IDENTIFICATION OF ERYTHROMYCIN A IN CULTURES OF STREPTOMYCES GRISEOPLANUS*

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SUMMARY. Erythromycin A has been identified in cultures of Streptomyces griseoplanus.

During 1967 an arrangement was made[1] to collaborate with workers at the University of Agricultural Sciences, Bangalore, India in the isolation and characterization of antibiotically active substances present in cultures of three actinomycetes which had been isolated from soils in that area (1). Preliminary studies including partial isolation of the antibiotic substances had been carried out, but none of the compounds involved had been identified at that time (1). Two papers were subsequently published describing the results of this work (2,3). Since these are incomplete in certain respects[2] and appeared in journals which may not be readily available, we wish to report the relevant collaborative studies carried out in our laboratory.

Only one of three organisms provided by the Bangalore group

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[1]By F.M.S., while on a George Ives Haight Travelling Fellowship in Natural Sciences awarded by the University of Wisconsin.

[2]The papers were published without our knowledge.

proved to be of interest because the other two either lost their ability to produce active materials or produced antibiotics which were too unstable for isolation. However, the actinomycete initially referred to as culture C-6 (1) and now identified as Streptomyces griseoplanus (2) proved to be more promising. This culture, on being received in our laboratory, was transferred to nutrient agar slants which were used as inoculum for shake flask cultures. These cultures were grown in 500 ml. Erlenmeyer flasks each containing 100 ml of a soybean meal-peptone-dextrose medium recommended by Rangaswami (4). After 5 days incubation at 30° on a rotary shaker the cultures were centrifuged and the broth worked up as indicated in Chart 1.

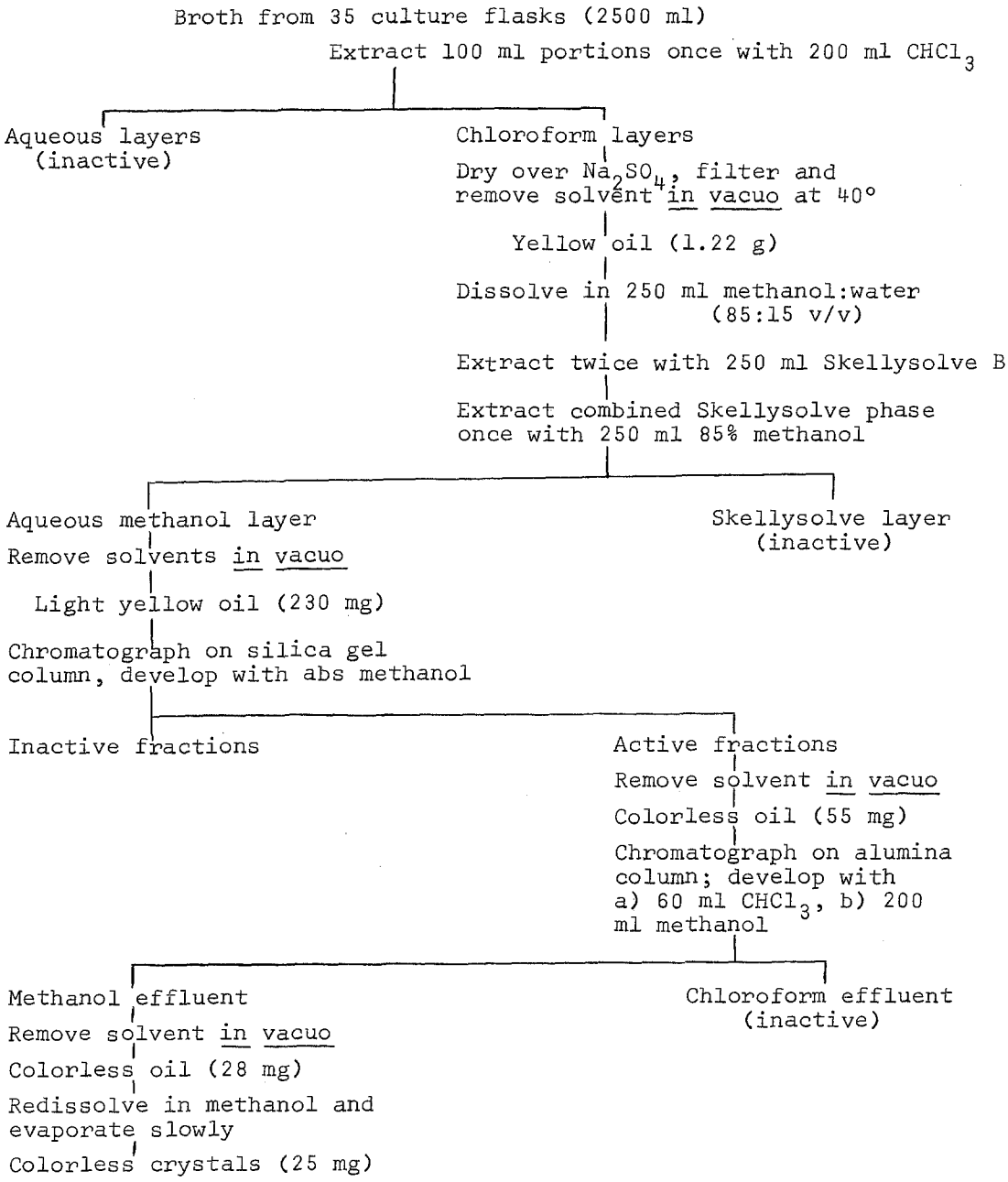
The purification procedure was guided by bioassays against a strain of Pseudomonas solanacearum (K-60)[3] by means of a filter disc assay.

The isolated product consisted of colorless crystals, m.p. 133-8°, $[\alpha]_D^{25°}$ -66.4° (0.5% in methanol), homogeneous by thin layer chromatography. The infrared spectrum showed large hydroxyl absorption (3476 cm^{-1}) and rather small carbonyl absorptions (1740 cm^{-1}, 1710 cm^{-1}), reminiscent of macrolide-type antibiotics. An examination of the melting points and optical rotation values tabulated for macrolides by Morin and Gorman (5) suggested that the C-6 antibiotic might be erythromycin A. An authentic sample of erythromycin A[4] was therefore obtained and compared with the isolated product from the C-6 culture. The infrared and proton magnetic resonance spectra of the

[3]Kindly provided by Professor Arthur Kelman, Department of Plant Pathology, University of Wisconsin, Madison.

[4]Abbott Laboratories, North Chicago, Ill.

CHART 1: Isolation of C-6 Antibiotic.



two samples were essentially superimposable. The mass spectrum of the C-6 compound showed a molecular ion at m/e 733 and large peaks at m/e 158 and 174. These are also the principal

peaks defining the mass spectrum of erythromycin A. No molecular ions were observed for either erythromycin B (m/e 717) or erythromycin C (m/e 719). The elemental analyses on the isolated product (C, 60.17; H, 9.39; O, 28.03) compared favorably with theoretical values for erythromycin A (C, 60.55; H, 9.21; O, 28.34). These data taken together definitely establish the identity of the C-6 antibiotic as erythromycin A[5].

During the purification studies summarized in Chart 1 no separation of activity into side fractions was observed. It appeared therefore that only one antibiotic was being produced by the culture or that any others that might have been formed were either inactive against the assay organism used or were formed in very minor amounts. As far as we are aware erythromycin has previsouly been found only as a metabolite of Streptomyces erythreus (6). Further studies have indicated that erythromycin A may be of value in the control of important plant diseases in India (2,3).

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[5]This result was reported to the Bangalore group in January, 1969