Note

SEPARATION OF ACTINOMYCINS AND BIOSYNTHETIC ANALOGUES BY NORMAL AND REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Chromatographic procedures for the separation and identification of actinomycins have been reviewed[1]. Only one report of HPLC has appeared[2]; this described reversed-phase separations of the C complex and of a biosynthetic mixture of actinomycin D (AMD) and congeners containing *cis*-4-chloroproline in place of one or both prolines. In the present study a wider range of naturally occurring actinomycins and analogues produced by directed biosynthesis[3] was chromatographed on both normal-phase and reversed-phase silica columns.

A Shimadzu LC-7A instrument equipped with an SPD-6AV UV-VIS detector (set at 445 nm) was used with a C-R6A Chromatopac data processor. The normal-phase column (from Rainin Instrument Co.) was 250×4.6 mm of Dynamax 150A 12 micron irregular silica and the reversed phase was the same bonded to C_{18}. The solvents were ethyl acetate-methanol (19:1) and acetonitrile-water (3:1) respectively, both run isocratically at 1 ml/minute. Sample injections were 20 μl of solutions containing 1 mg/ml of actinomycins in the same solvent as the mobile phase.

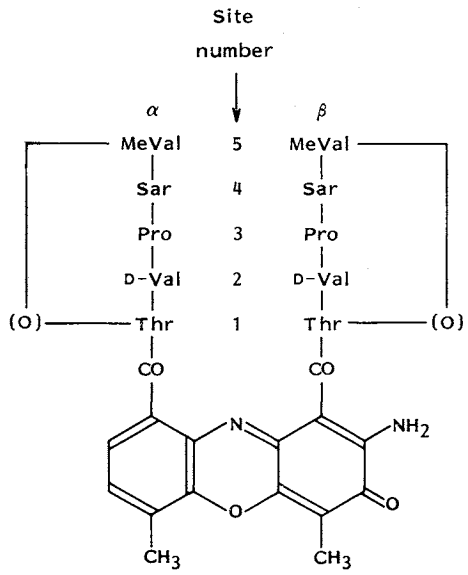
The structures of the actinomycins[4,5] are summarized in Fig. 1 and Table 1. For $X_{0\beta}$, $X_{0\delta}$ and V the replacements are at site 3' (β-peptide) rather than 3 (α-peptide); in other cases of aniso actinomycins this is not known or the isomeric mixture does not separate in these systems. Z_1 has multiple replacements: 1-γ-OH-Thr, 3-(4-keto-5-MePro), 3'-(3-OH-5-MePro), 5-MeAla. Abbreviations: Aze, azetidine-2-carboxylic acid; MeAla, *N*-methylalanine; MePro, methylproline (c, *cis*; t,

Table 1. Retention times of actinomycins[a] in minutes (RT) and relative to actinomycin D (RD) on reversed-phase C_{18} (ODS) and silica columns.

Actinomycin	Synonyms	Source (ref)	Structure (see Fig. 1)	ODS		Silica	
				RT	RD	RT	RD
D	IV, C_1, X_1	*	—	6.69	1.000	5.13	1.000
$X_{0\beta}$	I	*	3'-HyPro	4.50	0.673	8.62	1.680
$X_{0\delta}$		8	3'-*a*Hypro	6.05	0.904	5.98	1.166
II		9	3,3'-Sar_2	5.80	0.867	8.86	1.727
III		9	3-Sar	6.90	1.031	8.77	1.710
IIIA		6	3'-Sar	6.33	0.946	5.89	1.148
V	X_2	10	3'-(4-KetoPro)	6.56	0.981	4.44	0.865
C_2	VI	*	2'-D-*a*Ile	7.39	1.105	4.97	0.969
C_3	VII	*	2,2'-D-*a*Ile_2	8.12	1.214	4.97	0.969
Z_1		*	See text	4.74	0.709	7.02	1.368
Azα	Azetomycin II	11, 12	3,3'-Aze_2	5.48	0.819	7.19	1.402
Azβ	Azetomycin I	11, 12	3-Aze	5.91	0.883	6.76[b]	1.318
Pip 1β		13	3-Pip	8.00	1.196	4.43	0.864
Pip 2		13	3,3'-Pip_2	9.64	1.441	4.25	0.828
K_1c		14	3-c-4-MePro	7.46	1.115	4.68	0.912
K_2c		14	3,3'-c-4-$MePro_2$	7.06	1.055	4.43	0.864
K_1t		14	3-t-4-MePro	9.08	1.357	4.41	0.860
K_2t		14	3,3'-4-t-$MePro_2$	10.94	1.635	4.25	0.828

[a] Naturally occurring actinomycins are above the dotted line and biosynthetic analogues below.
[b] Second minor isomer (18%) observed at 5.64 minutes on silica column.
* Actinomycin samples supplied by Dr. E. KATZ, Georgetown University School of Medicine.

Fig. 1. Structures of actinomycin congeners are defined (see Table 1) in relation to AMD (shown) by amino acid replacements according to site number.



trans); MeVal, *N*-methylvaline; Pip, pipecolic acid; Sar, sarcosine.

Retention times for 18 actinomycins in the two HPLC systems are given in Table 1 and compared with those of AMD. In general, results accord with expectations based upon relative lipophilicities. An exception is K_2c, which behaves as less lipophilic than K_1c despite its extra methyl group. This may relate to the observation that K_2c is also anomalous conformationally[6]. Some striking separations of isomeric pairs were observed. For aniso isomers, representing replacement of an amino acid of AMD in the α versus β peptide, separation was observed in the case of sarcosine (III and IIIA) and azetidine-2-carboxylic acid (Azβ, see Table 1 footnote).

The data presented here have potential application to the identification of actinomycins which are occasionally isolated from new antibiotic cultures. Most mixtures are found to belong to one of three basic types: (i) I-V, (ii) C_1, C_2, C_3 and (iii) the Z complex (only Z_1 was available for this study). For positive identification of known compounds or structure determination of novel congeners the method can be used to collect samples of each component for Cf-252 plasma desorption mass spectrometry[7] and/or amino acid analysis. Preparative HPLC represents another possible extension of the methodology.

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