TRANS-3-(METHYLTHIO)-ACRYLIC ACID, A NEW METABOLIC PRODUCT FROM *STREPTOMYCES LINCOLNENSIS*

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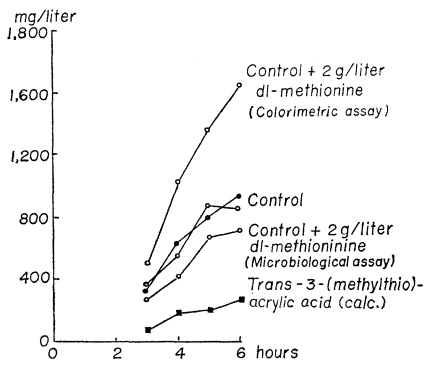
During development of the lincomycin* fermentation, we studied the effect of the addition of *dl*- and *l*-methionine to the culture medium. The microorganism used for these experiments was a mutant of *Streptomyces lincolnensis* var. *lincolnensis* sp. n.[1] The broth obtained in this way showed a significantly higher potency with a chemical assay method[2] than with the microbiological assay (*Sarcina lutea*)[3], as shown in Fig. 1. The chemical assay is based on a colorimetric determination of methyl mercaptan generated from lincomycin on acid hydrolysis[4].

The gas mixture obtained by hydrolysis of filtered broth was analyzed by vapor phase chromatography[5]. Methyl mercaptan was the only sulfur-containing compound present. Methionine itself does not form methyl mercaptan under the experimental conditions used. Therefore, methyl mercaptan originated not only from the lincomycin, but also from at least one other compound with a lower (or no) bioactivity.

Fig. 1. Comparison of colorimetric and microbiological assays of lincomycin with and without added *dl*-menthionine (2 g/liter).

Assays expressed as lincomycin base (mol. weight 406) and as methylthio-acrylic acid (mol. weight 118).



This material was isolated in the following manner: First, the filtered broth was extracted with *n*-butanol at pH 2.5. Then the chemically active compound was re-extracted into water at pH 10. Traces of lincomycin were removed by extraction with methylene chloride. The remaining aqueous solution was adjusted to pH 7 and evaporated to 1/10 of its volume. Addition of ten volumes of acetone precipitated impurities as an oil, which was discarded. The remaining solution on concentration gave crude crystals which were recrystallized successively from acetone/water, ethanol/water, and ethanol.

The purified compound melted at 140°C and was identified by IR, NMR, mass spectrum, and elemental analysis as *trans*-3-(methylthio)-acrylic acid. Analysis calculated for $C_4H_6O_2S$ (in percent): C 40.66, H 5.12, O 27.08, S 27.14; found: C 40.84, H 5.09, O 26.90, S 25.28. Molecular weight: 118 (mass spectrum). Chemical shifts and their assignment in δ (rel. TMS) of an NMR spectrum at 60 mc in d_6 acetone: CH_3 2.40, COOH 9.58, olefinic H 5.82 and 7.80, doublets with J=15 cps (indicates transconfiguration).

Fig. 1 shows the calculated course of synthesis of this compound during the fermentation.

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References

1) MASON, D.J.; A. DIETZ & C. DEBOER: Lincomycin, a new antibiotic. I. Discovery and biological properties. Antimicr. Agents & Chemoth.-1962: 554~569, 1963
2) PROSCOTT, G. C.: Automated assay for the antibiotic lincomycin. J. Pharmaceut. Sci. 55: 423~425, 1966
3) HANKA, L. J.; D. J. MASON, M. R. BURCH & R. W. TREICK: Lincomycin, a new antibiotic. Microbiological assay. Antimicr. Agents & Chemoth.-1962: 565~569, 1963
4) HOEKSEMA, H.; B. BANNISTER, R. D. BIRKENMEYER, F. KAGAN, B. J. MAGERLEIN, F. A. MCKELLAR, W. SCHROEDER, G. SLOMP & R. R. HERR: Chemical studies on lincomycin. I. The structure of lincomycin. J. Am. Chem. Soc. 86: 4223~4224, 1964
5) FARRUGIA, V. J. & C. L. JARREAU: Separation of isopropyl alcohol from aliphatic sulfides and thiols by gas chromatography. Anal. Chem. 34: 271~273, 1962

* Trade name of the Upjohn Company: Lincocin