Journal of Chromatography, 108 (1975) 405–408

CHROM. 8166

Note

Thin-layer chromatography of the cytochalasins

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(First received October 29th, 1974; revised manuscript received December 31st, 1974)

The "cytochalasins"[1] are a new class of mould metabolites that have unusual effects on mammalian cells in tissue cultures[2]. *Phoma* sp. (S-298) and *Helminthosporium dematioideum* have yielded cytochalasins A, B and F[3–5], while cytochalasins C, D and other zygosporin compounds have been isolated from cultures of *Metarrhizium anisopliae* and *Zygosporium masonii*[6–8]. Cytochalasin E was isolated from *Rosellinia necatrix* and *Aspergillus clavatus*[9] and its proposed structure has recently been revised[10]. Two new cytochalasins tentatively named as paspalin PI and paspalin PII (and later revised to cytochalasins H and J, respectively[11]) were reported by Pendse*,[12]. The literature on cytochalasins up to mid-1972 was reviewed by Binder and Tamm[13] and more recent work has been reviewed by Padhye and Pendse[14].

Although the isolation of these metabolites has involved some critical chromatographic separations, no systematic studies on their chromatographic profiles have been reported. In this paper, we describe the results of TLC studies on seven cytochalasins.

EXPERIMENTAL

Cytochalasins A, B, D and E were a kind gift from Dr. W. B. Turner of the Biochemical Research Department, ICI Pharmaceuticals Division, Alderley Park, Great Britain; cytochalasin C was kindly provided by Dr. H. Minato of the Shionogi Research Laboratory, Shionogi and Co. Ltd., Osaka, Japan; cytochalasin H (paspalin PI) and J (paspalin PII) were obtained from the Indian Drugs Research Association, Poona, India.

TLC separations were carried out on silica gel G plates (thickness 0.25 mm) prepared by using a Desaga-type applicator. The plates were activated at 110° for 40 min before use and solutions of the cytochalasins in dichloromethane (*ca.* 3 μl) were spotted on them.

Several developing solvent systems were used, of which eleven are considered here. The spots were made visible by using spray reagents commonly used for ketones[15], lactones[16,17] lactams[18] and indole derivatives[19–23].

* The work which yielded tentative structures of cytochalasins H and J was carried out in collaboration with Hoffmann-La Roche, Basle, Switzerland.

RESULTS AND DISCUSSION

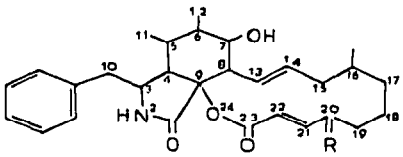
Many of the spray reagents used for ketones, lactones and indoles were not successful in the detection of cytochalasins. The reagents that reacted with these compounds were (a) ethanolic sulphuric acid[24]; (b) vanillin–phosphoric acid[24,25]; and (c) Dragendorff's reagent[26].

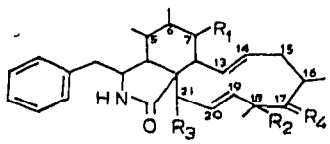
Examination under UV illumination showed fluorescence only for cytochalasin D (weak yellow) and H (yellow) on spraying with ethanolic sulphuric acid. The other spots were various shades of dull orange. The solvent system used by Korte and Vogel[18] for the separation of lactams was not successful with the present compounds, which is understandable in view of the large macrocycle fused to a highly substituted hydrogenated isoindolone unit in these metabolites.

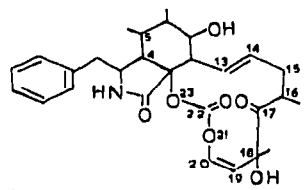
The solvent systems that yielded better separations were chloroform–methanol (95:5) (1), chloroform–diethylamine (90:10) (3), cyclohexane–ethyl acetate–diethylamine (6:3:1) (5) and benzene–acetone (70:30) (11) (Table I).

TABLE I

R_F VALUES OF CYTOCHALASINS ON SILICA GEL G PLATES







I, $\Delta^{6,12}$; R = O:cytochalasin A. II, $\Delta^{6,12}$; R = H, OH:cytochalasin B. F* $\Delta^{4,5}$; R = H, OH:cytochalasin F. III, $\Delta^{5,6}$; $R_1 = R_2 = OH$, $R_3 = OAc$, $R_4 = O$:cytochalasin C. IV, $\Delta^{6,12}$; $R_1 = R_2 = OH$, $R_3 = OAc$, $R_4 = O$:cytochalasin D. V, $\Delta^{6,12}$; $R_1 = R_2 = OH$, $R_3 = OAc$, $R_4 = H$:cytochalasin H (paspalin PI). VI, $\Delta^{6,12}$; $R_1 = R_2 = R_3 = OH$, $R_4 = H$:cytochalasin J (paspalin PII). VII, $\Delta^{4,5}$:cytochalasin E. Solvent systems: (1) Chloroform–methanol (95:5); (2) chloroform–methanol–formic acid (95:5:5); (3) chloroform–diethylamine (90:10); (4) diisopropyl ether–ethyl acetate (90:10); (5) cyclohexane–ethyl acetate–diethylamine (60:30:10); (6) benzene–chloroform (50:50); (7) *n*-butanol–formic acid–water (80:10:10); (8) benzene–methanol (70:30); (9) isopropanol; (10) acetone; (11) benzene–acetone (70:30). Spray reagents: (i) Ethanolic sulphuric acid; (ii) vanillin–phosphoric acid; (iii) Dragendorff's reagent.

No.	*Cytochalasin*	*R_F × 100*										
		Solvent system										
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
I	A	63	53	62	39	36	0	93	67	79	95	56
II	B	58	38	53	36	30	0	92	63	78	95	47
III	C	46	34	44	23	26	0	90	59	78	92	30
IV	D	45	32	42	19t**	20t	0	89	58	76	93	34
V	H	63	55	64	46	50	0	87	59	80	92	60
VI	J	44	38	56	34	35	0	75	55	72	90	46
VII	E	79	57	63	48	40	0	90	62	82	96	71t

* Not referred to in the table.
** t = tailing.

Cytochalasins A, B and F belong to the same class of cytochalasins, according to the recent classification[1]. Of these, cytochalasin A, which is a ketone, travels faster than the corresponding alcohol (cytochalasin B). The migration behaviour of cytochalasin F, which would be expected to be similar to that of cytochalasin B, could not, however, be compared as it was not available.

Cytochalasin C and D have almost identical R_F values in most of the solvent systems used. They could, however, be well distinguished from each other by spraying with ethanolic sulphuric acid and observing their fluorescence under UV light, cytochalasin C giving a dull orange colour and cytochalasin D weak yellow. Cytochalasin H (paspalin PI) shows a close resemblance in its migration behaviour and fluorescence characteristic under UV light to cytochalasin D. Cytochalasin H, however, lacks the ketonic function at the C17 position[12], resulting in slightly higher R_F values. The lower R_F values observed for cytochalasin J may be attributed to the presence of an acetyl group in its macrocycle.

Cytochalasin E, which is the fastest moving compound in all of the solvent systems, belongs to a different class of compounds, *viz.*, 21,23-dioxa-[13]-cytochalasins, and its higher R_F value may be due to the carbonic diester group in the large macrocycle.

It seems probable that ketonic functions in the macrocycle influence the migration behaviour of these compounds on silica gel. It was also considered to be a contributory factor to their antimicrobial properties by Betina and co-workers[27,28].

ACKNOWLEDGEMENTS

The authors thank Dr. J. R. Vakil and Mr. P. G. Deshmukh for helpful suggestions. One of us (C.R.J.) is grateful to the Director, Indian Drugs Research Association, Poona, India, for allowing him to work in the laboratories as a guest research worker.

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