Journal of Chromatography, 322 (1985) 386–390
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,506

Note

Thin-layer chromatographic investigation of the antimycin A antibiotic complex

JUDIT KÁDÁR PAUNCZ
Institute for Drug Research, H-1325 Budapest (Hungary)
(Received November 8th, 1984)

Antimycin A produced by Streptomyces species is active against fungi, insects and mammals and, moreover, is extremely toxic to fish, so it has been used for many years as a piscicide in fishery management. It is an inhibitor of cell respiration.

Antimycin A was isolated by Dunshee *et al.*[1] in 1949 and Watanabe *et al.*[2] as blastmycin (1957), and later its complexity was also revealed[3–5]. It is a complex of four major components designated as A_1, A_2, A_3 (blastmycin), A_4 and six minor ones. The sum of the minor components is usually less than 10%, mostly not more than 1% of the complex[6]. The antimycins are nine-membered ring dilactonic compounds with different alkyl substituents and acyloxy groups on the 7 and/or 8 side chain[4,5,7,8].

The ultraviolet (UV) spectra of the components in ethanolic solution show two bands, with absorption maxima at 227 and 320 nm and are practically identical. The antimycins exhibit fluorescence, with a peak at 420 nm, activated by a wavelength of 350 nm. A spectrofluorimetric assay has been developed by Sehgal *et al.*[9,10] for the determination of the sum of the compounds.

Lockwood *et al.*[3] and Liu and Strong[5] demonstrated by paper chromatography that antimycin A contains at least four different active substances. Paper chromatography has been used for the detection of the components. The components A_1, A_2, A_3 can be well separated, but A_4 only slightly. The method is time-consuming (analysis time about 24 h). The substances were detected microbiologically.

The components can also be separated by countercurrent distribution[11], but only partially.

Gas–liquid chromatography (GLC) of antimycin A antibiotics and their degradation products was investigated[12] and after pyrolysis, GLC of the pyrolyzate was used for the analysis of the antimycin components[4]. Straight GLC was not applicable because of the thermal instability of the antimycin A molecules.

Abidi[13] detected and determined the antimycin A components by high-performance liquid chromatography (HPLC) using electrochemical, fluorescence and UV detectors.

We have used reversed-phase thin-layer chromatography (RP-TLC) for the separation of the antimycin A components.

0021-9673/85/$03.30

EXPERIMENTAL

Stationary phases

The sorbent layers used were: (1) laboratory-made silica gel G (0.25 mm) and (2) precoated silica gel (Art. No. 5554, Kieselgel 60 F_{254}; E. Merck, Darmstadt, F.R.G.) for normal-phase TLC; (3) and (4) the above layers impregnated with 5% paraffin oil in hexane or petroleum ether (b.p. 40–70°C) and (5) silanized silica gel (Art. No. 13724, HPTLC precoated plates RP-18 F_{254}, E. Merck) for RP-TLC.

Mobile phases

The solvent systems used for normal-phase TLC are shown in Table I, those for RP-TLC in Table II.

All chemicals were of analytical grade used without further purification.

Procedure

The antimycin A complex (the Serva product and those prepared in our Institute) dissolved in ethanol were spotted (*ca.* 10–50 μg) on the layer. Chromatograms were developed for a distance of 14 cm, or 8 cm in the case of precoated plates. The fluorescent spots on the chromatograms were dried in a stream of air, located under an UV lamp, scraped off, dissolved with 5 ml ethanol. The resulting solution was centrifuged or filtered. The fluorescence of the compounds can be measured either in this solution or in one diluted 1:1 in ethanol or in 80% ethanol containing 0.01 *M* Tris [tris(hydroxymethyl)aminomethane] buffer, pH 8[10]. The fluorescence was measured at 420 nm using an activating wavelength of 350 nm.

RESULTS AND DISCUSSION

The components of the antimycin A complex did not separate in a range of solvents on silica gel by normal phase TLC. The R_F values of the complex in the chromatograms developed on precoated and laboratory-made silica gel thin-layer plates are shown in Table I.

TABLE I

TLC R_F VALUES OF ANTIMYCIN A COMPLEX ON SILICA GEL PLATES

Solvent		$R_F \times 100$	
		Precoated silica gel plate (Merck)	*Laboratory-made silica gel G plate*
Benzene–ethanol	(8:2)	60[9,10]	92
	(95:5)	50	60
n-Hexane–ethyl acetate	(5:3)	30[14]	65
n-Hexane–chloroform–methanol	(4:4:1)	48[15]	88
n-Hexane–2-butanone–acetone	(5:1:1)	28	40
Cyclohexane–ethyl acetate	(4:5)	67	74
Ethanol–conc. ammonia–water	(8:1:1)	82[16]	90
Benzene–chloroform–methanol–acetic acid	(7:5:1:0.7)	68[13]	85

TABLE II

R_F × 100 VALUES OF ANTIMYCIN A COMPONENTS IN REVERSED-PHASE TLC

Solvent		*Laboratory-made silica gel G plates impregnated with paraffin oil*					*HPTLC RP-18 plates (Merck)*				
		A_1	A_2	A_3	A_4	A_5	A_1	A_2	A_3	A_4	A_5
Acetonitrile–water	(1:1)	26	36	43	50	58					
	(2.5:1)						10	15	19	26	32
Acetonitrile–0.2 *M* sodium chloride	(2:1)	40	44	47	50	53					
	(2.5:1)						9	13	18	25	30
Acetonitrile–0.2 *M* sodium acetate (pH 4.1)	(1.6:1)	37	42	47	52	56					
	(2.5:1)						10	15	19	25	30
Acetonitrile–0.25 *M* ammonium acetate (pH 4.3)	(1.3:1)	38	44	51	57	63					
	(2.5:1)						12	17	23	31	37
Acetonitrile–0.25 *M* sodium dihydrogen-phosphate (pH 3.5)	(1.5:1)	16	19	22	25	28					
	(2.5:1)						12	17	22	29	36

Solvent		*Laboratory-made silica gel G plates impregnated with paraffin oil*					*Precoated plates (Merck) impregnated*				
		A_1	A_2	A_3	A_4	A_5	A_1	A_2	A_3	A_4	A_5
Methanol–water	(1.8:1)	29	37	46	55	65					
	(1.4:1)						17	24	34	42	48
Methanol–0.2 *M* sodium chloride	(1.8:1)	8	12	20	27	32	4	7	11	16	
	(2:1)	15	20	32	40	47	10	14	22	31	
Methanol–0.2 *M* sodium acetate (pH 4.1)	(2:1)	7	13	20	28	35	10	15	22	30	
	(2.6:1)	25	33	43	52	59					
Methanol–0.25 *M* ammonium acetate (pH 4.3)	(2.5:1)	21	27	35	44	52					
	(2:1)						8	13	20	29	
Methanol–0.25 *M* sodium dihydrogen phosphate (pH 3.5)	(2.5:1)	12	18	25	34	43					
	(2:1)						5	10	15	25	33
	(1.6:1)						14*	22*	30*	41*	

* On HPTLC RP-18.

However, the components can be separated by RP-TLC on laboratory-made silica gel G and on precoated silica gel plates, both impregnated with paraffin oil, as well as on silanized silica gel plates (RP-18). The plates were developed with acetonitrile or methanol containing (30–50%) water or sodium chloride, sodium acetate, ammonium acetate or sodium dihydrogen phosphate solution. The solvent systems and the R_F values of the antimycin components are shown in Table II. With increasing water content the R_F values decrease. Solvent systems containing acetonitrile are not suitable for separating the components on precoated silica gel plates impregnated with paraffin oil.

Fig. 1 shows chromatograms of the antimycin A complexes produced by microorganisms prepared in our Institute and that of the Serva product (Serva, Hei-

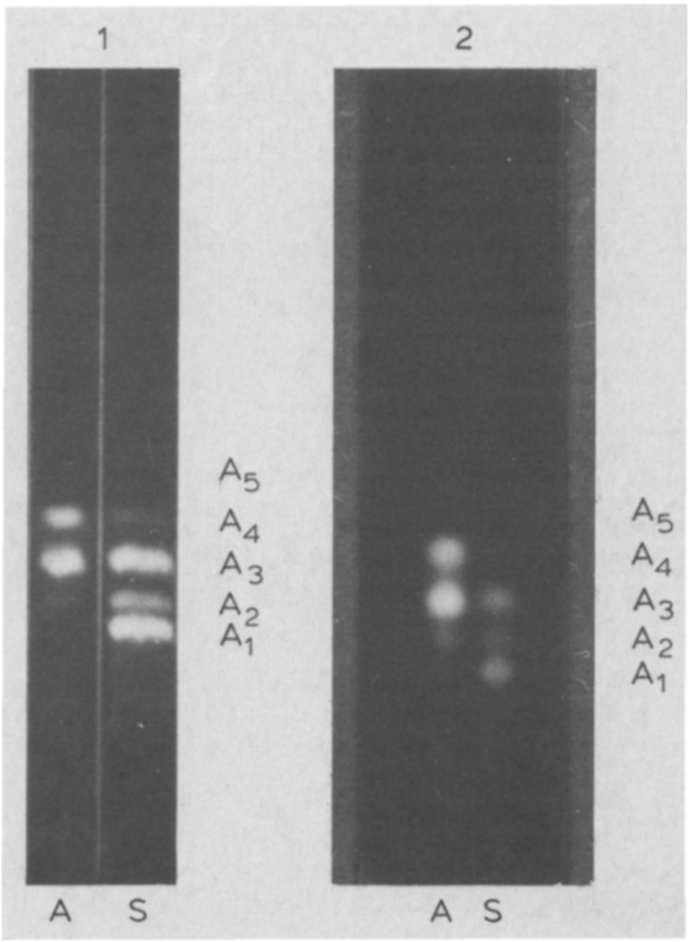

Fig. 1. Chromatograms of antimycin A complex: (1) on laboratory-made silica gel G plate impregnated with paraffin oil (5% in petroleum ether), developing solvent acetonitrile–water (1:1); (2) on HPTLC RP-18 plate (E. Merck), developing solvent acetonitrile–0.2 *M* sodium chloride (5:2). Detection at 366 nm. A, Antimycin A complex prepared in our Institute; S, antimycin A complex from Serva.

delberg, F.R.G.). The identity of the individual components dissolved from the spots was monitored by HPLC.

The separated antimycin A components fluorescing under an UV lamp (366 nm) can be measured *in situ* or dissolved from the silica gel with ethanol. The components of 10–30 μg complex can be well separated and each compound can be dissolved with 5 ml ethanol for further measurements. The recovery of the compounds from the silica gel is 98–100%. A linear relationship was observed between the fluorescence and the concentration of antimycin components between 0 and 10 μg/ml. The ratio of the components in the complex can be calculated from the results.

The impurities present can be detected on normal-phase silica gel plates as well as by RP-TLC. Contaminants and degradation products usually have lower R_F values on normal-phase silica gel and higher ones on reversed phases.

REFERENCES

1 B. R. Dunshee, C. Leben, G. W. Keitt and F. M. Strong, *J. Amer. Chem. Soc.*, 71 (1949) 2436.
2 K. Watanabe, T. Tanaka, K. Fukahara, N. Miyairi, H. Yonehara and H. Umezawa, *J. Antibiot., Ser. A*, 10 (1957) 39.
3 J. L. Lockwood, C. Leben and G. W. Keitt, *Phytopathology*, 44 (1954) 438.
4 G. Schilling, D. Berti and D. Kluepfel, *J. Antibiot.*, 23 (1970) 81.
5 W. C. Liu and F. M. Strong, *J. Amer. Chem. Soc.*, 81 (1959) 4387.
6 D. Kluepfel, S. N. Sehgal and C. Vezina, *J. Antibiot.*, 23 (1970) 75.
7 F. M. Strong, J. P. Dickie, M. E. Loomans, E. E. van Tamelen and R. S. Dewey, *J. Amer. Chem. Soc.*, 82 (1960) 1513.
8 E. E. van Tamelen, J. P. Dickie, M. E. Loomans, R. S. Dewey and F. M. Strong, *J. Amer. Chem. Soc.*, 83 (1961) 1639.
9 S. N. Sehgal, K. Singh and C. Vezina, *Anal. Biochem.*, 12 (1965) 191.
10 S. N. Sehgal and C. Vezina, *Anal. Biochem.*, 21 (1967) 266.
11 Y. Harada, K. Uzu and M. Asai, *J. Antibiot., Ser. A*, 11 (1958) 32.
12 T. Endo and H. Yonehara, *J. Antibiot.*, 23 (1970) 91.
13 S. L. Abidi, *J. Chromatogr.*, 234 (1982) 187.
14 M. Kinoshita, M. Wada and S. Umezawa, *J. Antibiot.*, 22 (1969) 580.
15 P. B. Hamilton, F. I. Caroll, J. H. Rutledge, J. E. Mason, B. S. H. Harris, C. S. Fenske and M. E. Wall, *Appl. Microbiol.*, 17 (1969) 102.
16 T. Ikekawa, F. Iwami, E. Akita and H. Umezawa, *J. Antibiot., Ser. A*, 16 (1963) 56.