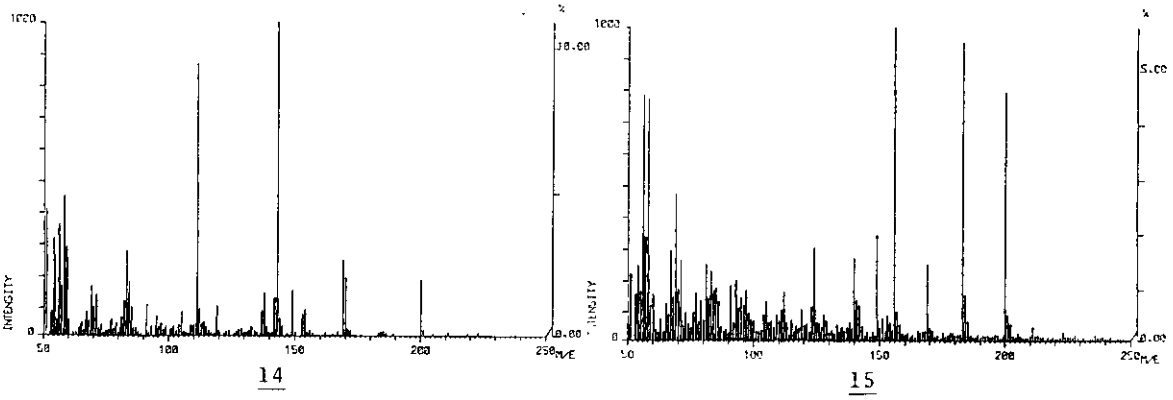
Saponification of the synthetic aldoxime esters (10,11) with 2N NaOH at room temperature gave the corresponding acids (4,5) respectively. Each acid (4,5) showed a separable single peak (retention time: 4: 3 min 18 sec; 5: 3 min 36 sec) on reverse phase high performance liquid chromatography (HPLC) (column: Nucleosil $7C_{18}$ (4 mm x 125 mm); eluate: 0.2% tetrabutylammonium bromide in 0.1M potassium phosphate buffer (pH 7.2)-methanol (95:5)). On the other hand, althiomycin was also degraded to 2-(hydroxyiminomethyl)thiazole-4-carboxylic acid (13) by hydrolysis with 2N NaOH at 60°C according to the Umezawa's procedure.[4] The hydrolyzate without purification showed the characteristic peak to the *syn*-form (4) at 3 min 18 sec on HPLC. However, after isolation and purification of the acid (13) as in the literature[4] (Dowex 50 x 8, H^+-form; eluate: water), it changed to the peak at 3 min 36 sec, which should be assigned to *anti*-form (5). This result suggested that an original configuration of the aldoxime in althiomycin must be *syn*-form and the configurational change occurred in the treatment with the acidic resin. On the basis of this assumption, the purification of 13 was carefully carried out in a neutral medium by preparative paper electrophoresis (1 mA/ 1 cm, 600 V, pyridine-acetic acid-water (30:4:966)(pH 6.9), 30 min, Toyo filter paper No 50) after the alkaline hydrolysis. The natural acid (13) thus obtained was virtually identical with the synthetic *syn*-form (4) in respects of nmr, uv, ir spectra (Table 2). In order to exclude a possibility of change of the configuration in the aldoxime during hydrolysis of the natural product, we then expose each synthetic aldoxime (4,5) to the same reaction condition applied to the natural compound (in 2N NaOH at 60°C for 2 h). Either aldoxime did not show any symptom of the configurational change during this procedure, giving each original single peak on HPLC.

The other point to have to be made clear is a difference of the methylation position of the aldoxime (4,5) depending on isomerism. The authentic *syn*-aldoxime (4) afforded *O*-methyl derivative (14) in the reaction with diazomethane in ether in the presence of a small amount of methanol, whereas *anti*-form (5) gave *N*-methyl, *i.e.*, nitrone derivative (15).[6] This exclusive formation of each methyl derivative

Table 2. Physicochemical properties of synthetic (4,5) and natural (13) 2-(hydroxyiminomethyl)thiazole-4-carboxylic acid.

	synthetic		natural
	4	5	13
mp(°C)(dec)	230-231	220-222	244-245
NMR(DMSO-d_6)	δ 8.42(d,1Hz) δ 8.35(d,1Hz)	δ 8.72(d,1Hz) δ 8.13(d,1Hz)	δ 8.39(d,1Hz) δ 8.32(d,1Hz)
UV $\lambda_{max}^{95\%EtOH}$(ε)	218 nm(24,900) 290 nm(9,700)	220 nm(17,500) 282 nm(9,030)	218 nm(22,200) 290 nm(7,360)
IR (cm^{-1})	3440,1700,1495	3200,1700,1490	3440,1720,1480
HPLC*	3 min 18 sec	3 min 36 sec	3 min 18 sec

* High performance liquid chromatography (column: Nucleosil $7C_{18}$ (4 mm x 125 mm); eluate: 0.2% tetrabutylammonium bromide in 0.1M potassium phosphate buffer (pH 7.2)-methanol (95:5)).

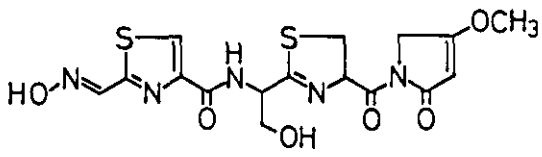


14 15

Fig. 2 Mass spectra of the methylation products (14,15).

was checked by TLC. The structure of each product was confirmed by electron-impact mass spectrometry. Thus, 15 showed a characteristic fragmentation to the nitrone at 183 $(M-OH)^+$ and 184 $(M-O)^+$ significantly,[9] which could not be found as prominent peaks in 14 (Fig. 2). These results were in agreement with the tendency of the methylation, which was reported by O.L.Brady *et al.*[6] In fact, the alkaline hydrolyzate (13) of the natural althiomycin gave the *O*-methyl derivative (14) on methylation with diazomethane, being opposite to the result by H.Umezawa *et al.*[4]

In conclusion, the configuration of the aldoxime involving in althiomycin is now clearly established as *syn*-form from the results of nuclear Overhauser enhance-



althiomycin (16)

ment and the methylation reaction.[10] Therefore, the total structure of althiomycin should be depicted as 16.

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REFERENCES

1. H.Yamaguchi, Y.Nakayama, K.Takeda, K.Tawara, K.Maeda, T.Takeuchi, and H.Umezawa, *J. Antibiot.*, 1957, 10, 195.
2. H.Fujino, T.Kinoshita, H.Suzuki, and H.Umezawa, *J. Antibiot.*, 1970, 23, 271.
3. D.J.Cram, O.Theander, H.Jager, and M.K.Stanfield, *J. Amer. Chem. Soc.*, 1963, 85, 1430.
4. H.Sakakibara, H.Naganawa, M.Ohno, K.Maeda, and H.Umezawa, *J. Antibiot.*, 1974, 27, 897.
5. H.Nakamura, Y.Iitaka, H.Sakakibara, and H.Umezawa, *J. Antibiot.*, 1974, 27, 894.
6. O.L.Brady, F.P.Dunn, and R.F,Goldstein, *J. Chem. Soc.*, 1926, 2386.
7. H.A.Kirst, E.F.Szymanski, D.E.Dorman, J.L.Occolowitz, N.D.Jones, M.O.Chaney, R.L.Hamill, and M.M.Hoehin, *J. Antibiot.*, 1975, 28, 286.
8. R.B.Moffet, '*Organic Syntheses*', IV, John Willey & Sons,Inc., New York, London, 1963, pp. 427 - 430.
9. R.Grigg and B.G.Odell, *J. Chem. Soc. (B)*, 1966, 218.
10. The signals in nmr spectrum of althiomycin itself (δ 8.36, δ 8.33) suggested directly the *syn*-aldoxime in reference to the signals 4 and 5 in Fig. 1.

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