Technical Papers

Paper Chromatographic Identification of the Actinomycins

Leo C. Vining and Selman A. Waksman
Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

The actinomycins, in common with many other antibiotics containing a polypeptide moiety, have been demonstrated to consist of a group of closely related compounds probably differing only in the kind, number, and arrangement of their amino acids (*1*).

By means of countercurrent distribution studies using solvent systems containing n-dibutyl ether, methyl butyl ether, and the sodium salts of aromatic sulfonic acids in water, Brockmann and coworkers (*2, 3*) were able to show that actinomycin C consisted of at least three components, C_1, C_2, and C_3. Actinomycin X was also separated into three components, X_0, X_1, and X_2, and was, as far as could be ascertained, indistinguishable from actinomycin B. Actinomycin I (*4*) appeared to be an almost homogeneous substance, identical with a sample supplied by our laboratory as actinomycin A (see later). Circular paper chromatography employing similar solvent systems also separated the actinomycins into their components, and parallel results were obtained (*3, 5*).

In view of the successful separation of basic antibiotics by paper chromatography with solvent systems containing p-toluenesulfonic acid (*6*), a similar method was applied to the actinomycins (*7*). Mixtures of n-butyl ether and benzene or ethyl acetate with aqueous solutions of a number of aromatic sulfonic acids were tested and found satisfactory. N-dibutyl ether, ethyl acetate, 2 percent naphthalene-β-sulfonic acid (3:1:4) was chosen as the most suitable. Excellent results were obtained with this system using either circular or ascending techniques. Whatman No. 1 paper was dipped in the aqueous phase and blotted between sheets of filter paper, and samples of the actinomycins to be tested were applied in acetone solution. The deep red color of the naphthalene-β-sulfonic acid salt facilitated detection of the zones.

The following samples of actinomycin were examined: (i) authentic actinomycin A produced from *S. antibioticus* 3435 (*8*) in 1940; (ii) actinomycin produced from *S. antibioticus* 3435 (*8*) in 1953; (iii) actinomycin B obtained from Hoffman LaRoche; (iv) an actinomycin produced from a species of *Streptomyces* obtained from Merck & Co., under their No. 6009; (v) an actinomycin, also designated as actinomycin B, produced from *Streptomyces* sp. 3687 (*8*) obtained from Delft, Holland; (vi) actinomycin C produced from *S. chrysomallus* 3657 (*8*); (vii) actinomycin D produced from *S. parvullus* 3677 (*8*), a new strain isolated in our laboratory in 1953 (*9*); (viii) an actinomycin produced from *Streptomyces* sp. 3491 (*8*) isolated in our laboratory in 1948; (ix) an actinomycin produced from *Streptomyces* sp. 3436 (*8*) isolated in our laboratory in 1944.

Resolution of the actinomycins into corresponding components was obtained by ascending paper chromatography. The results are summarized in Table 1. By circular paper chromatography, identical separations were achieved.

The actinomycins from the Merck and Delft strains showed no detectable differences from actinomycin B. In the actinomycin produced from strain 3491, the component of Rf 0.54 appeared to be present in much greater concentration than in actinomycin B, whereas that at Rf 0.30 was present in only trace amounts. The actinomycin A produced in 1953 differed from that produced from the same culture in 1940 in showing a trace of the component at Rf 0.02. Although it is possible that the composition of the actinomycin produced by this culture has changed slightly in the course of time, the methods of growing the culture and of isolating these two products were not identical, and there is some evidence to indicate that, as a result of repeated chromatography and recrystallization, the component at Rf 0.02 is removed from the material.

It has recently been verified that a sample supplied to H. Brockmann by this laboratory in 1949 as actinomycin A was, in fact, the actinomycin produced by culture 3436. At that time the heterogeneous nature of the actinomycins had not been recognized and, since the two products showed no outstanding differences in physical and chemical properties, they were assumed to be identical. As a result of the present study, although their similarity is confirmed, since each has the component at Rf 0.30 as the major fraction, they are shown to possess additional components in different proportions. Brockmann's actinomycin I is, therefore, identical, not with actinomycin A but with the actinomycin from strain 3436.

Table 1. Results of resolution of actinomycins into corresponding components.

Sample	Rf				
	0.02	0.30	0.47	0.54	0.60
Actinomycin A (produced in 1940)		xxx		xx	
Actinomycin A (produced in 1953)	x	xx		xxx	
Actinomycin B	x	xx		xxx	
Actinomycin from Merck culture 6009	x	xx		xxx	
Actinomycin from culture 3687	x	xx		xxx	
Actinomycin C		x	xx		xx
Actinomycin D		xxxx			
Actinomycin from culture 3491	x	x		xxxx	
Actinomycin from culture 3436	x	xxxx		x	

The actinomycin produced by *S. parvullus* behaves in our solvent system as a homogeneous substance and, since it is therefore different from any other actinomycin hitherto reported, we have proposed for it the name actinomycin D. Full details of the preparation and properties will be reported at the Symposium on Antibiotics, Washington, D.C., in Oct. 1954.

Whether those components from different actinomycins that have the same Rf value are, in fact, identical substances is at present under investigation. In view of the possible influences of the composition of the broth and the method of isolation on the proportion of the components present in each actinomycin, we have not assigned a distinguishing letter to the actinomycin produced by strain 3491.

We wish to express our appreciation to M. Tischler, of Merck & Co., who supplied the sample of authentic actinomycin A and the actinomycin from Merck strain 6009, to J. A. Aeschlimann of Hoffman LaRoche for actinomycin B, to the Northern Regional Research Laboratories for the *S. chrysomallus* culture, and to Dr. van der Laan of the Kon. Ned. Gist and Spiritusfabrik of Delft, Holland, for the actinomycin from strain 3687. The remaining actinomycins were isolated in this laboratory, and we are indebted to R. A. Manaker and F. J. Gregory for the preparation of many of the samples.

References and Notes

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7. The work reported in this paper was carried out under a grant from the Dorothy H. and Lewis Rosenstiel Foundation.
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Polarography with a Dropping Gallium Electrode

Paul A. Giguère and D. Lamontagne

Department of Chemistry,
Laval University, Quebec, Canada

The universal and almost exclusive use of the dropping mercury electrode in polarography depends on the unique properties of this metal. Gallium being the only other metal still liquid at ordinary temperature (mp 29.7°C), it could conceivably serve in a dropping electrode; however, its other properties are quite unsuited for that purpose, as was confirmed by the present unsuccessful attempt. Not only is gallium scarce and expensive, but also it is readily oxidized in air. The oxide coating, which in the solid prevents further oxidation, is continuously renewed in the molten state. As a result, liquid gallium wets glass, a condition most objectionable in a dropping electrode. Furthermore, it is less than half as dense as mercury, and its hydrogen overvoltage is quite small. Finally, it expands on freezing, as do antimony and bismuth.

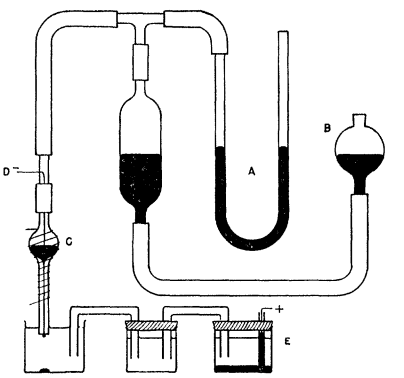

Fig. 1. Experimental arrangement of the dropping gallium electrode and electrolysis cell.

Since only about 4 ml (25 g) of metallic gallium, 99.99-percent pure (*1*) was available, a special capillary electrode was needed (Fig. 1). The capillary itself (bore diameter about 0.05 mm) had a small bulb *C* of some 10-ml capacity blown at a short distance from the tip to hold the supply of gallium; electric contact with this was secured through a fine platinum wire *D* sealed at the top. To make the liquid metal flow from the electrode, air pressure had to be applied over it by means of a leveling bulb *B* and a mercury reservoir. Although liquid gallium supercools readily, the polarographic cell was kept at 30°C in a constant temperature bath, and the electrode was wound with electrically heated resistance wire. The reference electrode *E* (saturated calomel) was of the external type to prevent the dropping gallium from mixing with the mercury of the anode. For working in absence of dissolved oxygen, some sodium sulfite was added to the electrolyte (0.1*N* KCl). The electric resistance of the entire circuit was about 2000 ohms. The current-voltage curves were recorded with a Sargent Model XI polarograph.

Despite all precautions, this gallium electrode always behaved erratically, especially with regard to dropping rate. This could never be reproduced from one run to the other with a given applied pressure. Therefore the figures in Table 1 must be taken only as a rough indication. Because the density of gallium is less than half that of mercury, while its surface is about 50 percent greater, one could expect that the drops falling from a certain capillary would be much larger than those of mercury; actually a factor of about 5 was observed. For the same reasons, the current intensity was proportionally higher. The measured rate of flow of gallium through the capillary electrode was appreciably greater than that calculated with the well-known equation (*2*) based on Poiseuille's