COMMUNICATIONS TO THE EDITOR

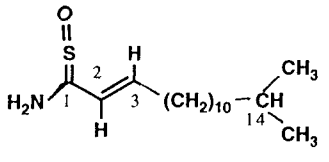
Sulfinemycin, a New Anthelmintic Antibiotic: Fermentation, Isolation and Structure Determination

Sir:

In the course of screening microorganisms for the production of anticoccidal and anthelmintic agents, a *Streptomyces* sp. designated LL-F35101 was isolated. This culture subsequently appeared to be identical with *Streptomyces albus* NRRL 3384, the producer of polyether antibiotic A204A.[1] Culture LL-F35101 was found to produce a new anthelmintic antibiotic, sulfinemycin, in additon to antibiotic A204A. This paper describes the fermenation conditions, the isolation procedures, and the chemical structure of this new anthelmintic antibiotic which contains a *trans* α,β-unsaturated thioamide S-oxide group which has not been previously observed in natural products.[2,3]

Culture LL-F35101 was grown in 500 ml Erlenmeyer flasks containing 100 ml of growth medium on a rotary shaker (200 rpm) at 28°C for 3 days. The growth medium consisted of (g/liter): Glucose 10, Dextrin (Soluble Starch) 20, Yeast Extract 5, N-Z Amine A 5 and $CaCO_3$ 1. This broth was used to inoculate the fermentation medium (5% v/v) which consisted of (g/liter): Dextrin 30, Proflo (Cottonseed Flour) 15 and $CaCO_3$ 1. The fermentations were carried out at 28°C for 5 days. Tank fermentations were carried out in 300-liter vessels. The inoculum, first grown in flasks as described above, was used to seed a 10-liter second stage innoculum which was aerated at 1 vvm and agitated at 450 rpm. This second stage innoculum was then used to inoculate a 300-liter fermentation medium which was aerated at 0.66 vvm and agitated at 220 rpm. The tank fermentation medium was the same as described above for flasks and was grown at 28°C and harvested after 4 days.

The procedure for the isolation of sulfinemycin is summarized in Fig. 1. The fermentation broth (pH 7.5), from two combined tank fermentations totaling 450 liters, was extracted with 225 liters of ethyl acetate. The organic phase was concentrated *in vacuo* to give 323 g of crude residue. A 10 g portion of this residue was dissolved in 100 ml of methylene chloride and adsorbed onto a dry packed column (5.7 cm × 71.1 cm) of silica gel. The column was then serially eluted with 2 liters of methylene chloride, 1 liter each of methylene chloride-ethyl acetate at ratios (v/v) of 75:35, 50:50 and 30:70, and finally 2 liters of ethyl acetate. Fractions of 25 ml were collected and were analyzed by thin layer chromatography (Analtech silica gel GHLF, Cat. No. 21511, CH_2Cl_2/EtOAc, 3:7). Sulfinemycin was detected on the TLC plates at an Rf value of 0.40. by UV_{254} quenching and Mazur spray reagent.[4] The fractions (96~105) containing sulfinemycin were pooled and concentrated *in vacuo* to yield 2.01 g of partially purified product. Further purification of the antibiotic was accomplished by preparative TLC using the same TLC system as described above. Pure sulfinemycin, 752 mg, was extracted with ethyl acetate from the zones of the developed plates.



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Sulfinemycin was obtained as a yellow amorphous powder. It was soluble in methanol, chloroform, and methylene chloride, but insoluble in ether and heptane. The molecular weight of sulfinemycin was 285 with a molecular formula of $C_{16}H_{31}NOS$ which was based upon elemental analysis and mass spectral data. Electron impact (EI) exact mass data of the fragment ions were also consistent with this composition. Selected physicochemical properties of sulfinemycin are listed in Table 1. A trace impurity with a molecular weight of 299 was also observed in the sample by mass spectrometry which probably corresponded to a CH_2 homolog of sulfinemycin.

The molecular formula suggested two or three unsaturated bonds depending on the oxidation state of sulfur. The ^{13}C NMR spectrum showed a low field carbonyl-like carbon (188.8 ppm) and two olefinic carbons (116.0 and 138.5 ppm). All of the other 13 carbons in the molecule were sp^3 type (Table 2). Terminal branching of the aliphatic chain was apparent from two

Fig. 1. Process for the isolation and purification of sulfinemycin.

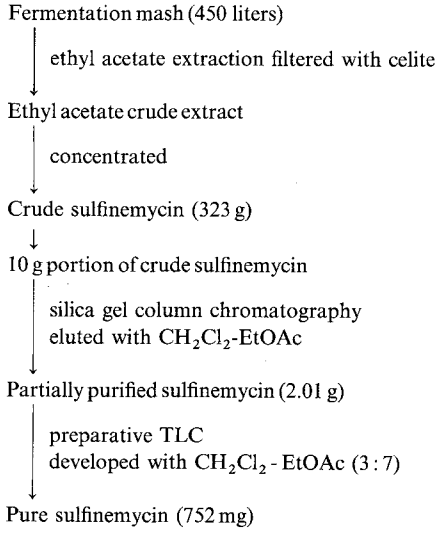


Table 1. Physico-chemical properties of sulfinemycin.

Appearance	Yellow amorphous powder
MP	84°C, Degrades at 105°C
$[\alpha]_D^{26}$	0° (*c* 0.325, $CHCl_3$)
Elemental	Calcd for $C_{16}H_{31}NSO$: C 67.96, H 10.69, N 4.63, S 10.82 Found: C 67.37, H 10.69, N 4.91, S 11.23
MW	285 (EI-, FD-, FAB-MS)
UV/MeOH nm	210, 247, 343
IR (KBr) cm^{-1}	3230, 3020, 2920, 2850, 1650, 1470, 960, 952, 930
Rf (TLC)	0.40 (adsorbant: Analtech silica gel GHLF, Cat. No. 21511, Solvent: CH_2Cl_2-EtOAc, 3:7, Detection: UV_{254} quenching and Mazur spray reagent)

superimposed C–CH_3 doublets at 0.85 ppm (J=6.7 Hz). Additional ^1H and ^{13}C NMR assignments of the molecule are given in Table 2. In order to account for the UV chromophore (λ_{max} 247 and 343 nm), the carbonyl-like carbon, the double bond, sulfur, nitrogen and oxygen were all believed to be involved. This assumption was supported by the EI mass spectrum (Fig. 2) which has an intense fragment ion C_3H_4NOS (*m/z* 102.0002) resulting from the loss of a saturated neutral radical $[C_{13}H_{27}]^{\cdot}$ from the molecular ion. Therefore, the C_3H_4NOS fragment ion was derived from the unsaturated chromophoric portion of the molecule (Fig. 3).

The chromophore was identified as an α,β-unsaturated thioamide S-oxide. A *trans* olefin was deduced from the ^1H NMR spectrum which had resonances at δ 5.80 (d, J=15.8 Hz) and δ 6.53 (d, t, J=15.8, 6.8 Hz). The ^{13}C NMR resonance at 188.8 ppm was consistent with the estimated value for the C-1 of an α,β-unsaturated thioamide S-oxide. The mass spectral fragmentation pattern and thermal degradation results discussed in the

Table 2. ^1H NMR and ^{13}C NMR assingments of sulfinemycin.

^1H NMR ($CDCl_3$, 300 MHz)					^{13}C NMR ($CDCl_3$, 75 MHz)		
Position*	δ (ppm)	Multiplicity	J (Hz)	No.	Position*	δ (ppm)	No.
2	5.80	d	15.8	1	1	188.8	1
3	6.53	d,t	15.8, 6.8	1	2	116.0	1
4	2.21	q	6.8	2	3	138.5	1
5	1.46	m		2	4	33.2	1
6~12	1.26	s		14	5~12	28.0~30.0	8
13	1.15	m		2	13	39.1	1
14	1.51	m		1	14	27.4	1
15, 16	0.85	d	6.7	6	15	22.7	1
NH_2	7.65	s		2	16	22.7	1
				31			16

* See structure **1** for numbering system.

Fig. 2. Electron impact mass spectrum of sulfinemycin.

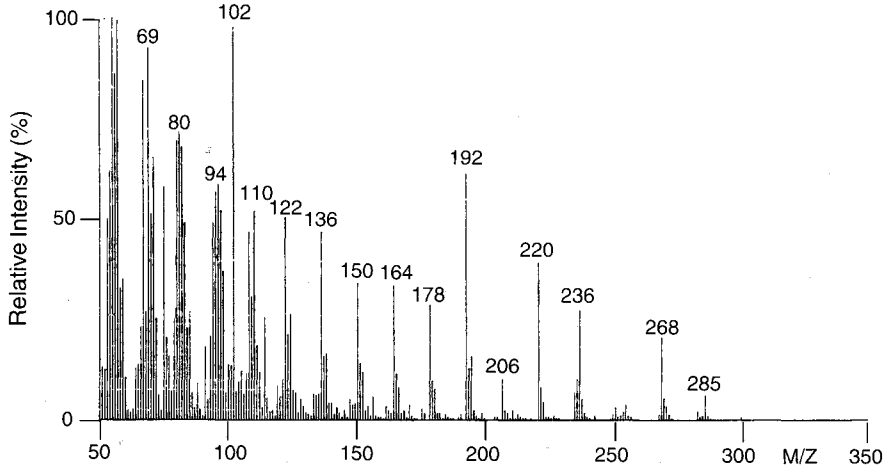
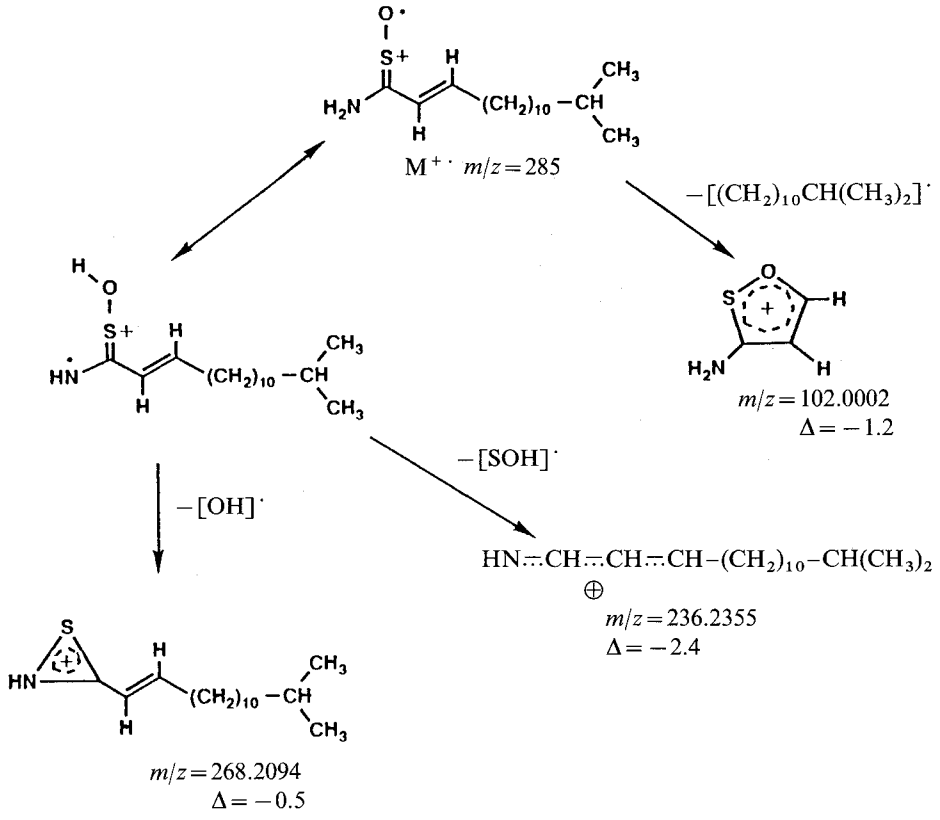
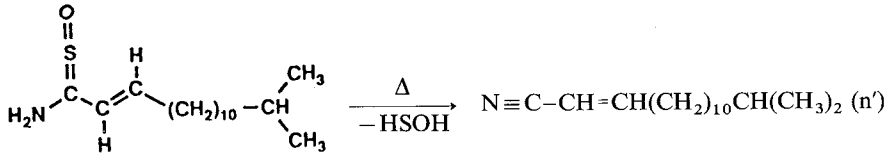


Fig. 3. Electron impact fragmentation processes for sulfinemycin.



(Δ=Experimental Mass−Predicted Mass in millidalton units)

Fig. 4. Thermal degradation of sulfinemycin and subsequent electron impact fragmentation of the conjugated nitrile hydrocarbon.



$$\xrightarrow[e^-]{EI} [M'-CH_3]^+ + [M'-CH(CH_3)_2]^+ + [N\equiv C-CH=CH(CH_2)_n]^+$$

$$n = 1, \ldots, 10$$

following sections were also consistent with this assignment.

Fig. 3 describes for sulfinemycin unusual mass spectral fragmentation processes in which product ions are stabilized by the formation of conjugated (*m/z* 236.2355) and aromatic systems (*m/z* 268.2094 and 102.0002). The ions at *m/z* 268 and 236 were formed *via* an intramolecular rearrangement and subsequent fragmentation with loss of [OH]· and [SOH]·, respectively. These unusual rearrangement ions are consistent with similar rearrangement processes reported for aromatic sulfines.[5] The ion at *m/z* 102 is formed *via* an intramolecular cyclization reaction which parallels the chemistry of intermediates formed in the reactions of α,β-unsaturated sulfines.[6,7] The other ions observed in the mass spectrum can be rationalized as arising from the fragmentation of a conjugated nitrile hydrocarbon which presumably was formed by thermal degradation of the parent compound (Fig. 4). The molecular ion for the conjugated nitrile (M′) was not observed, but rather the $(M'-CH_3)^+$ and $[M'-CH(CH_3)_2]^+$ ions as well as the homologous series $[N\equiv C-CH=CH(CH_2)_n]^+$, where $n=1, \cdots, 10$. The elemental composition for all the ions were confirmed by exact mass measurements.

Thermal studies of sulfinemycin with a differential scanning calorimeter and a thermogravimetric analyzer demonstrated that the compound melted at 84°C and degraded at temperatures above 105°C. The degradation

product generated from heating sulfinemycin over 115°C was monitored by IR spectrometry. The IR absorptions attributed to the original S=O group (960, 952, 930 cm^{-1}) and the NH_2 group (3230, 3020 cm^{-1}) disappeared and an absorption at 2220 cm^{-1} appeared consistent with that of a conjugated nitrile. The formation of the conjugated nitrile by thermal degradation was also consistent with the mass spectral data. The room-temperature IR spectrum of sulfinemycin resembles that of CH_3CSONH_2 as reported by WALTER and KUBERSKY.[8]

The tautomeric form of the NH_2CSO group was assumed to be the thioamide S-oxide group ($H_2N-\overset{|}{C}=S=O$) and not the iminosulfenic acid group ($HN=\overset{|}{C}-SOH$) based upon previous studies of these functional groups[2]. The *syn* or *anti* stereochemistry around the C=S bond of the $H_2N-\overset{|}{C}=S=O$ group was not determined.

Sulfinemycin has poor activity (MIC's of 128 μg/ml) against a limited number of Gram-positive bacteria, *e.g.*, *Staphylococcus aureus* (SSC 82-24), *S. aureus* (SSC 82-57) and *S. epidermis* (ATCC 12228) and is not active against any of the Gram-negative bacteria tested. It has marginal activity at 100 ppm in the diet of gerbils at four days, eliminating 52% of the helminth, *Trichostronglus colubriformis*.

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