

Separation of actinomycins by thin-layer chromatography

Actinomycins are chromopeptides with antibiotic and cytostatic activity produced by species of Streptomyces¹. Chemically, actinomycins are characterized by a chromophoric phenoxazinone group, which is identical for all known actinomycins, and two pentapeptidic chains. The variation in the amino acid composition of the peptide chains gives rise to the different actinomycins².

The separation of actinomycins has been achieved by countercurrent distribution³, column chromatography⁴ and paper chromatography⁴⁻⁶.

In the course of studies on the biosynthesis of actinomycins it became necessary to separate rapidly the actinomycins of the C group² from those of the F group⁷. (Actinomycin C₁ has two molecules of D-valine in the peptide chain, while in actinomycin C₂ one molecule of D-valine is substituted by one molecule of D-alloisoleucine and in actinomycin C₃ two molecules of D-alloisoleucine are present. Actinomycins F₁ to F₄ are identical to those of the C group, but for the presence of three or four sarcosine molecules instead of two as in the case of the C group.)

Separation has been achieved (Table I) by chromatography on layers of alumina (Merck, G grade) or silica gel (Merck, G grade). Localization of the antibiotic is easily accomplished since actinomycins show a bright orange color (E_{\max} 440 to 450 m μ) and strongly absorb under U.V. light (E_{\max} 240 m μ). Identification of each actinomycin was achieved by semi-quantitative analysis of the amino acid content of the peptide chains⁸.

The actinomycins may be recovered from the plates by elution with methanol; as determined colorimetrically, 74% and not more than 50% of the substance applied was recovered from the silicagel and alumina layers, respectively.

If the chromatographic run and the elution of the antibiotic from the plates were performed in the dark or in dim light, the recovery of actinomycins from both types of layer became almost quantitative.

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TABLE I
SEPARATION OF ACTINOMYCINS C AND F

| Layer | Solvent | Distance* travelled cm | R _F | | | | | | |
|------------|--|------------------------------|----------------|----------------|----------------|----------------|---------|----------------|----------------|
| | | | C-group | C ₁ | C ₂ | C ₃ | F-group | F ₁ | F ₃ |
| Alumina | Ethyl acetate- <i>sym</i> -tetrachloroethane-water (3:1:3, v/v, bottom layer) | 12.5 | — | 0.44 | 0.51 | 0.58 | — | 0.21 | 0.35 |
| | Ethyl acetate- <i>di-n</i> -butyl ether-water (3:1:3, v/v, top layer) | 15 | — | 0.40 | 0.46 | 0.53 | — | 0.23 | 0.29 |
| | Ethyl acetate- <i>di-n</i> -butyl ether-water (2:1:2, v/v, top layer) | 17 | — | 0.28 | 0.30 | 0.33 | — | 0.10 | 0.13 |
| Silica gel | Benzene-ethyl acetate-methanol (10:2.5:1, v/v) | 15 | 0.24 | — | — | — | 0.13 | — | — |
| | Benzene-ethyl acetate-methanol (6:4:1, v/v) | 16 | 0.43 | — | — | — | 0.33 | — | — |
| | Butan-1-ol-methanol-water (6:1:3, v/v) | 14.5 | 0.63 | — | — | — | 0.53 | — | — |
| | Butan-1-ol-acetic acid-water (10:1:3, v/v) | 15 | 0.70 | — | — | — | 0.50 | — | — |
| | Ethyl acetate-propan-2-ol-water (5:2:1, v/v) | 15.5 | 0.95 | — | — | — | 0.75 | — | — |

* Migration time ranged from 30 to 60 min.

NOTES