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Thin-layer chromatography of mycophenolic acid and related compounds

Mycophenolic acid is an antibiotic that can be isolated from penicillium cultures[1,2]. Recent reports of antiviral and antitumor properties[3] of mycophenolic acid have renewed interest in the compound. Such interest has led to the total synthesis of mycophenolic acid by BIRCH AND WRIGHT[4] and to the development of a quantitative gas chromatographic assay by GAINER AND WESSELMAN[5]. Several reports are in the literature which include the chromatography of mycophenolic acid[6–9], but much of the work has involved general screening of antibiotics and has not been specific for mycophenolic acid. WILLIAMS *et al.*[10] were the first to report thin-layer chromatographic (TLC) data for mycophenolic acid along with their work with paper chromatography (PC).

This paper reports the results of extensive evaluation of TLC systems for mycophenolic acid. In addition, compounds related to mycophenolic acid are included in this study.

Experimental

Chemicals. All chemicals were of reagent grade and were used without further purification.

Developing systems. All solvents were mixed on a v/v basis immediately before being put into the developing chamber.

Spray reagent. A 1% solution of $FeCl_3 \cdot 6H_2O$ in methanol (w/v) was used.

Equipment. Pre-coated 250-μ Silica Gel F_{254} and pre-coated 0.1-mm Cellulose Powder MN 300 F_{254} thin-layer plates were supplied by Brinkmann Instruments, Inc. Micropipets (Microcaps®) from Drummond Scientific Company were used for making sample applications.

TABLE I

SOLVENT SYSTEMS FOR TLC ON SILICA GEL PLATES

No.	*Solvent systems*	*Development time (min)*
1	Chloroform–methanol (9:1)	52
2	Benzene–ethyl acetate–acetic acid (80:20:5)	85
3	*n*-Pentylacetate–*n*-propyl alcohol–acetic acid–water (4:2:1:1)	142
4	Benzene–*n*-propyl alcohol–acetic acid (9:6:1)	98
5	Acetonitrile–water (4:1)	53
6	Chloroform–acetic acid (20:1)	55
7	Benzene–acetic acid (20:1)	48
8	Ethyl acetate–acetic acid (20:1)	54
9	Ethyl ether–acetic acid (20:1)	72
10	Methylene chloride–acetic acid (20:1)	55
11	Petroleum ether–ethyl ether–acetic acid (80:30:5)[a]	50
12	Petroleum ether–ethyl ether–acetic acid (80:30:5)[b]	170

[a] Plate developed three times in this system.
[b] Plate developed once; no liner was used in the development tank.

Procedure. The TLC chamber was lined with Whatman No. 1 filter paper and allowed to equilibrate with the developing solvents overnight in order to achieve complete equilibrium before use, unless otherwise stated. A sample of each compound was dissolved in methanol at a concentration of 10 mg/ml, and 10 μl of this solution were spotted on a thin-layer plate, using a micropipet. The plate was developed at room temperature, allowing the solvent front to move 15 cm from the point of application and then removed from the chamber and dried at room temperature. The plate was then visualized under a UV lamp in both the short (254 nm) and long (366 nm) wavelength regions unless otherwise specified.

Results and discussion

Seven different solvent systems were examined for use in the chromatography of monosodium mycophenolate on cellulose-coated thin-layer plates. The results of this investigation indicated that mycophenolic acid and related compounds cannot be satisfactorily chromatographed on cellulose if simple solvent systems are employed.

The TLC results obtained by using silica gel plates are quite meaningful. Table I shows the solvent systems utilized, along with corresponding development

TABLE II

R_F × 100 VALUES ON SILICA GEL PLATES IN VARIOUS SOLVENT SYSTEMS

Solvent systems are as expressed in Table I. Compounds: A = Mycophenolic acid; B = ethyl mycophenolate; C = methyl mycophenolate; D = normycophenolic acid; E = cyclic acid hydrolysis product with the assigned name 3,4-dihydro-5-methoxy-2,6-dimethyl-9(7H)-oxo-2H-furo <3,4-h> benzopyran-2-propionic acid.

Solvent system	*Compound*				
	A	*B*	*C*	*D*	*E*
1	27	70	70	17	17
2	45	68	64	40	25
3	67	71	71	65	57
4	77	81	81	76	70
5	53	79	79	47	47
6	34	49	47	29	16
7	11	21	20	9	2
8	55	65	64	51	45
9	67	83	80	63	39
10	43	54	54	38	25
11	43	67	58	34	11
12	41	65	56	32	10

times. In Table II, the R_F values determined for the compounds studied are listed, along with the appropriate solvent systems. Monosodium mycophenolate and disodium mycophenolate behaved similarly to mycophenolic acid in all solvent systems investigated.

The solvent system which gives the best overall separation of the compounds studied was utilized in developing the plate photographed in Fig. 1. Ferric chloride is specific for phenolic hydroxy groups, so the lack of color for the spot in lane E after spraying the plate with ferric chloride was a good indication that acid hydrolysis of mycophenolic acid resulted in cyclization involving the hydroxy group. The fact that

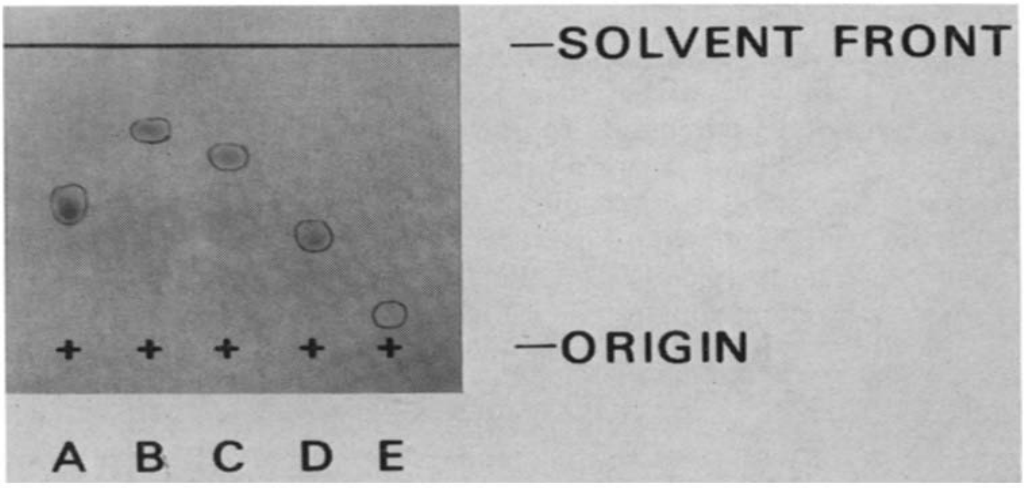

Fig. 1. Thin-layer chromatogram of mycophenolic acid and related compounds. Solvent system No. 12, Table I. Lanes correspond to compounds A–E, Table II.

the two related esters, ethyl mycophenolate and methyl mycophenolate, can be quantitatively separated from one another by TLC gives credence to the method.

All of the systems investigated are satisfactory for identification of mycophenolic acid by comparing R_F values of main spots in sample lanes with the R_F value of the spot in the reference standard lane. However, some of the systems are suitable for following stability of mycophenolic acid in addition to control.

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