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THIN-LAYER CHROMATOGRAPHIC CLASSIFICATION OF ANTIBIOTICS EXHIBITING ANTITUMOR PROPERTIES*

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SUMMARY

A classification system for 151 antibiotics exhibiting antitumor properties using thin layer chromatography (TLC) and bioautography was developed. TLC classification is based on mobility of the compounds in a solvent system using a certain adsorbent, rather than on misleading R_F values. The solvent systems and conditions used are presented.

INTRODUCTION

The use of chromatographic classification, in conjunction with bioautography, for presumptive identification of antibiotics is well documented[1–5]. Because of the orientation of our laboratories, it was necessary to use thin-layer chromatography (TLC) to classify new antibiotics exhibiting antitumor properties.

Different adsorbents and different solvent systems were used to classify 151 antitumor antibiotics. Bioautography was perfected on five microorganisms and is under development in two mammalian cell lines. This work describes TLC classification and bioautography with microorganisms; bioautography with mammalian cells will be described elsewhere.

EXPERIMENTAL

Materials

The antibiotics used in this study were received from Dr. J. Douros, NCI, and are listed in Table I. Silica gel 60 and cellulose precoated TLC plates (EM Labs., Elmsford, N.Y., U.S.A.) were used without preactivation. All solvents were distilled-in-glass (Burdick and Jackson, Muskegon, Mich., U.S.A.) except acetic acid (Analyzed Reagent; J. T. Baker, Phillipsburgh, N.J., U.S.A.) and pyridine (Fisher Scientific, Pittsburgh, Pa., U.S.A.). The work was carried out in a TLC laboratory equipped

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TABLE I

LIST OF ANTIBIOTICS, THEIR SOLUBILITY, SUBGROUPS AND MICROORGANISMS

DMF = dimethylformamide, DMSO = dimethylsulfoxide. BS = *Bacillus subtilis*, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, SC = *Saccharomyces cerevisiae*, PN = *Penicillium notatum*, and — = no activity detected.

NSC No.	*Name*	*Subgroup*	*Solubility*	*Microorganism used*
85680	Acrylamide	II-4	Methanol	BS
31083	Actinobolin	II-4	Methanol	BS
87221	Actinomycin C2	IV-3	Methanol	BS
87222	Actinomycin C3	IV-3	Methanol	BS
3053	Actinomycin D	IV-3	Methanol	BS
53396	Actinogan	I-2	Water	
124668	Actinorubin	I-4	Water	BS
3056	Adenosine	II-4	Methanol	—
123127	Adriamycin	I-4	Water	SA
153353	Alanosin, monosodium salt	I-3	Water	
143647	L-Alanosine	V	Methanol	
5340	Amicetin	III-2	Methanol	BS
110344	3-Amino-2,3,6,-L-hexopyronase·HCl	I-4	Water	
115712	cAMP	II-3	Water–pyridine	RC
141537	Anguicine	IV-4	Methanol	
76712	Anisomycin	III-2	Methanol	SC
99843	Antibiotic 1037	III-5	DMF	PN
56310	Antibiotic B-17498X	IV-3	Methanol, heat	PN
32743	Antibiotic E73	IV-1	Methanol	PN
104947	Antibiotic M5-18903	III-5	Methanol	BS
106410	Ascomycin	III-4	Methanol	PN
109229	Asparaginase	I-1	Water	—
526595	Aureolic acid (=Mithromycin)	III-2	Methanol	BS
91583	Azacolutin complex	II-1	DMSO	SC
102816	5-Azacytidine	II-2	Water	BS
91768	Azalomycin F-complex	II-1	Methanol	BS
742	Azaserine	II-2	Methanol–water	PN
10709	Azastreptonigrin	III-3	Methanol	BS
56654	Azotomycin	II-1	Water	BS
91770	Blasticidin-S	II-1	Water	BS
125066	Bleomycin A1	I-2	Water	BS
146942	Bleomycin A2	I-2	Water	—
11930	Bluensomycin sulfate	L-3	Water	BS
94219	Candicidin	I-5	DMF	PN
51601	Carbomycin	IV-3	Methanol	BS
157365	Carzinostatin	I-2	Water, heat	SA
5159	Chartreusin-2 (hydrate)	III-4	Chloroform	—
3069	Chloramphenicol	IV-3	Methanol	SA
131187	Chromomycin A2	III-5	Methanol	BS
18335	Cinerubin B	IV-1	Chloroform	BS
71936	Cinnamycin	I-4	Chloroform	BS
110326	Copiamycin (acetyl)	I-5	Methanol	—
63948	Cordycepin	III-4	Water	SC
107412	Coumermycin A1	V	DMF	SA
89671	Cyanein	IV-3	Methanol	BS
144184	Cyclomycin complex	V	Chloroform	SA
185	Cycloheximide	IV-1	Methanol	PN
91770	Cytovirin	II-1	Water	BS
7365	DON	II-2	Methanol–Water	BS
72151	Daunomycin	I-4	Water	BS

TABLE I *(continued)*

NSC No.	*Name*	*Subgroup*	*Solubility*	*Microorganism used*
51097	Duazomycin A	II-1	Methanol–Water	BS
71935	Duramycin	I-4	Water	BS
88465	Enteromycin	IV-3	Water	SA
525661	Flammulin	I-2	Water	—
103811	Formycin A	II-3	Methanol	BS
106486	Formycin B	II-2	Water	BS
58368	Fumagillin	IV-2	Methanol	SA
106193	Fusarubin	IV-2	Methanol	BS
56192	Fusidic acid	III-4	Methanol	—
132346	Gelbecidine	IV-4	Chloroform	SA
102866	Gliotoxin	IV-1	Methanol	SA
528943	Gougerotin	I-2	Water	EC
34533	Griseofulvin	IV-1	Methanol	—
521778	Hadacidin	I-4	Water	—
70929	Hedamycin	I-5	Chloroform	SA
400979	Iiludin S	IV-3	Methanol	SA
107455	Indole-3-carboxaldehyde	II-1	Methanol	EC
109023	3H-Indole	II-2	Methanol	BS
83340	Iyomycin B_1	III-1	Methanol	EC
94217	Iyomycin complex	I-4	Water	—
62778	Kanchanomycin	V	Methanol	SA
100858	Kasugamycin	I-3	Water	BS
119573	Kundrymycin	V-4	Methanol	SA
105338	Lasgosin	III-2	DMF	SC
110345	L-Lyxo-hexopyranoside	II-4	Methanol	—
170105	Macromonycin	I-2	Water	—
125176	Mikamycin	IV-3	Methanol	BS
105759	Mithramycin	II-3	Methanol	BS
143020	Mithramycin-Mg	III-1	Methanol	BS
24559	Mithramycin	III-1	Methanol	BS
77471	Mitocromin	II-4	Methanol	SA
69559	Mitogillin	I-1	Water	—
26980	Mitomycin C	III-5	Chloroform	BS
117032	Mitosper	I-1	Water	—
101492	Mycorhodin	IV-2	Methanol	BS
63445	Narangomycin	IV-4	Methanol	BS
65423	Nebularin	III-3	Water	—
69556	Neocarzinostatin	I-2	Water, heat	—
110903	Nisin	I-4	Methanol	—
52141.	Nonactin	IV-1	DMF	—
70664	Nucleoside fraction of septacidin	I-5	Water	—
63926	Oligomycin	IV-2	Water	PN
76911	Olivomycin	III-5	Methanol	BS
88466	Oosporein	I-5	Methanol	—
60745	PA 147	I-5	Water, heat	
52947	Pactamycin	III-5	Methanol	BS
135962	Palmitoyl-cytidine	II-3	Methanol	—
73382	Peptinogan	I-2	Water	—
61586	Phleomycin	I-3	Water	BS
56410	Porfiromycin	IV-3	Methanol	BS
47147	Prodigiosin	IV-1	Chloroform	—
3055	Puromycin	III-3	Methanol	—
140395	Pyrazomycin	II-2	Water	BS

(Continued on p. 294)

TABLE I *(continued)*

NSC No.	*Name*	*Subgroup*	*Solubility*	*Microorganism used*
53398	Restrictocin	I-1	Water	—
133099	Rifamide	IV-3	Methanol	BS
133100	Rifamycin SV	III-5	Methanol	BS
55202	Roseolic acid	I-2	Water	BS
83142	Rubidomycin·HCl	I-4	Water	BS
105023	Rubiflavin	II-2	Methanol	BS
99282	Rubradirin	IV-4	Methanol	BS
83950	Rufochromomycin	II-4	Methanol	BS
117572	Ryanodine	IV-4	Methanol	SA
31812	Sancyclin	I-3	Water	EC
65346	Sangivamycin	II-3	Methanol	—
100844	Saramycetin	II-3	CH_2O	—
46401	α-Sarcin	I-2	Water–methanol	—
14347	Sarkomycin (sodium salt)	II-2	Methanol–chloroform	—
65104	Septacidin	I-3	DMF	—
149789	Sistomycosin	I-3	DMF	—
59729	Sparsomycin	III-1	DMF	BS
100559	Spectinomycin	I-4	Water	BS
71901	Statolon	II-6	DMF	—
122716	Stendomycin salicylate	I-5	Chloroform	SA
2360	Streptolydigin	III-5	Methanol	BS
45383	Steptonigrin	II-4	Chloroform	BS
145384	Streptonigrin methyl ester	III-3	Methanol	BS
76779	Streptorubin	IV-1	Methanol	—
48810	Streptovaricin A	IV-4	Chloroform	BS
156215	Streptovaricin B	IV-2	Chloroform	BS
19990	Streptovaricin C	IV-2	Chloroform	SA
156216	Streptovaricin D	IV-2	Chloroform	BS
156219	Streptovaricin G	IV-2	Chloroform	—
39147	Streptovitacin A	IV-2	Methanol	—
37917	Steptozotocin	III-4	Methanol	BS
85998	Streptozotocin·HCl	III-5	Water	SA
105972	Thiosangivamycin	II-3	Methanol–chloroform	SA
105972	Thiosangivamycin	II-3	Methanol–chloroform	PN
138780	T-2 Toxin	IV-1	Methanol–water	PN
63701	Toyocamycin	IV-3	Methanol	EC
126730	Trienine	I-2	Methanol	PN
73832	Tuberin	IV-1	Methanol	—
126728	Verrucarin A	IV-1	Methanol	—
49842	Vinblastine (sulfate, hydrate)	III-3	Water	EC
88468	Viridogrisein	IV-3	Methanol	BS
146208	Zorbamycin	I-4	Water	EC
172254		V	Methanol	—
26697		I-3	Methanol	—
58972		V	Methanol	SA
72942	Noformycin	I-4	Water	—
75603		I-1	Water	—
102810		V	Methanol	SA
103645		V	Chloroform	—
104524		III-5	Methanol	—
108408		V	Methanol–chloroform	—
114573	Viundrymycin	IV-4	Chloroform	SA
116328	Threomycin	V	Water	—
135015		I-2	DMF	—

with special soft fluorescent lights (General Electric F40G0) which have no voltage output at wavelengths shorter than 500 nm. Spots were located on the TLC plates in a view box equipped with long (366-nm) and short (254 nm) UV lights. White light was used for colored compounds.

Method

Fresh solutions of the drugs were prepared from individual standards dissolved in appropriate solvents (Table I) and spotted on silica gel or cellulose plates with Drummond Microcaps. The plates were then developed with four different solvent systems (Fig. 1).

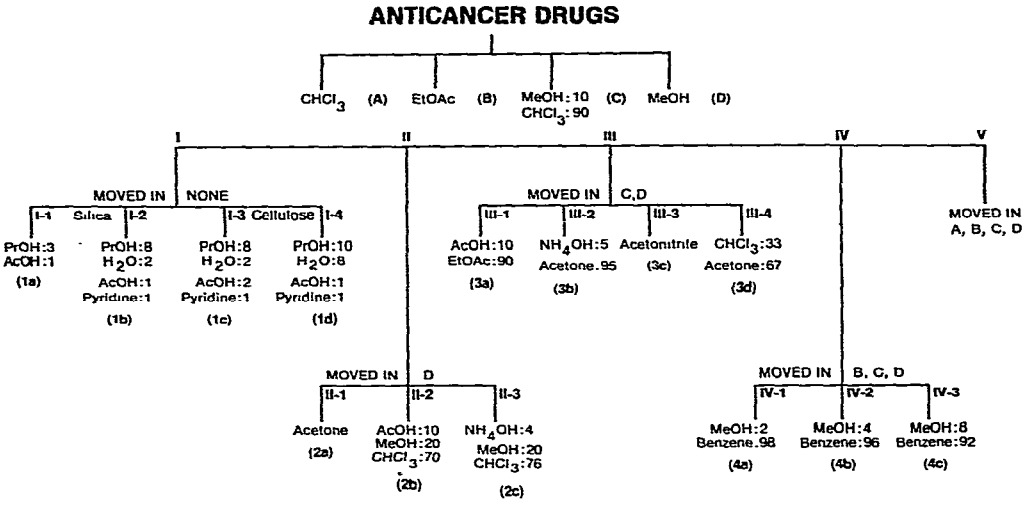

Fig. 1. Schematic drawing illustrating the choice of solvents and plate type for the classification of antibiotics exhibiting antitumor properties.

The four solvent systems employed were: (A) chloroform; (B) ethyl acetate; (C) methanol–chloroform (10:90); and (D) methanol. The five groups obtained by these four primary solvents were further analyzed by 14 additional solvent systems to give 19 subgroups.

After development, the chromatograms were scanned under UV light and the R_F values of the antibiotics were determined.

Bioautographic procedure

The organisms used for performing bioautographs were *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), *Saccharomyces cerevisiae* (ATCC 2601), and *Penicillium notatum* (ATCC 9478). Inocula were prepared by growing and harvesting these organisms according to Isaacson[6]. The bioautograph plate consisted of a base layer overlayed with a second layer seeded with the microorganism. The composition of the second layer varied with the microorganism: antibiotic No. 2 (base, Difco) and antibiotic medium No. 1 (Difco) for *S. aureus*; antibiotic medium No. 4 (Difco) for *B. subtilis*; trypticase soy agar for *E. coli*; YM agar (Difco) for *S. cerevisiae*; and Czapek Dox agar for *P.*

TABLE II

LIST OF ANTIBIOTICS (GROUP I) THAT DID NOT MOVE IN SOLVENTS A–D USING SILICA GEL PLATES AND THEIR R_F VALUES

+ = No exact R_F value available; S = streaking.

Subgroup	*Name or NSC No.*	$R_F \times 100$				
		Silica gel	*Cellulose*		*Silica gel*	
		A, B, C, D	*Ia*	*Ib*	*Ic*	*Id*
I-1	Asparaginase	0	0	0	0	0
	Mitogillin	0	0	0	0	0
	Mitosper	0	0	0	0	0
	Restrictocin	0	0	0	0	0
	75603	0	0	0	0	0
I-2	Actinogan	0	0	0	0	0
	Bleomycin A1	0	0	0	0	0
	Bleomycin A1	0	0	0	0	0–35s
	Bleomycin A2	0	0	0	0	85
	Carzinostatin	0	0	0	0	0, 82
	Flammulin	0	0	0	0	0–30s
	Gougerotin	0	0	0	42	0
	Macromomycin	0	0	0	0	75s, 80
	Neocarzinostatin	0	0	0	0	80
	Peptinogan	0	0	0	0	10
	Roseolic acid	0	0	0	0	86
	α-Sarcin	0	0	0	0	0–35s
	Trienine	0	0	0	0	73
	116328	0	0	0	0	68, 90
I-3	Alanosin (monosodium salt)	0	0	+	0	40
	Bluensomycin sulfate	0	0	0	50, 30	38
	Kasugamycin	0	0	+	0	60
	Phleomycin	0	0	+	0	25s, 82
	Sancyclin	0	0	87	0	16
	Septacidin	0	0	0	85	80
	Sistomycosin	0	0	0	80	77
	26697	0	0	0	0s	s
I-4	Actinorubin	0	0	+	10s	0–41s, 80
	3-Amino-2,3,6-L-hexopyronase·HCl	0	0	+	60	82
	Adriamycin	0	0	75	73	87
	Cinnamycin	0	0	+	+	85
	Daunomycin	0	0	78	77	90
	Duramycin	0	0	+	—	80, 85, 45
	Hadacidin	0	0s	+	40	64
	Iyomycin complex	0	0	+	21s	100
	Nisin	0	0s	20	0–30s	0–50s
	Spectinomycin	0	0	+	23	60
	Zorbamycin	0	0	0–10s	25, 5, 82, 99	0–64s
	72942	0	0	+	18	0–50s
I-5	PA 147	0	10	+	+	86
	Candicidin	0	60s	10	80	0–75s
	Copiamycin (acetyl)	0	94	+	+	88
	Hedamycin	0	0–1s	+	+	50, 82, 0

TABLE II *(continued)*

Subgroup	*Name or NSC No.*	$R_F \times 100$				
		Silica gel	*Cellulose*		*Silica gel*	
		A, B, C, D	*1a*	*1b*	*1c*	*1d*
	Nucleoside fraction of Septacidin	0	21–50s	+	0	63, 87
	Oosporein	0	45–50s	+	0	45–50s
	Stendomycin Salicylate	0	83	+	+	70

notatum. The vacuum-dried chromatogram was then placed on a Whatman grade 1 filter paper resting on the previously seeded agar plate for approximately 3 h to allow elution of test antibiotics. The incubation condition for bioautograms was at 37° for approximately 24 h, except for *S. cerevisiae* and *P. notatum*, which were incubated at 28° for 24–48 h.

TABLE III

R_F VALUES OF ANTIBIOTICS (GROUP II) THAT MOVED IN METHANOL ON SILICA GEL PLATES

s = Streaking.

Subgroup	*Name or NSC No.*	$R_F \times 100$			
		Solvent system			
		D	*2a*	*2b*	*2c*
II-1	Azacolution complex	0, 40	0	0	0
	Azalomycin F-complex	14, 50	0	0	0
	Azotomycin	86	0	0	0
	Cytovirin	0, 14	0	0	0
	Duazomycin	s	0	0s	0s
	Indole-3-carboxaldehyde	75	0	0	0
	Statolon	0–33s	0	0	0
II-2	5-Azacytidine	59	0	29	0
	Azaserine	s	0	10	0
	DON	s	0	10	0
	Formycin B	76	0	31	0
	3H-Indole	13	0	0	92
	Pyrazomycin	78	0	25	0
	Rubiflavin	10s	0	0	58, 98
	Sarkomycin (sodium salt)	68	0	9, 73	0
II-3	cAMP	74	28	0	39
	Formycin A	67	0	29	12
	Palmitoyl-cytidine	94	0	77	33
	Sangivamycin	71	0	26	21
	Thiosangivamycin	75	0	42	18
II-4	Acrylamide	86	65, 86	52	10
	Actinobolin	36	0–25s	11, 13	10, 18
	Adenosine	35	30	21	61
	L-Lyxo-hexopyranoside	18	0, 22	50	58
	Mitocromin	79	10	100, 95, 82, 72	100, 71, 41, 40
	Rufochromomycin	82	0–9s, 10	68s	0, 6, 15
	Steptonigrin	86	21	15s, 63	21

RESULTS

The criterion for the classification of the antibiotics was the movement of each in a specific solvent system on a specific TLC plate, silica gel, cellulose, etc. Analysis of 151 compounds in primary solvent systems A, B, C, and D yielded five main groups. Group I consisted of antibiotics which did not move in solvents A, B, C, and D; Group II, antibiotics that moved in solvent D; Group III, antibiotics that moved in solvents C and D; Group IV, antibiotics that moved in solvents B, C, and D; and Group V, antibiotics that moved in solvents A, B, C, and D. Of the 151 antibiotics tested, 45 were in Group I; 27 in Group II; 28 in Group III, 41 in Group IV; and 10 in Group V. Groups I, II, III, and IV were further classified in 14 secondary solvent systems yielding 19 subgroups. Note that Group I was tested in silica gel, giving Groups I-1 and I-2, and in cellulose, giving Groups I-3 and I-4. R_F values of all antibiotics in both primary and secondary solvent systems are listed in Tables II–VI.

Bioautographic evaluation of the developed TLC plates is described in the

TABLE IV

R_F VALUES OF ANTIBIOTICS (GROUP III) THAT MOVED IN SOLVENT SYSTEMS C AND D ON SILICA GEL PLATES

s = Streaking.

Subgroup	*Name or NSC No.*	$R_F \times 100$					
		Solvent system					
		C	*D*	*3a*	*3b*	*3c*	*3d*
III-1	Iyomycin B_1	0–10	0–10	0	0	0	0
	Mithramycin	16	92	0	0	0	0
	Mithramycin-Mg	18	92	0	0	0	0
	Sparsomycin	16	67	0	0	0	0
III-2	Aureolic acid	16	92	0	0	96	0
	Amicetin	60	80	0	14–15	0	0
	Anisomycin	15	40	0	13	0	0
	Logosin	17	90	0	13	0	0
III-3	Azastreptonigrin	34	91	23	0	0	35, 0
	Nebularin	18	71	0	29	24	0
	Puromycin	15	40	0	0–12	0	81
	Steptonigrin methyl ester	11	88	0–35	0	0–15	0
	Vinblastine (sulfate, hydrate)	72, 41	55	0	92	0	21
III-4	Ascomycin	90, 0	70	96, 0	96, 0	34, 0	0
	Chartreusin-2 (hydrate)	40	70	50, 26	0	0–25	18–37
	Cordycepin	13	60	17	45	21	0
	Fusidic acid	0	24	85	0	96	36
	Steptozotocin	20, 0	80	45, 0	34, 23	0	30, 20
III-5	Antibiotic M5-18903	60, 50	88	—	96	98	25
	Antibiotic 1037	13	70	32	45	75	16
	Olivomycin	50	90	—	98	98	41
	Chromomycin A2	47	95	45	94	98	48
	Mitomycin C	18	78	18	74	75	23
	Olivomycin A	50	95	26–50	93	98	40
	Pactamycin	44	70	12	93	93	78
	Steptolydigin	0–45	85, 77	0–20	55	0–14	28
	Steptozotocin·HCl	62, 25, 0	80	19	21	0–16	11

TABLE V

R_F VALUES OF ANTIBIOTICS (GROUP IV) THAT MOVED IN SOLVENT SYSTEMS B, C AND D ON SILICA GEL PLATES

s = Streaking.

Subgroup	*Name or NSC No.*	$R_F \times 100$					
		Solvent system					
		B	*C*	*D*	*4a*	*4b*	*4c*
IV-1	Antibiotic E73	50	76	81	20	40	—
	Cinerubin	s	94	s	30	46	92
	Cycloheximide	73, 50	—	80	—	62, 37	86, 662
	Gliotoxin	75	90, 80, 70	87	10	30	40
	Griseofulvin	52	92	83	10	16	50
	Verrucarin A	80, 75	98, 95	90	10	14	50
	Nonactin	86	30	85	40	40	50
	Prodigiosin	46	71, 33	50	12	17	35, 15
	Steptorubin	63	90	71	10s	31, 17s	50, 36s
	T-2 Toxin	76	79	88	10	10	—
	Tuberin	42	43	83	12	18	29
IV-2	Fumagillin	38	0–43	89	0	10	—
	Fusarubin	68	80	82	0	17	43
	Mycorhodin	38	95	89	0	10	22
	Oligomycin	86	90	89	0	10	15
	Streptovitacin A	15	40	80	0	11	30
	Streptovaricin B	48	78	92	0	10	14
	Streptovaricin C	48	71	92	0	10	18
	Streptovaricin D	69	88	92	0	11	33
	Streptovaricin G	52	76	92	0	10	14
IV-3	Antibiotic B-14798X	42	43	83	0	0	15
	Actinomycin C2	24	50, 40, 30	86	0	0	20
	Actinomycin C3	25	55, 45, 35	86	0	0	18
	Actinomycin D	24	50, 40, 30	86	0	0	20
	Carbomycin	30	72, 58, 54	92	0	0	16
	Chloramphenicol	42	43	83	0	0	15
	Cyanein	40	33	88	0	0	10
	Enteromycin	13	17, 04	87	0	0	10
	Illudin	56	44	88	0	0	13
	Mikflmycin	75, 25, 17	82, 70, 0–50	82, 40	0	0	22
	Porfiromycin	12	32	78	0	0	14
	Rifamide	14, 0	62, 25, 0	90	0	0	10
	Toyocamycin	10	23	80	0	0	50
	Viridogrisein	0–15	80	85	0	0	21
IV-4	Anguidine	50	14	88	0	0	0
	Gelbecidine	0–16	81	91	0	0	0
	Narangomycin	18	65	78	0	0	0
	Rubradirin	63, 0	98, 75, 0	87, 0	0	0	s
	Ryanodine	50	14	88	0	0	0
	Streptovaricin A	24	43	95	0	0	0
	Kundrymycin	0–11	18–27	82	0	0	0

TABLE VI

R_F VALUES OF ANTIBIOTICS (GROUP V) THAT MOVED IN SOLVENT SYSTEMS A–D ON SILICA GEL PLATES

Name or NSC No.	$R_F \times 100$			
	Solvent system			
	A	*B*	*C*	*D*
L-Alanosine	45	40	80	75
Coumermycin	0–10	10–18	0–23	100
Cyclamycin complex	0–14	0–12, 20–60	30, 90	60, 80
Kanchanomycin	0–20	0	0, 60, 80	0–24
17254	96	96	95	95
58987	28	41	77	93
102810	10, 20	91	93	91
Bostrycoidin	10	0–60	80	0–36
108408	60	88	100	85
135015	0–60	40	75	75

experimental section. Good inhibition zones could be observed in each case with the indicated test organism. It was essential to place filter paper between the seeded agar and the TLC plate and a minimum of 1 h diffusion time was necessary for every antibiotic. The formation of inhibition zones was observed periodically during incubation. This periodic observation was especially necessary with the test organism *Penicillium notatum*, in order to detect inhibition at optimum time.

DISCUSSION

The objective of this study was to develop a classification system to be used for possible identification of known active antitumor principles produced in new fermentation broth. The system is based on both TLC and bioautography.

The solvent systems initially chosen for the class of antibiotic agents were divided into 3 groups: (a) two non-polar solvents, an aromatic (benzene) and an aliphatic (hexane); (b) two solvents of intermediate polarity, chloroform and ethyl acetate; and (c) two polar solvents, non-protic (acetone) and protic (methanol). Because hexane and benzene did not result in migration of an appreciable number of compounds, they were dropped. Acetone, which did not give results significantly different from methanol, was also dropped. The four solvent systems used gave five main groups, which were further analyzed with 14 different solvent systems to give 19 antibiotic subgroups (see Fig. 1). Table I lists all the groups and subgroups in alphabetical order.

TLC classification is based on mobility of the compounds in a solvent system using a certain adsorbent, rather than on potentially misleading R_F values. R_f values are used in this classification system only to demonstrate movement. R_F values of less than 0.05 were regarded as "no movement" because such a small distance of migration cannot be differentiated well in a bioautographic system from "no movement" and would result in erroneous classification of the bioactive material in question.

Certain compounds streaked in all the solvent systems used when chromato-

graphed. Such streaking occurred with Hedamycin, Actinogen, Bleomycin A-1, α-Sarcin, Actinorubin, Zorbamycin, Coumermycin, and Kanchanomycin. Others streaked only in certain solvent systems; these are Actinobolin, Mitochromin, Rufochromomycin, Puromycin, Streptolydigin, and Cyclomycin. The streaking behavior of a material can be used as an identifying characteristic.

The method of classification described here is simple, easy, and could be used in any TLC laboratory.

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