TOPICS IN MICROBIAL CHEMISTRY

F. M. Strong

CHEMISTRY OF MICROBIAL PRODUCTS

Topics in Microbial Chemistry

ANTIMYCIN, COENZYME A, KINETIN AND KININS

By F. M. STRONG

t of Biochemistry
University of Wisconsin, Madison

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In recognition of the importance of cooperation between chemist and microbiologist the E. R. Squibb Lectures on Chemistry of Microbial Products were established with the support of The Squibb Institute for Medical Research in 1955. The lectures are presented annually in the fall at the Institute of Microbiology, Rutgers, the State University of New Jersey, New Brunswick, New Jersey.

PREFACE

The title *Topics in Microbial Chemistry* was chosen for these lectures because a good part of my scientific activities during the past twenty-five years has been concerned in one way or another with the chemistry and biochemistry of microorganisms. This came about through my association, starting in 1932, with Professor W. H. Peterson, who was particularly interested in bacterial nutrition and the relation of bacterial growth factors to vitamins (reference 1, p. 42). The bacterial nutrition work led to an assay method for riboflavin (reference 2, p. 42) which was so successful that much later work was oriented along similar lines.

No attempt will be made in these lectures to provide a comprehensive review of the topics considered. The object rather will be to present a capsule view of several individual research projects and to illustrate how the use of certain experimental techniques contributed to such progress as was made. The substances to be considered, antimycin, coenzyme A, and kinetin, are quite unrelated, but they do

have in common the fact that they are physiologically active at very great dilutions and are obtainable from microorganisms.

F. M. STRONG

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CHAPTER

1

ISOLATION, STRUCTURE, AND PROPERTIES OF ANTIMYCIN A

PRODUCTION AND ISOLATION

In 1945 Leben and Keitt, working in the Department of Plant Pathology at the University of Wisconsin, noted that a plate culture of the apple scab organism, *Venturia inequalis,* was markedly inhibited by a white, slow-growing, actinomycete contaminant (3). Following the classical pattern of Sir Alexander Fleming, they isolated the contaminant, succeeded in growing it in pure culture, and demonstrated that the active inhibitory substance could be recovered in crude form from extracts of the culture filtrates. Since the antibiotic appeared to be new, and was particularly effective against fungi, it was named *antimycin.*

The problem was brought to the Department of Biochemistry at this point for help in obtaining the active substance in pure form and determining its chemical nature. To this end a simplified culture medium was prepared from glucose, soybean oil meal, and calcium carbonate, and a series of 70-liter fermentations was carried out (Table 1.1).

TABLE 1.1

Culture Methods for Antimycin Fermentation

Medium	40 grams of soybean oil meal, 20 grams of glucose, and 1.5 grams of calcium carbonate per liter
Conditions	70 liters of medium in a 160-liter iron tank, 80- to 96-hour fermentation, 24–28°, continuous stirring, aeration rate of 50–100 liters per minute
Inoculum	Prepared from a dried soil culture of the *Streptomyces* species by growing on the soybean medium in 2-liter shake flasks

After considerable work the antibiotic was isolated in crystalline form by the purification procedure shown in Fig. 1.1 (4). The recrystallized product is shown in Fig. 1.2.

Although it looks simple and straightforward, we had a lot of trouble developing the purification procedure. Many of the fractions were dark-colored, tarry messes, the bioassay often gave variable results on similar samples, activity sometimes mysteriously disappeared along the way, and not infrequently the starting material itself—the original fermentation broth—was contaminated, inactive, or lost because of excessive foaming. Even today we are still plagued with difficulties in producing and purifying antimycin, and our greatest single problem in studying its chemical nature and biological properties has been to get enough of it.

Isolation by Other Workers

For these reasons we have been very pleased that antimycin has now been obtained in several other laboratories. Almost simultaneously with our own original isolation work, Burger, Teitel, and Grunberg at the Hoffmann-La Roche laboratories in Nutley, New Jersey, crystallized

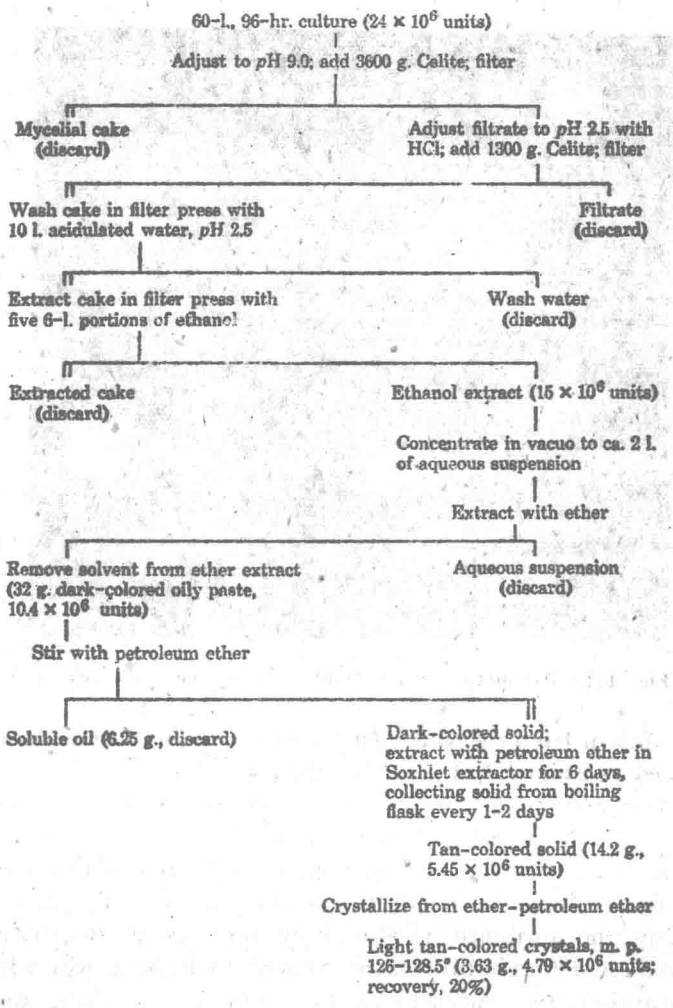

Fig. 1.1. Flow sheet of procedure for obtaining crude crystalline antimycin. (Modified from Dunshee et al. (4).)

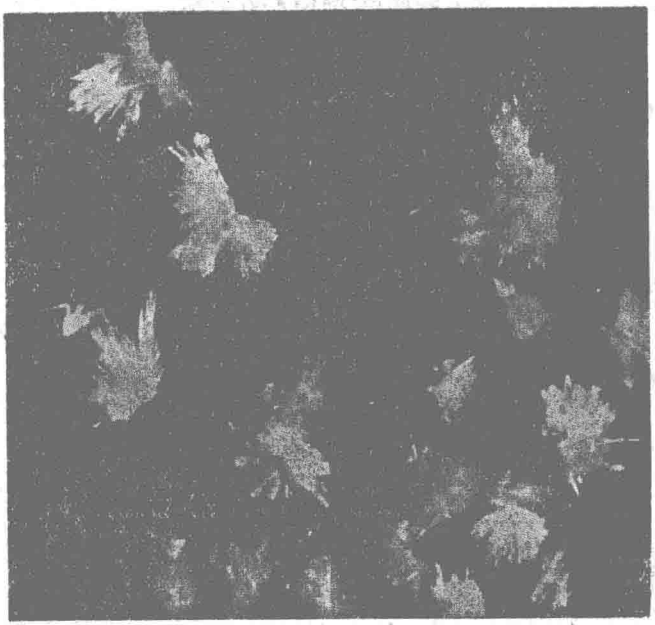

Fig. 1.2. Antimycin crystals from ether-petroleum ether (×28).

antimycin from two different *Streptomyces* organisms designated as isolates X-41 and X-4992 (5).

Somewhat later the Wisconsin group screened several thousand organisms for production of antibiotics effective against a series of plant pathogens. Of three cultures selected as being sufficiently promising to warrant further study, the antibiotic produced by one, isolate 102, when obtained in crystalline form, proved to be identical with antimycin (6). Accordingly this product was designated antimycin A-102. The original culture of Leben and Keitt was then called isolate 35 and the antibiotic antimycin A-35 (6).

A third group of workers, Harada et al., at Kyowa Fermentation Industry Co., Ltd., Tokyo, Japan, found that antimycin was identical with an antibiotic isolated by them and named *antipiriculin* A (7). In this case the isolated antimycin was recovered from the *mycelium* by extracting with ethyl acetate and adding petroleum ether to the extract.

Very recently an antibiotic called "blastmycin," which appears to consist largely of one of the components of the antimycin complex, has been obtained in pure form by Yonehara of the University of Tokyo [1] (8). Still another antimycin-like antibiotic called *virosin* has also been reported (9).

Antimycin-Producing Organisms

The original isolate 35 of Leben and Keitt (3, 6) was placed, according to the classification of Waksman and Henrici (10), as an undetermined species in the genus *Streptomyces.* A culture of this organism is now deposited in the permanent collection of microorganisms as NRRL 2288 with the Culture Collection Section, Fermentation Division of the Northern Regional Research Laboratory,[2] at Peoria, Illinois (11). The characteristics of this culture and of isolate 102 were later described more closely, and the two were shown to be different (12, 13).

Cultures X-41 and X-4992 of the Hoffmann-La Roche group (5) have not been described in a formal publication, but X-4992 is distinguished from other antimycin-producing

[1] A sample of blastmycin was very kindly placed at our disposal by Dr. Yonehara.

[2] Now called the Northern Utilization Research Division, Agricultural Research Service, United States Department of Agriculture.

organisms by the fact that it also forms large amounts of actinomycin B.

Harada and co-workers compared their antimycin-producing cultures 48B3 and 21A2 with NRRL 2288 and showed they were not the same. Both 48B3 and 21A2 belonged to the same species of *Streptomyces,* and since this differed from known species it was named *Streptomyces kitazawaensis* Harada *et* Tanaka nov. sp. (14, 15). Both cultures also produced a new antitumor substance named *carzinocidin* (16).

Cultural characteristics of strain 455D1 of Watanabe et al. (8), which produces blastmycin, were described by these authors, and the organism was named *Streptomyces blastmyceticus.* Several other antimycin-producing strains were described by Sakagami et al. (9).

Antimycin Supplies

Thus at least seven organisms, all apparently different, produce antimycin. However, in spite of this favorable circumstance, our supplies are still pitifully meager. Although no precise record has been kept, I estimate that the sum total of the high-purity crystalline antimycin (m.p. 140° or above) that we have had for study and for distribution to other investigators up to the present time probably has not exceeded 20 grams.[3,4]

There are several reasons for this situation. One of the

[3] However while this manuscript was in preparation, a larger supply was very kindly made available to the author by Dr. Y. Harada.

[4] An antibiotic also called antimycin is now being produced and marketed by Borkent-Pharmacie, of Haarlem, Holland, but the author has been unable to obtain a sample of it. Probably it is different from our antibiotic

main ones is that relatively little effort has been devoted to the development of high-yielding cultures or to working out the optimum conditions for the fermentation. Another difficulty has been one which must be common to most academic people, namely, a lack of sufficient pilot-plant facilities. The industrial worker usually does not suffer from this particular bottleneck, but he in turn is necessarily limited in the time he can give to problems of purely "academic" interest, e.g., the structure of toxic antibiotics.

Such problems may have high scientific merit, and are eminently suitable for university research, but to obtain the purified antibiotic or other microbial product in sufficient amounts is very difficult for the university worker. It would be a great help if some way could be found to provide academic laboratories with the equipment for working up relatively large quantities of material—let us say on a scale of about 100 to 500 gal. per batch. Such quantities are practically always needed in working with physiologically active natural products, which characteristically occur only in trace amounts. Possibly certain items of pilot-plant equipment could be donated by the industry—items which for one reason or another are no longer suitable for the daily grind of industrial use but which would be perfectly adequate for occasional runs in connection with an academic research project.

Of course it is equally essential that personnel competent to operate such large-scale equipment be available, and unfortunately this also is frequently not the case in the university laboratory. No doubt the best solution, for the academic worker, would be for industry to prepare the product for him, but this often is not feasible.

MULTIPLE NATURE OF ANTIMYCIN

To return to our main subject, still another difficulty soon presented itself, namely, that the crystalline antimycin so laboriously obtained was by no means pure, but that it consisted in fact of a mixture of three or four separate components. This, of course, is more the rule than the exception among antibiotics, and in fact among many other groups of natural products. Criteria of purity which would ordinarily be adequate for organic chemicals often fail in such cases, and antimycin is an excellent example. The product was recrystallized to constant melting point, 141–142° from four different solvents, and several preparations showed consistent physical properties and analytical values, yet paper strip chromatography, when properly carried out, revealed the presence of four active components (Fig. 1.3). As seen in Fig. 1.3, the crude crystalline antimycins produced by different cultures may vary in the proportion of the individual components which they contain. One organism, the Hoffmann-La Roche culture X-41, is said to produce only one form of the antibiotic, and this if true would be a great advantage for chemical studies. Efforts to produce antimycin with this organism are currently in progress.

When examined by similar techniques, blastmycin appeared to consist almost exclusively of one component, although traces of a second active substance were also detectable (17). In this preliminary study it has not yet been determined which of the components of antimycin A-35 or A-102 corresponds to blastmycin.

Slight differences between blastmycin and antimycin in regard to elementary composition, infrared spectrum, Millon's color test, toxicity to mice, and antifungal potency