NOTE

APPLICATION OF HIGH-VOLTAGE PAPER ELECTROPHORESIS FOR SEPARATION AND IDENTIFICATION OF ANTIBIOTICS*

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For the screening studies of new antibiotics, separation and identification using paper[1,2,3] and thin-layer[4,5,6] chromatography have been reported by many researchers. In general, thin-layer chromatography is effective in rapidly separating antibiotics soluble in organic solvents, and paper chromatography is useful for separating water-soluble antibiotics, although it takes much more time and mobility of the antibiotic may be influenced by the purity of the sample applied, especially by inorganic salts. We have found that high-voltage paper electrophoresis for 15 minutes gives a good separation of antibiotics, especially water-soluble basic or amphoteric antibiotics, even with crude preparations.

The commercial apparatus, Model HV 5000-3 of Savant Instruments, Inc., was used in these experiments. The sample solution was spotted on strip of Toyo No. 51 filter paper (60 cm × 15 cm) on a transverse line 16 cm from one end and the strip uniformly sprayed with an electrolyte solution (pH 1.8) of formic acid - acetic acid - water (22 : 75 : 900 by volume), similar to the technique reported by ATFIELD and MORRIS[7] for the separation of amino acids. A constant current at 3,300 V (about 40 mA/15 cm) was then applied to the strip for 15 minutes. After electrophoresis, the strip was dried with flowing warm dry air to remove electrolyte solution. Antibiotics on the paper were detected by bioautography using test organisms such as *Staphylococcus aureus*, *Micrococcus flavus*, *Bacillus subilis* or *Escherichia coli*. In some cases, ninhydrin and SAKAGUCHI reactions and ultraviolet light were used for the detection of the antibiotics. Under standard conditions, alanine moved toward the cathode *ca.* 10 cm. The mobilities of antibiotics relative to alanine were calculated as Rm values. The relative mobilities (Rm) of 92 antibiotics are listed in two groups according to their extraction properties in Tables 1 and 2.

Most antibiotics assigned to group II are adsorbed on weak cation-exchange resins such as Amberlite IRC-50 or strong cation-exchange resins such as Amberlite IR-120. Interestingly, antibiotics having an Rm value

Table 1. Relative mobilities of antibiotics extracted by solvent process. (Group I)

Ahtibiotics	Rm *	Detection
Spiramycins I, II, III	0.88	*Staph. aureus*
Pluramycin A	0.84	*Staph. aureus*
Bacitracin	0.83	ninhydrin
Amicetin	0.77	UV light
Lincomycin	0.77	*Staph. aureus*
Pyridomycin	0.71	UV light
Methymycin	0.63	*Staph. aureus*
Mitomycin C	0.63	UV light
Narbomycin	0.59	*Staph. aureus*
Picromycin	0.59	*Staph. aureus*
Tetracycline	0.58	UV light
Oxytetracycline	0.58	UV light
Oleandomycin	0.57	*Staph. aureus*
Chlortetracycline	0.55	UV light
Tertiomycins A, B	0.51	*Staph. aureus*
Erythromycin	0.50	*Staph. aureus*
Carbomycin	0.50	*Staph. aureus*
Leucomycins	0.46	*Staph. aureus*
Josamycin	0.46	*Staph. aureus*
Tylosin	0.45	*Staph. aureus*
Azomycin	0.11	*M. flavus*
Noboviocin	0	UV light
Coumermycin A_1	0	UV light
Trichomycin	0	UV light
Pentamycin	0	UV light
Nystatin	0	UV light
Amphotericin B	0	UV light
Actinomycin C	0	UV light
Chromomycin A_3	0	UV light

* Rm=mobility relative to alanine as 1.0.

* The most of this work was reported at the 11th Kanto Branch Meeting of Pharmaceutical Society of Japan, held on November 11, 1967 in Tokyo. The Abstracts of Papers (in Japanese), pp. 5~8.

Table 2. Relative mobilities of antibiotics extracted by adsorption process. (Group II)

Antibiotics	Rm	Detection	Antibiotics	Rm	Detection
Gentamicins C_1, C_2	2.20	ninhydrin	BD-12	1.27	*B. subtilis*
Neamine	2.15	ninhydrin	BY-81	1.27	*B. subtilis*
Neomycins B, C	2.04	ninhydrin	Kikumycin B	1.15	*B. subtilis, E. coli*
Paromomycins I, II	1.99	ninhydrin	Kikumycin A	1.00	*B. subtilis, E. coli*
Paromamine	1.92	ninhydrin	Nojirimycin	1.00	*Staph. aureus*
Kanamycin B	1.92	ninhydrin	Bleomycins	0.53～1.10*	*B. subtilis*
Kanamycin C	1.85	ninhydrin			
Kanamycin A	1.82	ninhydrin	Glebomycin	0.97	*B. subtilis*
Capreomycins I, II	1.59	ninhydrin	Cordycepin	0.90	UV light
Streptothricin (A-249)	1.53	*B. subtilis*	Tubercidin	0.90	UV light
Hygromycin B	1.50	ninhydrin	Aristeromycin	0.90	UV light
Phleomycins	0.69～1.50*	*B. subtilis*	Angustmycin A (Decoynin)	0.82	UV light
Blasticidin S	1.48	UV light	Netropsin	0.79	*E. coli*
Dihydrostreptomycin	1.47*	Sakaguchi	Actinobolin	0.79	UV light
Streptomycin	1.45*	Sakaguchi	Angustmycin C (Psicofuranine)	0.79	UV light
Destomycin A	1.43	ninhydrin			
Destomycin B	1.40	ninhydrin	Formycin	0.79	UV light
Cycloserine	1.39	ninhydrin	Sangivamycin	0.78	UV light
Hydroxystreptomycin	1.38*	Sakaguchi	Toyocamycin	0.75	UV light
Viomycin	1.37	ninhydrin	Trehalosamine	0.74	ninhydrin
Alboverticillin	1.33	ninhydrin	Kasugamycin	0.74	ninhydrin
Mannosidostreptomycin	1.30	*B. subtilis*, Sakaguchi	Puromycin	0.72	UV light
			Ferrimycin A	0.71	*B. subtilis*
Gougerotin	1.30	UV light	HON	0.58	ninhydrin
Polymyxin B	1.30	ninhydrin	Danomycin	0.49	Barton ($FeCl_3$)
Colistin	1.30	ninhydrin	Polyoxin A	0.45	UV light
Actinospectacin	1.30	ninhydrin	O-Carbamyl-D-serine	0.43	ninhydrin
Angustmycin B (adenine)	1.30	UV light	Gramicidin J	0.37	ninhydrin
			Formycin B	0.04	UV light
SF-701	1.28	*B. subtilis*	Oxoformycin	0	UV light

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greater than 1.0 are efficiently purified with Amberlite IRC-50 and the most antibiotics with Rm <1.0 are purified with Amberlite IR-120.

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