MYXOTHIAZOL, AN ANTIBIOTIC FROM *MYXOCOCCUS FULVUS* (MYXOBACTERALES)

II. STRUCTURE ELUCIDATION

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Myxothiazol is shown to be 4-(6-carbamoyl-3,5-dimethoxy-4-methylhexa-1E,5E-dienyl)-2′-(1,6-dimethylhepta-2E,4E-dienyl)-2,4′-bithiazole by spectroscopic (mainly ^{1}H NMR, ^{13}C NMR and mass spectroscopic) and chemical methods.

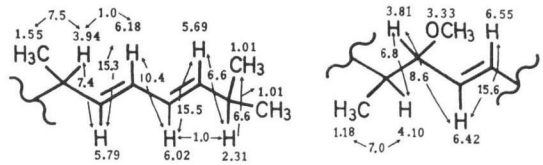
The highly potent biological activity of myxothiazol has been described in the preceding paper[1]. Here we wish to report the structure elucidation of this compound.

Results and Discussion

NMR Spectroscopy

The ^{1}H NMR data for myxothiazol, **1**, are shown in Table 1. Homonuclear double irradiation and spectral analysis allowed the partial structural assignments shown in Fig. 1 to be made. From the spectral analysis the magnitude of the vicinal coupling constants indicated that the protons on the double bonds have trans orientations.

Fig. 1. ^{1}H shifts and coupling constants of fragments I and II.



Fragment I Fragment II

Proton noise-decoupled and single frequency off-resonance decoupled (SFORD) ^{13}C spectra were recorded. A proton coupled ^{13}C spectrum was recorded by gating the heteronuclear proton decoupler to give spectra with nuclear-OVERHAUSER-enhancement. Numerous selective proton decoupling experiments gave the cross correlation between the ^{1}H and ^{13}C shifts. These results are shown in Table 2.

The integration of the ^{1}H spectrum, multiplicity of the carbon signals in the SFORD spectrum and the number of signals in the ^{13}C spectrum is in agreement with the molecular formula from the high-resolution mass spectrum of $C_{25}H_{33}N_3O_3S_2$.

Correlations between the carbon signals and their directly bonded protons indicated that the proton at 3.81 ppm is bound to a carbon carrying an oxygen function from the carbon chemical shift and $^{1}J_{CH}$ value (85.2 ppm and 146 Hz, respectively). Of the two methoxy carbon signals only the one at 56.8 ppm (A) showed a long-range J_{CH} of 4 Hz characteristic of a vicinal coupling through oxygen[2]. The other methoxy carbon signal (B, 55.1 ppm) showed no such coupling and must be geminal to a quaternary carbon. As the third oxygen atom occurs as a carbonyl function, see below, the methoxy group A is bound to the carbon carrying the proton with the shift of 3.81 ppm.

Treatment of **1** with acidified methanol resulted in the production of a compound of molecular

Table 1. ^1H NMR data of **1** in $CDCl_3$.

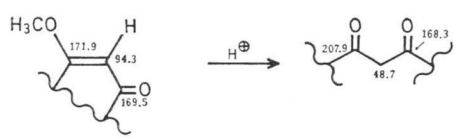
Peak No.	Shift (ppm)	Multiplicity[2]	No. of protons	Coupling (x−y)[1] (Hz)[5]
1	1.01	d	6	(1∼ 4) 6.6 (2∼ 9) 7.0
2	1.18	d	3	(3∼ 8) 7.5 (4∼13) 6.6
3	1.55	d	3	(7∼ 9) 6.8 (7∼17) 8.6
4	2.31	d, sp	1	(8∼14) 7.4 (13∼15) 15.5
5	3.33	s	3	(14∼16) 15.3 (15∼16) 10.4
6	3.58	s	3	(17∼18) 15.6 (4∼15)[3] 1
7	3.81	d, d	1	(8∼16)[3] 1
8	3.94	d, q	1	
9	4.10	d, q	1	
10	4.94	s (broad)[4]	1	
11, 12	5.6∼5.8 broad		2 (exchangeable)	
13, 14, 15	5.69, 5.79, 6.02	1)	4	
16, 17, 18	6.18, 6.42, 6.56	1)	2	
19	7.11	s	1	
20	7.85	s	1	

1) Deduced from spectral analyses.
2) sp=septet, q=quartet, d=doublet, and s=singlet.
3) From a 270 MHz spectrum.
4) Irradiation causes sharpening of the resonance at 3.58 ppm.
5) x and y refer to the peak numbers.

formula $C_{24}H_{31}N_3O_3S_2$, as determined from the high resolution mass spectrum. The ^1H and ^{13}C spectra of this compound, **2**, are given in Tables 3 and 4, respectively. Comparison with **1** indicates that the three double bonds remain intact and only fragment II is affected. From the ^{13}C spectra the methoxy group B is lost with the appearance of a signal at 207.9 ppm, a shift characteristic of a saturated carbonyl carbon. Similarly the singlet at 4.94 ppm in the ^1H spectrum and the corresponding carbon signal at 94.3 ppm of **1** is lost with the appearance of a methylene group. Simple shaking of **2** with D_2O causes this methylene carbon signal to disappear from the spectrum, indicating that the CH_2 protons are readily exchangeable. The changes in shifts of the methine and methyl carbon signals of fragment II and the above indicate that the following has occurred (Fig. 2).

It follows from the proton double resonance experiments (Table 1) and peak simulation that the methoxy group B attached to the double bond was coupled to the olefinic proton with $^5J_{HCCOCH}$ of 0.5±0.15 Hz. Comparison with literature values[3] indicate that compounds with the E configuration have $^5J_{HCCOCH}$ values of the order of 0.3 to 0.5 Hz, while the values for the corresponding compounds with the Z configuration are not detectable (<0.1 Hz). Hence the magnitude of this coupling found here suggests an E

Fig. 2.



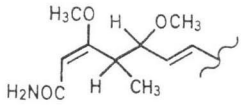
1 **2**

Table 2. ^{13}C NMR data of **1** in $CDCl_3$.

Shift (ppm)	Assignment C-No.	Multiplicity		Couplings (Hz)		Correlation with proton shifts[2]
		SFORD	Coupled[1]	$^1J_{CH}$	$^nJ_{CH}$	
14.4	24	q	q	127.4	3.6	1.18
20.9	15	q	t	130.0		1.55
22.3	13, 14(2)	q	q	126.9	3.9	1.01
31.1	12	d	quin	134.0	3.6	2.31
39.7	19	d	m	132.6		4.10[3]
41.3	7	d	q or d, d	130.1	3.6, 5	3.94[3]
55.1	25	q		144.8		3.58
56.8	23	q	d	141.0	4.0	3.33
85.2	18	d	m (1/2, 20)	146		3.81[3]
94.3	21	d	m (1/2, 20)	153.3		4.94
115.2	2	d	d	187.1	2.2	7.11
115.6	5	d		191.9		7.85
126.0	16	d	m (1/2, 10)	155.0		6.56
126.6	10	d	m (1/2, 14)	~154		6.02
131.4	17	d	m (1/2, 14)	154.8		6.42
132.0	9	d	m (1/2, 14)	154.8		6.18
132.6	8	d	m (q) (1/2, 14)	~154		5.79
142.5	11	d	m (q) (1/2, 20)	~154		5.69
149.0	4	s	d		4.3	
154.5	1	s	(q)=d, d, d		5.1	
162.7	3	s	d		7.5	
169.5	22	s	(1/2, 4)			
171.9	20	s	m (1/2, 16)			
176.3	6	s	m (1/2, 16)			

1) Symbols are given in Table 1; quin=quintet, m=multiplet, and (1/2 Number)=peak width at half height.
2) This is the correlation between the carbon shifts and the directly bonded proton shifts.
3) The order of these signals was also deduced from the off-resonance effects of the selective decoupling experiments.

Fig. 3. Fragment II as deduced from the NMR and mass spectra.



configuration for the double bond carrying methoxy group B.

When **1** was reduced to **3**, $C_{25}H_{39}N_3O_3S_2$, the ^1H and ^{13}C spectra, Table 5, indicated that three olefinic bonds from fragments I and II had been lost but that the fourth bond carrying methoxy group B had been retained. In the mass spectra of both **1** and **3** a loss from the molecular ion of a fragment $C_6H_{10}NO_2$ (see mass spectral data below) was found, which suggested fragment II terminates as Fig. 3.

This is also compatible with the ^{13}C spectra of **1**, **2** and **3**, the carbonyl shift in **1** and **3** is 169.3±0.2 (peak width at half-height=4 Hz) and in **2** is 168.4 ppm. The same carbonyl in $CH_3C(O)CH_2C(O)NH_2$ is found at 169.7 ppm (Table 6). Similarly the magnitude of the small coupling to this carbonyl in **2** is identical with $^2J_{CH}$ in $CH_3C(O)CH_2C(O)NH_2$.

Thus the remaining moiety in **1**, connecting fragments I and II, is $C_6H_2N_2S_2$. The nature of

Table 3. ^{1}H NMR data of **2** in $CDCl_3$.

Shift (ppm)	Multiplicity[2]	No. of protons
1.02	d	6
1.19	d	3
1.55	d	3
2.33	d, sp	1
2.96	d, d	1
3.34	s	3
3.56	s	2, exchangeable
3.94	d, q	1
4.03	d, d	1
5.54~6.40	1)	5, 1 of which is exchangeable
6.40~6.80	1)	2
7.00	broad	1, exchangeable
7.14	s	1
7.89	s	1

1) The splitting patterns were very similar to those of **1**.
2) See footnote 2) of Table 1.

Fig. 4. ^{1}H and ^{13}C chemical shifts (ppm) and coupling constants (Hz) for the bithiazole fragment of **1**.

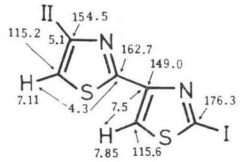
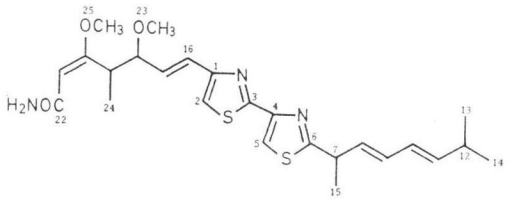


Fig. 5. Complete structure of myxothiazol, **1**, and the numbering system used for the ^{13}C shift assignments.



1

Table 4. ^{13}C NMR data of **2** in $CDCl_3$.

Shift (ppm)	Assignment C-No.	Multiplicity		Couplings (Hz)	
		SFORD	Coupled[1]	$^{1}J_{CH}$	$^{n}J_{CH}$
11.5	24	q	q	130	3.8
20.7	15	q	q	128	3.8
22.1(2)	13, 14	q	q	125	4.0
31.0	12	d	d, d	128	3.6
41.1	7	d		~128	
48.7[2]	21	t		122~126	
51.9	19	d		128	
56.8	23	q	d	142	4
82.6	18	d	m	~145	
115.8	2	d		190	
116.4	5	d	d	186	2.2
126.5	16	d			
126.9	10	d		~158	
129.0	17	d		~158	
131.8	9	d			
132.4	8	d			
142.3	11	d		154	
148.7	4	s	d		4
153.5	1	s	t or q		5
162.9	3	s	d		6
168.3	22	s	t		6
176.2	6	s	t or quin		6
207.9	20	s			

1) See footnote 1) of Table 2.
2) Deuterium exchange makes this signal impossible to detect.

Table 5. ^{1}H and ^{13}C NMR data of **3** in $CDCl_3$.

^{1}H data			^{13}C data		
Shift (ppm)	Multiplicity	No. of protons	Shift (ppm)	Assignment: C-No.	Multiplicity SFORD
0.86	d	6	14.37	24	q
1.16	d	3	21.24	15	q
1.26	m	7	22.52(2)	13, 14	q, q
1.41	d	3	27.16	16	t
1.88	m	4	27.37(2)	9, 10	t, t
2.88	m	2	27.80	12	d
3.32	m	2	31.21	8	t
3.40	s	3	37.61	19 or 7	d
3.58	s	3	37.78	17 or 11	t
4.30	quin	1	38.54	7 or 19	d
4.94	s	1	38.74	11 or 17	t
5.26	s (broad)	2 (exchangeable)	54.94	25	q
6.90	s	1	57.41	23	q
7.80	s	1	82.82	18	d
			92.77	21	d
			113.37	2	d
			114.15	5	d
			148.78	4	s
			158.51	1	s
			162.25	3	s
			169.14	22	s
			174.27	20	s
			177.89	6	s

this was derived from ^{13}C spectra with selective ^{1}H decoupling. For **1** irradiation at 7.85 and 7.11 ppm in the ^{1}H spectrum removes the small couplings to the quaternary carbon signals at 149.0 and 162.7 ppm in the ^{13}C spectrum, respectively. The quaternary carbon signal at 154.5 ppm that appears to be a quartet is, in fact, a doublet of doublets of doublets arising from coupling with protons at 6.56 and 6.42 ppm, and with one of the protons at either 7.85 or 7.11 ppm. The magnitude of $^{1}J_{CH}$ of the signals at 115.2 and 115.6 ppm of 189.5±3 Hz is characteristic of a sp^2 hybridised carbon adjacent to a nitrogen or sulphur atom, but not adjacent to two heteroatoms.

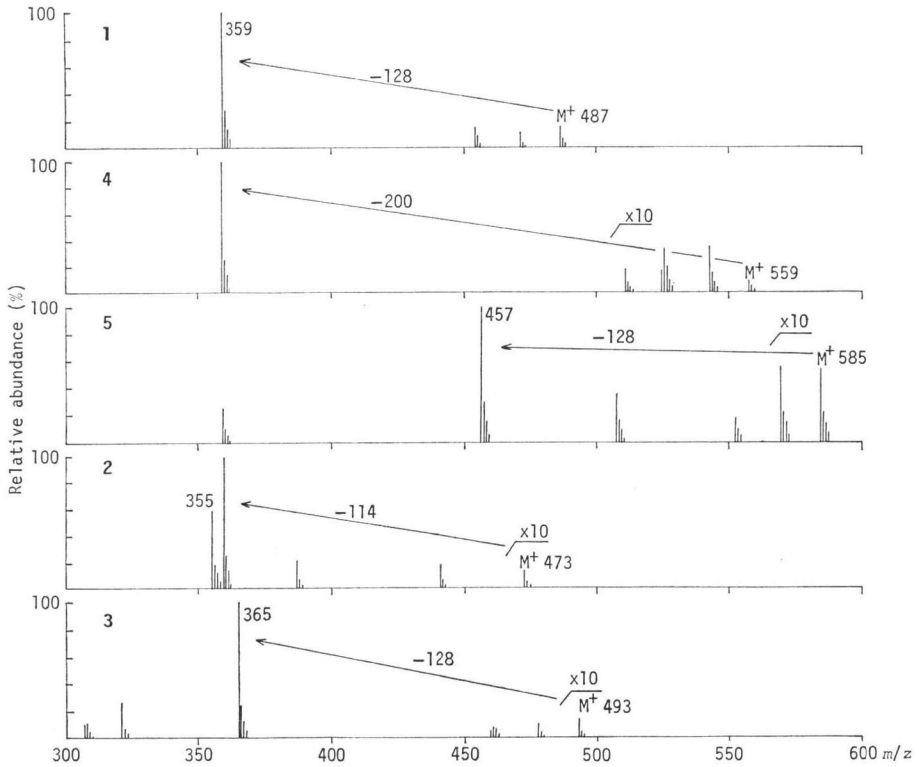
These data, together with a comparison with the data for thiazole (Table 6) suggest the structure shown, with the attachment of fragments I and II as indicated, in Fig. 4. Alternative arrangements of the thiazole rings appear to be incompatible with the ^{13}C shifts and magnitudes of the longer range J_{CH} values.

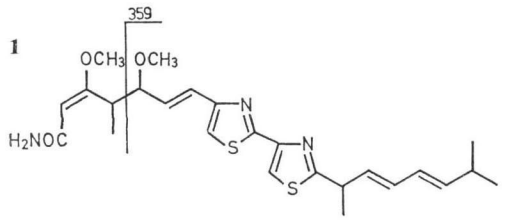
Thus all the NMR spectral data are compatible with the structure of **1** being as shown in Fig. 5. For convenience in the assignment of the ^{13}C signals in **1** to **3** the carbon atoms are numbered as shown. This should not be confused with the numbering in the systematic nomenclature of this type of compound. The complete ^{13}C NMR assignment for **1** to **3** are given in Tables 2, 4 and 5, respectively.

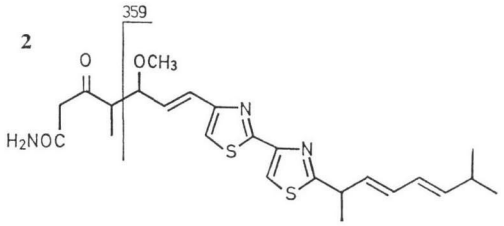
Mass Spectroscopy

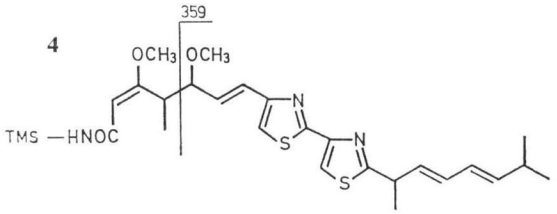
The molecular formula of **1** and the derivatives **2** to **5** were deduced directly from the high resolution

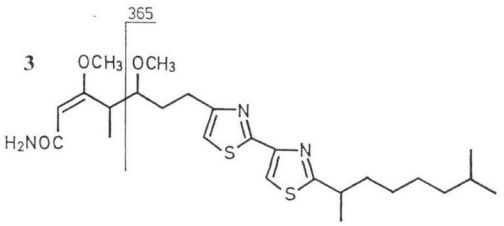
Fig. 6. The mass spectra of the compounds **1** to **5** taken at 200°C and 70 eV, except **1** (20 eV) with MS 9.











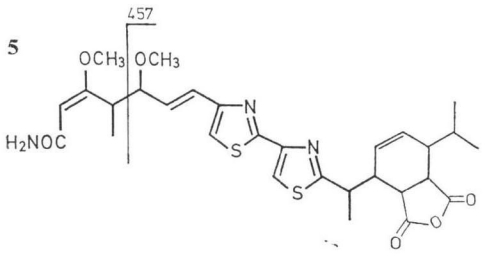


Table 6. ^{13}C NMR data for the model compounds acetoacetamide and thiazole.

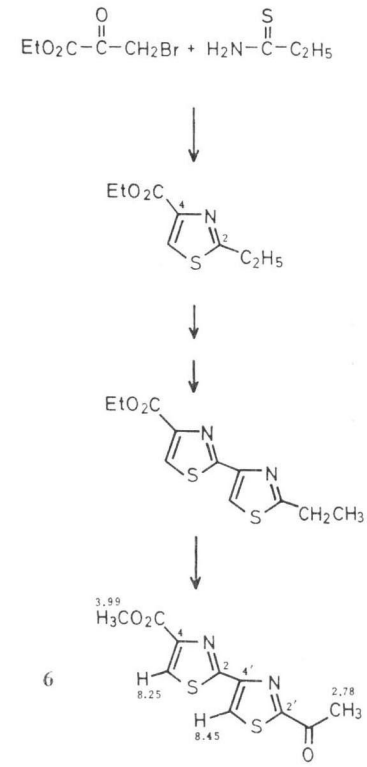
$^{1}CH_3{}^{2}CO\text{-}{}^{3}CH_2\text{-}{}^{4}CO\text{-}NH_2$ 

Shifts (ppm)	Shifts (ppm)*
C-1 30.5	C-2 153.6
C-2 204.3	C-4 143.8
C-3 50.4	C-5 119.6
C-4 169.7	

Couplings (Hz)	Couplings (Hz)
(C1-H1) 128.1	(C2-H2) 212.54
(C3-H3) 128.9	(C2-H4) 15.15
(C3-H1) 6.2	(C2-H5) 6.14
(C2-H1) 6.0	(C4-H4) 186.96
(C2-H3) 6.0	(C4-H2) 14.96
(C4-H3) 6.0	(C4-H5) 7.06
	(C5-H5) 190.10
	(C5-H4) 16.29
	(C5-H2) 1.55

* The ^{13}C shifts were assigned by cross correlation with the ^{1}H NMR spectrum.

Fig. 7. The total synthesis and ^{1}H shifts of **6**.



6

and/or normal mass spectra (Fig. 6). The spectrum of **1** and of both the hexahydro derivative **3** and the DIELS-ALDER adduct **5** show very stable ions which are accounted for by loss of $C_6H_{10}NO_2$ (128 mass units) from M^+. The production of the same stable ion (*m/z* 359) in **2** and **4**, as in **1**, is accounted for by the change in side-chain constitution of fragment II (or a derivative). In all cases we postulate an allylic-like fragmentation of the 4-substituent of the bithiazole system; thus **3** fragments to an ion (*m/z* 365) in which the additional six protons (compared with **1**) are all incorporated. From its mass and because of its stability it is clear that the aromatic system is contained in this fragment ion.

For **5** the loss of $C_4H_2O_3$ (maleic anhydride by a retro DIELS-ALDER process) from the basic ion ($M^+ - C_6H_{10}NO_2$) at *m/z* 457 occurs to an extent of only 25%. An alternative route for fragmentation is found only in the spectrum of **2**. Besides the loss of $C_5H_8NO_2$ leading to the basic ion at *m/z* 359, there exists an ion at *m/z* 355 with 59% relative intensity. Two fragmentation pathways are possible for its generation; thus methanol is lost at first from the molecular ion to give the ion at *m/z* 441 followed by loss of the β-ketoamide function, and secondly the β-ketoamide function is lost, which is shown by the appearance of the ion at *m/z* 387. This is followed by methanol elimination to give the ion at *m/z* 355.

Direct evidence for the bithiazole system or the diene fragment I in **1** is not available from the mass spectroscopic data.

Chemical proof of the bithiazole system:

To prove the presence of the bithiazole system suggested by the NMR spectral data of **1** to **3**, we isolated after ozonisation of **1** the methyl ester of saramycetic acid (**6**). The free acid has been ob-

tained by oxidation of saramycetin, an antifungal antibiotic from a streptomyces strain[4]. Although all the spectroscopic data for this compound agree with the postulated structure, it was thought necessary to prove the structure of **6** by an independent total synthesis.

Condensation of propiothioamide with ethyl bromopyruvate gave the monothiazole, (**7**). Thioamidation of **7** yielded[5], after additional condensation with ethyl bromopyruvate, the bithiazole **8**. Oxidation of the 2'-ethyl substituent to the desired acetyl function was performed by an ETARD reaction with chromyl chloride in tetrachloromethane. Transesterification to the corresponding methyl ester gave a crystalline compound identical in all respects to **6**.

We, therefore, postulate that myxothiazol has the constitution shown in Fig. 5. Work related to the absolute configuration of the three asymmetric centres of **1** is in progress and will be published elsewhere.

Experimental

General

Melting points were recorded on a Kofler hot stage apparatus and are uncorrected. Mass spectra were obtained on an A.E.I. MS 9 spectrometer operating at 70 and 12 eV. IR spectra were recorded on a Perkin-Elmer 297 spectrophotometer. Optical rotations were determined with Perkin-Elmer Polarimeter 141. The NMR spectra were measured on a Varian XL-100-12 spectrometer operating in the FOURIER transform mode at 100.06 MHz for ^{1}H and 25.16 MHz for ^{13}C, at ambient temperature. The instrument was locked to the deuterium resonance (15.4 MHz) of the solvent and was controlled with a Varian 620-L computer equipped with a moving head disc together with complementary software. If not otherwise stated, column chromatography was performed on silica gel (Merck, Darmstadt), with a particle size of 10 μm at 5~10 bar.

All homo- and heteronuclear-decoupling experiments were performed using the Gyrocode^{R} module of the instrument. Chemical shifts were measured relative to internal tetramethylsilane (TMS) and are reported, according to the IUPAC convention, on the δ-scale with positive values being used to denote shifts to high frequency of the reference.

Spectral analyses were carried out with a program based upon LAOCOON III.. All calculations were carried out in single precision on a PDP-10 computer.

Myxothiazol (1)

The isolation procedure together with certain spectroscopic data are given in the preceding paper. NMR and mass spectral data are given in Tables 1 and 2 and Fig. 6.

Desmethyl-myxothiazol (2)

To a solution of **1** (100 mg) in methanol was added 1 ml of 1 N HCl and the solution was stirred for 10 hours at room temperature. Ethyl acetate was added and after extraction with bicarbonate, water and saturated sodium chloride solution, the organic phase was concentrated *in vacuo*. Chromatography on silica gel (10 μm, 5 bar) with 6% isopropanol - dichloromethane as the eluant afforded 90 mg (93%) **2** as a viscous oil. $[\alpha]_D$ −22.8° (methanol). High resolution MS found: *m/z* 473.1805 and $C_{24}H_{31}N_3O_3S_2$ requires: *m/z* 473.1807.

UV (methanol): λ_{max} (ε): 313 (10,500), 234 (47,000). IR ($CHCl_3$): $\tilde{\nu}$=3400, 3340, 3200, 3100, 3000, 2960, 2920, 2860, 1705, 1670 (broad), 1550, 1485, 1450, 985 and 970 cm^{-1}. MS: see Fig. 6. NMR: see Tables 3 and 4.

Hexahydro-myxothiazol (3)

To a solution of **1** (100 mg) in 40 ml isopropanol was added 80 mg $Pd/BaSO_4$. **1** was reduced for 3~4 hours with hydrogen at 2 atm. The catalyst was filtered off and the solution was concentrated under reduced pressure.

Chromatography on silica gel (10 μm, 10 bar), with 10% isopropanol - dichloromethane gave 82 mg (81%) of **3** as a viscous oil. All attempts to crystallize **3** failed. $[\alpha]_D$ +45.1° (methanol).

High resolution MS: found: m/z 493.2432 and $C_{25}H_{39}N_3O_3S_2$, requires: m/z 493.2433.

UV: λ_{max} (ε) 296 (10,100, methanol). IR ($CHCl_3$): $\tilde{\nu}$=3530, 3420, 2960, 2930, 2850, 1670, 1620, 1585 and 1450 cm^{-1}. MS: see Fig. 6. NMR: see Table 5.

Trimethylsilyl-myxothiazol (**4**)

Five mg of **1** and bis (trimethyl-silyl)-trifluoroacetamide (0.1 ml, Merck, Darmstadt) were heated for 30 minutes in a sealed tube at 60°C. The crude reaction mixture was used for the mass spectrometric study.

DIELS-ALDER adduct of **1** and maleic acid anhydride (**5**)

For the mass spectrometric study **1** (5 mg) and maleic acid anhydride (3 mg) were heated under reflux with 5 ml of benzene under nitrogen for three hours. The solvent was evaporated *in vacuo* and the crude reaction mixture was then chromatographed on a small silica gel column (0.5 cm, 5 cm) with 6% *iso*-propanol - dichloromethane. An oil (0.5 mg) of **5** was isolated. This was directly used for the mass spectrometric study. λ_{max} 313 nm (ethanol), (Fig. 6).

2′-Acetyl-4-carbomethoxy-2,4′-bithiazole (**6**); (Saramycetic acid methyl ester)

Compound **1** (36 mg) was dissolved in 25 ml of methanol. The solution was cooled to −70°C with dry ice - acetone, and ozone was introduced for 30 seconds into the solution at a rate of 40 liters per hour. The solution was refluxed for 30 minutes after addition of 0.3 ml hydrogen peroxide (30% solution) and 0.6 ml formic acid. The solvent was evaporated *in vacuo*, methanol was added and removed under vacuum. The crude reaction mixture was chromatographed on silica gel (10 μm, 10 bar) with 7% isopropanol - dichloromethane. Compound **6** was isolated after recrystallization from *n*-heptane - dichloromethane, mp 196~197°C (8 mg).

High resolution MS: found: 267.9965 and $C_{10}H_8N_2O_3S_2$, requires: 267.9976.

UV: λ_{max} 289 nm (log ε 4.14, methanol).

IR: $\tilde{\nu}$=3120, 3000, 2960, 2925, 2850, 1725, 1695, 1500, 1425, 1410, 1300 cm^{-1}.

MS: M^+=268 (75%), characteristic fragments at m/z (%): 238 (20.5), 210 (31.6), 195 (11.6), 158 (58.3), 136 (26), 57 (21), 43 (100), m*=164.5 (268→210).

NMR: ^1H ($CDCl_3$) δ=8.45 (s, 1H, H-5′), 8.25 (s, 1H, H-5), 3.99 (s, 3H, ester-CH_3), 278 (s, 3H, methyl ketone).

4-Carboxyethyl-2-ethyl-thiazole (**7**)

Thiopropionamide (500 mg, λ_{max}=262 nm, log ε=4.12), prepared by the method described by C. S. RAO *et al.*[5] in dichloromethane, was condensed with ethyl bromopyruvate (1.09 g) in 50 ml ethanol for 3 hours at reflux temperature. The solution was concentrated *in vacuo*, 100 ml dichloromethane was added and the precipitated material was filtered off. After concentration, the product was isolated by chromatography on silica gel with dichloromethane - 10% isopropanol, mp. 37°C, yield 1.2 g.

$C_8H_{11}NO_2S$ (171.2): found: C 51.76, H 5.95, N 7.60; calcd: C 51.88, H 5.99, N 7.56.

UV: λ_{max}=233 nm, log ε=4.28, (isopropanol).

IR ($CHCl_3$): $\tilde{\nu}$=3125, 2985, 2945, 2875, 1720, 1485, 1460, 1445, 1390, 1370, 1340, 1325, 1310, 1175, 1095, 1045, 1015, 960 cm^{-1}.

MS: M^+=m/z 185 (37.8%), characteristic fragments m/z (%): 142 (5.4), 141 (24.3), 140 (75.6), 139 (33.3), 115 (6.7), 114 (10.8), 113 (100), 112 (17.6), 111 (27.6), 59 (5.4), 58 (12.2), 57 (36.5).

NMR: ^1H ($CDCl_3$) δ=8.16 (s, 1H, arom.), 4.44 (q, 2H, ester-CH_2), 3.29 (q, 2H, 2-ethyl-CH_2-), 1.46 (t, 3H, 2-ethyl-CH_3), 1.42 (t, 3H, ester-CH_3).

4-Carboxamido-2-ethyl-thiazole (**8**)

Compound **7** (500 mg) was dissolved in 20 ml of methanol in a pressure tube and cooled with dry ice and acetone to −70°C. About 1 ml of ammonia was then introduced. The tube was sealed and the solution was allowed to come to room temperature and stand for 24 hours. The tube was opened, the solvent evaporated and the crude mixture was dissolved in a small amount of hot water. Compound **8** crystallized upon cooling (yield 350 mg, mp. 122°C).

$C_6H_8N_2OS$ (156.1): found: C 46.30, H 5.32, N 17.88; calcd: C 46.15, H 5.16, N 17.94.
UV: λ_{max}=230 nm (methanol).
IR ($CHCl_3$): $\tilde{\nu}$=3520, 3400, 3125, 3000, 2970, 1680, 1570, 1360, 960 cm^{-1}.
MS: M^+=156 (100%), characteristic fragments *m/z* (%): 113 (30.9), 101 (45.2), 58 (47.7).
NMR: ^1H ($CDCl_3$) δ=8.08 (s, 1H, H-arom.), 7.2 and 6.3 (2H, broad, -NH_2), 3.06 (q, 2H, ethyl-CH_2) 1.43 (t, 3H, ethyl-CH_3).

2-Ethyl-4-thiocarboxamido-thiazole (**9**)

P_2S_5 (125 mg) and sodium bicarbonate (190 mg) were added to a solution of **8** (100 mg) in 20 ml dry acetonitrile. After stirring at room temperature for 10 hours the desired product was detected (Rf 0.54 with 5% isopropanol - dichloromethane). After working up and chromatography on silica gel (10 μm, 10 bar with 8% isopropanol - dichloromethane) **9** was isolated (40 mg).

$C_6H_8N_2S_2$ (172.1): found: C 41.92, H 4.71, N 16.19; calcd: C 41.86, H 4.68, N 16.18.
UV: λ_{max}=253 nm (methanol).
IR ($CHCl_3$): $\tilde{\nu}$=3470, 3350, 3115, 2980, 1580, 1500, 1455, 1370, 965 cm^{-1}.
MS: M^+=172 (100%), characteristic fragments *m/z* (%): 139 (61.4), 117 (18.2), 113 (15.9).
NMR: ^1H ($CDCl_3$) δ=8.65 and 7.70 (2H, broad, -NH_2), 8.35 (s, 1H, H-arom.), 3.06 (q, 2H, -CH_2-), 1.44 (t, 3H, -CH_3).

4-Carboxyethyl-2′-ethyl-2,4′-bithiazole (**10**)

Compound **9** (30 mg) was dissolved in 10 ml of ethanol. Ethyl bromopyruvate (36 mg) was added and the solution was refluxed for 2 hours. The solvent was evaporated *in vacuo* and chromatography on silica gel (10 μm, 10 bar, 6% isopropanol - dichloromethane) gave crystalline **10** (40 mg, mp. 79/80°C).

High resolution MS: found: 268.0340 and $C_{11}H_{12}N_2S_2O_2$, requires: 268.0340.
UV: λ_{max}=289 nm (log ε 4.14, methanol).
IR ($CHCl_3$): $\tilde{\nu}$=3125, 2980, 2935, 2875, 1720, 1540, 1490, 1440, 1365, 1340, 1315, 1295, 1165, 1095, 1065, 1015, 995, 875 cm^{-1}.
MS: M^+=*m/z* 268 (100%) and characteristic fragments *m/z* (%): 223 (25.9), 196 (74.1).
NMR: ^1H ($CDCl_3$) δ=8.18 (s, 1H, H-arom., H-5), 8.05 (s, 1H, H-5′), 4.47 (q, 2H, ester-CH_2-), 3.10 (q, 2H, ethyl-CH_2-), 1.45 (t, 3H, ethyl-CH_3), 1.44 (t, 3H, ester-CH_3).

2′-Acetyl-4-carboxymethyl-2,4′-bithiazole (**6**)

Compound **10** (20 mg) dissolved in 5 ml of tetrachloromethane (dried over P_2O_5) was brought to −5°C. Two drops of chromyl chloride (Merck, Darmstadt), were added and the red solution was then stirred for one hour at 0°C and an additional hour at room temperature. With a small quantity of ice the chromyl ester was destroyed and the solution was extracted with dichloromethane. After evaporation of the organic solvent the crude reaction product was dissolved in 5 ml methanol containing 4% sulfuric acid. Heating for two hours under reflux gave, after chromatography on silica gel (10 μm, 10 bar) with 7% isopropanol - dichloromethane, the desired methyl ester **6** (10 mg), which was identical in all respects with the compound isolated after ozonolysis of myxothiazol under the conditions described above.

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