of RNA bases, thymine analogs like 5-fluorodeoxyuridine, 5-bromouracil, and 6-azathymine which interfere with DNA synthesis (*6*, *7*) did not appreciably inhibit induction of two-dimensional growth in the gametophytes (Fig. 1*d*). A slight decrease observed in the length of the protonema and in the width, cell number, and surface area of the gametophytes grown in thymine analogs may be due to a nonspecific growth inhibition by the added compounds. These results imply that inhibition of RNA synthesis leads to an inhibition of two-dimensional growth of the gametophytes, while effects of interfering with DNA synthesis are exerted as a general retardation of growth. Indeed, when grown in a medium containing ribonuclease (0.8 to 1.0 mg/ml), two-dimensional differentiation in the gametophytes of this same species was completely blocked (*8*).

Further work showed that the nonspecific growth inhibition due to 5-fluorodeoxyuridine was completely reversed by thymidine, thymidylic acid, cytidine, and cytidylic acid; thymine, cytosine, and the purines were ineffective in reversing this inhibition (Table 2). The inhibition of two-dimensional growth induced by analogs of adenine and guanine was annulled by the corresponding purine bases and their derivatives; pyrimidines were ineffective in completely reversing this inhibition, although in some cases partial reversals did occur. Similarly, inhibition caused by antagonists of cytosine and uracil was reversed by the corresponding pyrimidine bases or their derivatives, but not by the purines (Fig. 1, *a* and *c*). Thus there is a requirement for the purine and pyrimidine bases of RNA in the induction of a two-dimensional growth pattern in the gametophytes of *Asplenium*; the requirement for the purines, adenine and guanine, was not met by the pyrimidines, cytosine, uracil, or thymine, and a requirement for cytosine and uracil was not satisfied by the purines or by thymine.

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Ethylation: Biological Formation of an S-Ethyl Homolog of Lincomycin

Abstract. Streptomyces umbrinus *var.* cyaneoniger *produces the antibiotic lincomycin. When grown in the presence of ethionine an additional antibiotic is produced. Physical and chemical analyses and incorporation of radioisotopes indicate that the new antibiotic is the* S-*ethyl homolog of lincomycin.*

Ethionine has been reported to be a naturally occurring compound (*1*), and several examples of biological ethylation with it as an ethyl donor are known (*2*). The reactions occurring represent transethylation in place of normal transmethylation, and the ethyl group occurs attached to either an oxygen or a nitrogen atom in the final product. We now report an example of biological ethylation in which a new antibiotic, a homolog of the antibiotic lincomycin (*3*), was formed when *Streptomyces umbrinus* var. *cyaneoniger* (var. nov.) (*4*) was grown in the presence of ethionine. In this case the ethyl group is attached to a sulfur atom in the final product.

Streptomyces umbrinus var. *cyaneoniger,* isolated from soil, was grown under conditions of aeration and agitation at 28°C for 4 days on a medium consisting of soybean meal, 2 percent; glucose, 2 percent; corn steep liquor, 1 percent; sodium chloride, 0.3 percent; and calcium carbonate, 0.2 percent (*5*). An antibiotic, found in the culture broth, was isolated as the crystalline hydrochloride salt. It had the following properties: m.p. 152°–156°C; $[\alpha]_D$ +131 (*c*, 1.01, H_2O); *pK* 7.4 (neutralization equivalent 434); and essentially no absorption in ultraviolet and visibile light. Analysis (percentage): C, 48.25; H, 7.98; N, 6.24; S, 7.10; Cl, 8.13; O, 21.56; *O*—CH_3, 0.45; *N*—CH_3, 2.6; *C*—CH_3, 4.8; acetyl, 0.83. Calculated (percentage) for $C_{18}H_{34}N_2O_6S \cdot HCl$: C, 48.78; H, 7.97; N, 6.32; S, 7.24; Cl, 8.01; O, 21.68. These properties, together with the mobility on paper chromatography and the infrared absorption spectrum (*6*) indicate that the antibiotic is identical with lincomycin (*3*).

In other similar fermentations of *Streptomyces umbrinus* var. *cyaneoniger,* 0.006 percent DL-ethionine was added after 48 hours of growth. At harvest time a second antibiotic was detected in the culture broth by paper bioautography against *Corynebacterium xerosis* on *p*H 7.9 nutrient agar medium. By developing on No. 1 Whatman paper in the system, *n*-butyl alcohol, water, isoamyl alcohol, and dichloroacetic acid (100 : 75 : 50 : 1), the R_F values of lincomycin and the new compound were 0.50 and 0.65, respectively. A concentrate containing the two antibiotics was prepared by the procedure used for the isolation of lincomycin as follows. The antibiotics were adsorbed from the culture filtrate by activated charcoal (1.5 percent weight/volume) and eluted with a mixture of acetone and water (9 : 1). The eluate was concentrated to an aqueous phase and adjusted to *p*H 10.5. This was extracted with two equal volumes of *n*-butyl alcohol. The combined *n*-butyl alcohol extracts were extracted twice with one-fifth volume of water adjusted to *p*H 2.5 with hydrochloric acid. The aqueous phase was lyophilized. The residual solid was about 90 percent pure antibiotic, containing lincomycin and the new compound in the ratio of about 10 : 1. The antibiotics were present, as shown by paper chromatography, in essentially the same ratio in the culture broth. Crystallization from acetone and methanol (5 : 1) resulted in no resolution because the antibiotics cocrystallized.

Lincomycin and the new compound were resolved by partition chromatography on diatomaceous earth, in the system composed of ethyl acetate and 0.5*M* phosphate, *p*H 6.2. The distribution coefficients were 0.05 and 0.09 for lincomycin and the new compound, respectively. Most of the new compound, which contained less than 2 percent lincomycin as judged by paper chromatography, was eluted with an amount of developing fluid equal to 3 to 5.5 times the void volume of the column. This portion of eluate was extracted with water adjusted to *p*H 2.5 with hydrochloric acid. The extract was

Table 1. Incorporation of labeled precursors into lincomycin and a new compound, the *S*-ethyl homolog of lincomycin. The labeled compound and unlabeled ethionine (100 μg/ml) were added 48 hours after inoculation. The compounds had the following specific activities: L-methionine-C^{14}-methyl, 20 mc/mmole; L-ethionine-ethyl-l-C^{14}, 1.2 mc/mmole; and DL-methionine-2-C^{14}, 0.6 mc/mmole.

Product	Specific activity (μc/mg)
L-Methionine-methyl-C^{14}	
Lincomycin	5.3
New compound	2.6
L-Ethionine-ethyl-1-C^{14}	
Lincomycin	0
New compound	1.4
DL-Methionine-2-C^{14}	
Lincomycin	0
New compound	0

neutralized to *p*H 4.5 with Amberlite IR-45 (Rohm and Haas Co.) and lyophilized. The residue was dissolved in methanol (200 mg/ml), and five volumes of acetone were added. After storage overnight at 4°C, the crystalline hydrochloride salt was collected and recrystallized twice in the same way. It had the following properties: m.p. 160° to 162°C; $[\alpha]_D$ + 141 (*c* 1.02, H_2O); *pK* 7.3 (neutralization equivalent 438); and no characteristic absorption in ultraviolet and visible light. Analysis (percentage): C, 49.16; H, 8.23; N, 6.40; S, 7.27; Cl, 8.01; O, 21.16; *O*—CH_3, 0.5; *N*—CH_3, 2.6; *C*—CH_3, 5.8; acetyl, 0.7. Calculated (percentages) for $C_{19}H_{36}N_2O_6S \cdot HCl$: C, 49.91; H, 8.17; N, 6.13; S, 7.02; Cl, 7.76; O, 21.01.

The nuclear magnetic resonance spectra of the two antibiotics, as the hydrochloride salts in deuterium oxide, were essentially the same except for one mutually exclusive feature in each not shared by the other. In both spectra, determined at 60 Mc/sec, there was a signal (3H) at 2.99δ which was assigned to a protonated *N*-methyl (*N*–CH_3) function (*7*). This is consistent with the chemical functional group analyses which indicate that each antibiotic probably contains 1 mole of *N*-methyl. Lincomycin had a singlet peak (3H) at 2.17δ corresponding to *S*-methyl (*8*). This signal was absent in the new compound, and instead there was a quartet at 2.65δ (2H, J = 6 cy/sec, J being the spin coupling constant) (*7*) and a triplet at 1.27δ (3H, J = 6 cy/sec) which was interpreted as *S*-ethyl (*S*–CH_2–CH_3) (*8*). These data suggest that lincomycin contains a methyl thioether group and that the new compound is the homologous ethyl thioether compound. This interpretation is permissible on the basis of the elemental analyses. If this constitutes the only difference between the two antibiotics, then the new compound should also contain one more *C*-methyl group than lincomycin. This was substantiated by functional group analyses (*9*). The structure of lincomycin was recently reported (*10*), and the foregoing conclusions concerning the functional groups in it are correct.

In a study of the radioactive-labeling pattern in the two antibiotics produced in the presence of certain radioactive precursors, C^{14} from L-methionine-methyl-C^{14} was incorporated into both antibiotics (Table 1). However, the amount found in the new compound was one-half that in lincomycin. This is the proportion expected if the conclusions previously made concerning the relation between the structures of the antibiotics are correct. The groups labeled would be one *N*-methyl and one *S*-methyl in lincomycin and one *N*-methyl in the new compound. Neither antibiotic contains any *O*-methyl. Radioactivity (C^{14}) from L-ethionine-ethyl-1-C^{14} was incorporated into the new compound but not into the lincomycin, suggesting the presence of an ethyl group in the new compound and its absence in lincomycin. Label from DL-methionine-2-C^{14} was incorporated into neither antibiotic. The last observation rules out the possibility that methionine was incorporated as a unit into the antibiotics, thus restricting the reactions to alkylation.

These data suggest that the *S*-ethyl homolog of lincomycin is formed by a process of *S*-transethylation with ethionine replacing methionine as alkyl donor. However, label from S^{35}-methionine was incorporated into both lincomycin and the new compound, and the possibility that the new compound arises by a process of ethylthiolation has not been excluded. To our knowledge, neither biological *S*-transethylation nor ethylthiolation have previously been reported.

The production of the new compound was dependent upon the presence of ethionine as a specific source of *S*-ethyl groups. Neither *S*-ethyl-L-cysteine nor L-rhamnose diethylmercaptal could serve as a source of *S*-ethyl groups. Out of 32 compounds tested only D-, L-, and DL-ethionine caused the formation of the new compound. High concentrations of L-methionine (0.5 mg/ml) prevented the ethionine-stimulated production of the new compound.

After the completion of this work, the isolation of an *S*-ethyl homolog of lincomycin was reported from the culture filtrate of *Streptomyces lincolnensis* var. *lincolnensis* fed DL-ethionine (*11*).

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5. We thank M. Dann, A. C. Dornbush, Dr. J. S. Schultz, Dr. S. O. Thomas and staffs, and the organic chemical section of our laboratories, for large-scale extractions, microbiological assays, large-scale fermentations, preliminary microbiological work, and micro and spectroscopic analyses, respectively.
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Nervous Control of Ciliary Activity

Abstract. *Electrical stimulation of the visceral ganglion of the mussel* Mytilus edulis *caused cilia on the lateral epithelium of the gill to beat faster. This effect was blocked by cocaine, physostigmine, hyoscyamine, and acetylcholine but not by tubocurarine. These agents did not block the cilioexcitatory effect of serotonin.*

The beating of cilia on the lateral epithelium of the gill of the mussel *Mytilus edulis* provides the force for propelling water through the gills. This