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## Note

# Reversed-phase high-pressure liquid chromatography of actinomycins

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The actinomycin complexes are mixtures of closely related chromopeptides which differ only in limited areas of their peptide moieties[1]. In the C complex[2], for example, two of the ten amino acid sites may be occupied by two D-valines, ($C_1$ = D), two D-*allo*isoleucines ($C_3$) or one of each ($C_2$). Chromatographic procedures for the separation of such mixtures have played an important role in their subsequent structural elucidation[2] and comparative biological evaluation[3]. The most potent methods have involved partition chromatography. The high degree of lipophilicity of actinomycins has necessitated the use of a solubilizing agent in the aqueous phase. The earliest reported separation of an actinomycin complex (C) was achieved by counter-current distribution with urea in the aqueous phase[4]. Improved separations were obtained with sodium naphthalene-β-sulfonate or sodium *m*-cresotinate in the aqueous phase and dibutyl ether–butanol mixtures as the organic phase[5]. These and related solvent systems were successfully applied to paper[5–7] and cellulose column[5] chromatography, and the actinomycin A, B, C and X complexes were separated by these procedures. Preparative paper chromatography has also been utilized[8], and, for colummn chromatography, Sephadex G-25 has been described as a support for the aqueous phase[9]. Adsorption chromatography on silicic acid columns[10] and preparative thin-layer chromatography on silica gel[11] have also been employed.

In considering the application of high-pressure liquid chromatography to actinomycins it was apparent that the classical partition systems might present technical difficulties. This communication describes the satisfactory use of a reversed-phase system in a high-pressure liquid chromatography apparatus.

## APPARATUS

A commercially available high-pressure liquid chromatograph from Waters Ass., Framingham, Mass., U.S.A., ALC202/6000 p.s.i., was used throughout this investigation. The instrument has a flow capability of 0–9.9 ml/min delivered by a positive displacement reciprocating pistons pump at pressures up to 6000 p.s.i. The chromatograph is equipped with a UV detector operating at 254 nm. Analytical columns were 6 ft.×1/8 in. stainless steel with an I.D. of 2.3 mm. Retainers for the column packing consisted of porous PTFE plugs and end fittings of 5-μ porous stainless frits embedded in reducing unions. Two reversed-phase column packings

were used: Bondapak $C_{18}$/Corasil and Bondapak phenyl/Corasil (Waters Ass.). These consist of a Corasil base with, respectively, an octadecylsilicone and phenylsilicone permanently bonded to the surface.

## MATERIALS AND METHODS

Four actinomycin preparations were used in this investigation: (i), actinomycin D (Merck) which is identical with $C_1$; (ii), actinomycin C complex (provided by

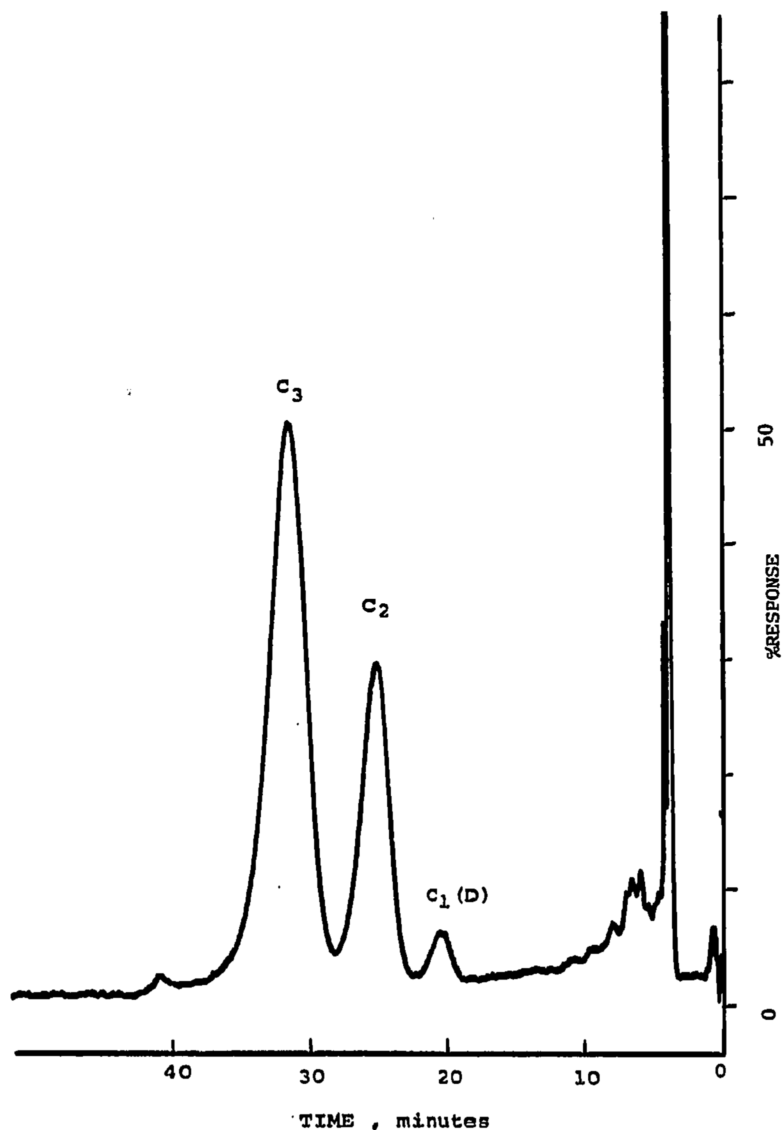


Fig. 1. Actinomycin C complex, 1 μl. Column: Bondapak $C_{18}$/Corasil, 6 ft. × 2.3 mm I.D. Solvent: acetonitrile–$H_2O$ (1:1), 22°. Flow: 1.0 ml/min, 1000 p.s.i. Attenuation: 08.

the National Cancer Institute) comprising $C_1$, $C_2$ and $C_3$; (iii), actinomycin $C_3$ (Squibb); and (iv), an actinomycin complex produced by *Streptomyces parvullus* in the presence of *cis*-4-chloro-L-proline, which contains a component identical with D and several additional components. Structural studies of the latter actinomycins, which contain *cis*-4-chloro-L-proline, will be reported elsewhere. Solutions (1%) of the various actinomycin samples in methanol were prepared and stored in the dark at −4° prior to use.

Columns were packed by vibration and tapping. The preferred polar solvent

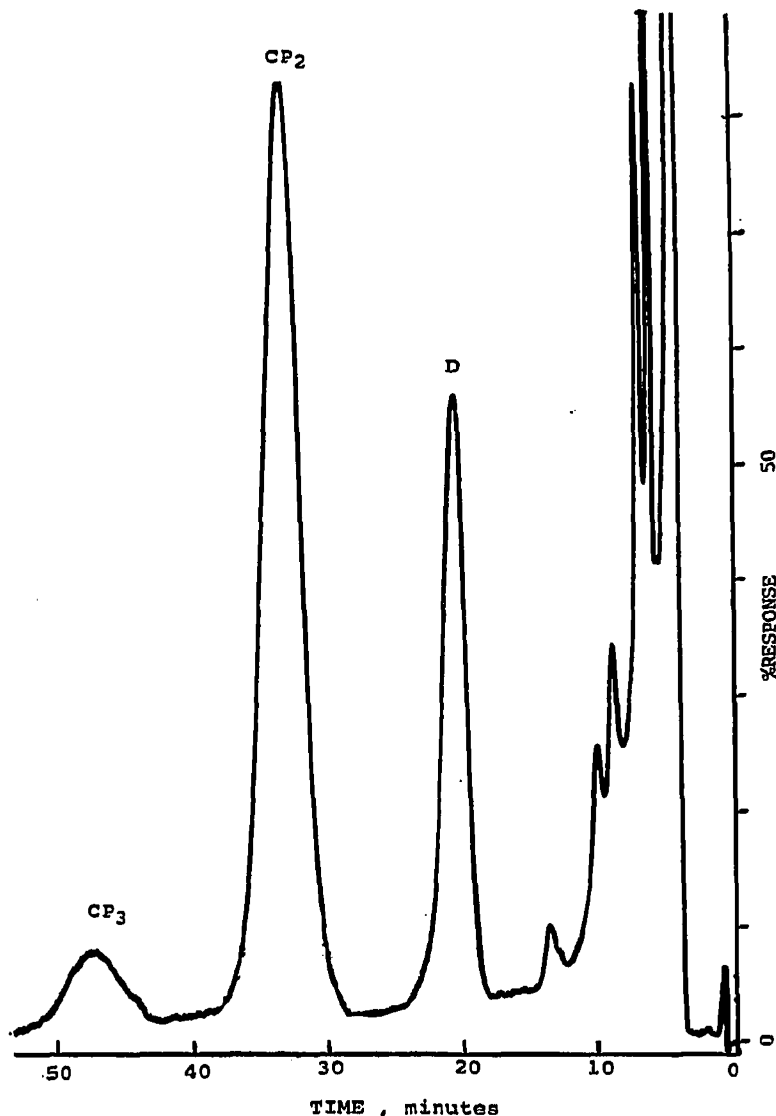


Fig. 2. Actinomycin complex from *S. parvullus* with *cis*-4-chloro-L-proline, 2 µl. Conditions as for Fig. 1.

system was a mixture of water and freshly distilled acetonitrile (1:1). Injections were made with a Precision Sampling 5-$\mu$l HP syringe.

## RESULTS AND DISCUSSION

Of the two column packings investigated, Corasil $C_{18}$ provided a superior resolution of actinomycin mixtures. Actinomycins D and $C_3$ gave single peaks and their retention times were used to identify the corresponding components in the complexes. The baseline separation obtained with the C complex is shown in Fig. 1. Fig. 2 illustrates the separation of the three major components (designated $CP_3$, $CP_2$ and D) of the actinomycin complex produced by *S. parvullus* in the presence of *cis*-4-chloro-L-proline. This organism normally produces essentially a single actinomycin (D)[12] and the new actinomycins observed here contain *cis*-4-chloro-L-proline in place of one or both proline residues normally present (details to be reported elsewhere). In addition to the three major components, this complex contains a number of minor, more polar components which emerge earlier and are not well separated under these conditions. The uniformity of the separated major components was confirmed by their chromatography in the same system using the recycling technique[13]. Preparative samples up to 10 mg were separated in the same system using a sample injection valve (Disc Inc. 706L) with a 125-$\mu$l capacity loop.

The technique described here represents the most rapid method thus far described for the separation of mixtures of closely-related actinomycins. It can be applied both to preparative separations and to analytical comparisons of various actinomycin complexes both qualitatively and quantitatively.

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