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Identification of Low-Molecular-Weight Nucleic Acid-Related Substances Secreted by *Streptomyces aureofaciens*

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Streptomyces aureofaciens growth in chemically defined medium is actively associated with the secretion of low-molecular-weight nucleic acid-related substances and is linked to low availability of phosphate. Thirteen pure compounds were isolated, of which seven were identified.

A previous paper (1) described the development of a chemically defined medium for the growth of a nonantibiotic producer *Streptomyces aureofaciens* strain associated with the active secretion of a complex mixture of low-molecular-weight nucleic acid-related substances. This note describes the fractionation of the mixture with the purification of 13 substances and identification of 7 compounds as purine and pyrimidine bases, nucleosides, and orotic acid.

Fractionation and purification of the fermented deproteinized filtrate was achieved by classical ion-exchange column chromatography (Fig. 1). The identification of the pure compounds was made by classical paper chromatography (2, 6) and UV spectrophotometry (1), with 76 natural and nonnatural purine and pyrimidine bases and derivatives serving as reference substances for standard and differential hydrolysis (3, 5).

Figure 1 shows the main fractionation and purification of the crude fermented *S. aureofaciens* broth (1) and shows the partial identification steps for the pure compounds.

Tables 1 and 2 show the well-defined and purified compounds present in the main anionic

and cationic fractions of the crude fermented broth. From eight anionic components, only component no. 2 could be identified with one of the 76 standard reference substances. It seems to be identical to orotic acid. The other seven substances could not be identified with any of the reference substances. However, some characteristics are as follows. (i) They do not contain phosphorus. (ii) They probably do not contain either D-ribose or D-deoxyribose. (iii) Compounds 1, 10, 13, and 14 are easily hydrolyzable, suggesting a purine-like derivative structure. (iv) Compounds 3, 4, and 6 behave as pyrimidine-like derivatives or as simple nitrogen bases. Components 4 and 6 are so similar that they can be taken as the same compound appearing in different fractions.

All six well-defined cationic components could be identified, despite their low concentrations in the deproteinized filtrate (Table 2).

In the previous paper (1), the *S. aureofaciens* low-molecular-weight substances, related to nucleic acid, were presumed to be secondary metabolites actively secreted by the microorganism in all growth phases as a response to low phosphate level, available in the chemically de-

TABLE 1. Anionic fraction components

Fraction	Compound no.	R_f^a		Avg spectral maxima (nm) by S_1 and S_2	Identification or characteristics	Concn in broth filtrate (mg/liter)
		S_1	S_2			
A ₁₁	1	0.51	0.53	240; 288	Purine-like derivative	11.0
A ₁₁	2	0.60	0.53	280	Orotic acid	
A ₂₁	3	0.60	0.61	260	Simple nitrogen base or pyrimidine-like derivative	
A ₂₁	4	0.72	0.77	250; 295	Simple nitrogen base or pyrimidine-like derivative	
A ₃₁	6	0.74	0.75	250; 295	Probably compound 4	
A ₄₃	10	0.71	0.76	248; 298	Purine-like derivative	
A ₅₁	13	0.66	0.74	248; 290	Purine-like derivative	
A ₅₂	14	0.68	0.65	268	Purine-like derivative	

^a S_1 , Paper chromatography system 1 (6); S_2 , system 2 (2).

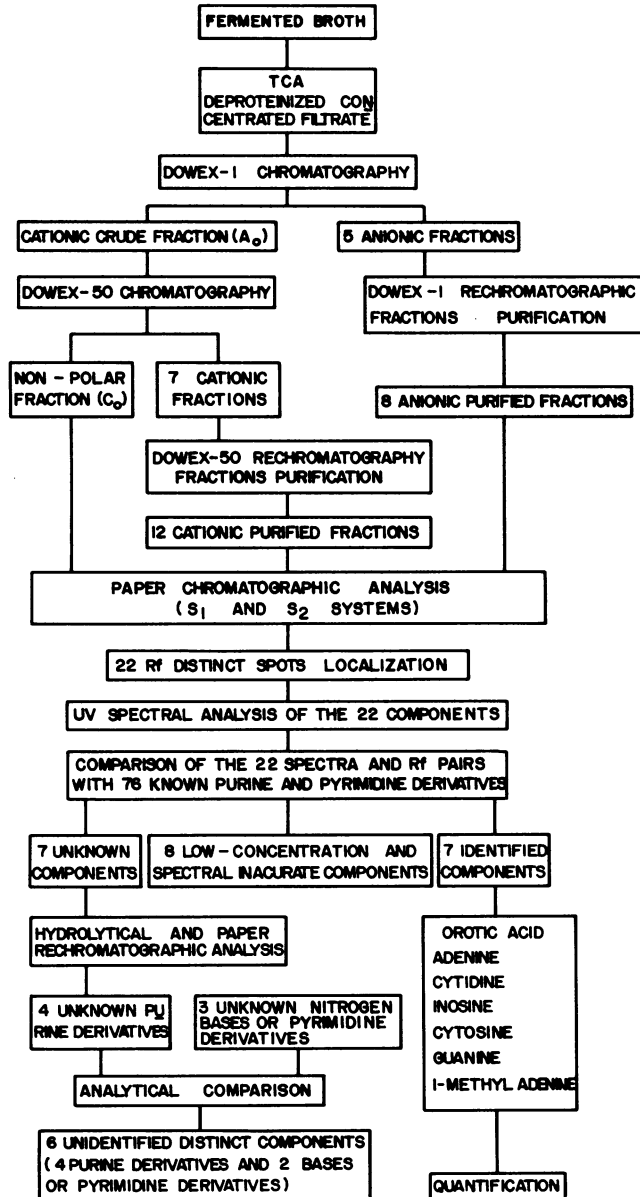


FIG. 1. Purification and identification scheme.

TABLE 2. Cationic fraction components

Fraction	Compound no.	R_f^a		Avg spectral maxima (nm) by S ₁ and S ₂	Compound identification	Concn in broth filtrate (mg/liter)
		S ₁	S ₂			
C ₁₂	15	0.29	0.30	264	Adenine	0.2
C ₂₁	16	0.49	0.54	277	Cytidine	0.3
C ₃₁	17	0.34	0.36	247	Inosine	0.5
C ₄₁	18	0.48	0.45	275	Cytosine	1.9
C ₅₃	20	0.26	0.18	249	Guanine	0.1
C ₆₂	22	0.35	0.43	260	1-Methyladenine	0.1

^a S₁, Paper chromatography system 1 (6); S₂, system 2 (2).

finer medium, specifically developed for such a purpose. On the other hand, Simuth and Zelinka (4) have proposed RNA degradation by active RNase as their explanation for the accumulation of hypoxanthine, cytosine, and guanosine by chlortetracycline-producing *S. aureofaciens* industrial strains. RNA degradation by active ribonuclease as the explanation for their observation. The absence of phosphorus in all of our identified and unidentified well-separated compounds could be explained either as a consequence of nucleotide enzymatic degradation, as supposed by Simuth and Zelinka (4), or by the lack of enough phosphate for normal nucleotide synthesis, as we have presumed (1). The latter hypothesis fit well our observation that orotic acid was the most abundant identified substance of the studied complex mixture, since it is very difficult to think of orotic acid as an RNA

degradation product. Another corroborative observation is the absence of uracil and uracil derivatives in all of the fractions studied.

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