LETTERS TO THE EDITORS

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Antibacterial and Antifungal Activity of Benztropolone

FOLLOWING the suggestion of Dewar[1], it is now generally accepted that the mould metabolic products, stipitatic acid, puberulic acid and puberulonic acid are derivatives of tropolone[2]. Erdtman and Gripenberg[3] have shown that α-, β- and γ-thujaplicins from *Thuja plicata*, the western red cedar tree, are, respectively, α-, β- and γ-*iso*propyltropolones. γ-Thujaplicin was found to possess fungicidal activity; when spores of *Pullularia pullulans* were suspended in a 0·02 per cent solution, 100 per cent survived after 1 hr., 11 per cent after 3 hr. and none after 6 hr. and 24 hr. exposure. Two of us (J. W. C. and A. R. S.) have recently described a synthesis of 3 : 4-benztropolone[4].

We have found that 3 : 4-benztropolone is fungistatically active; at the highest concentration tested, specific fungicidal properties towards a few species were observed. The compound has weak bactericidal activity. We are indebted to Prof. H. Erdtman for a specimen of γ-thujaplicin, which was included in our tests for comparative purposes. Preliminary solubility tests showed that under the conditions of the spore germination and fungicidal tests the saturation point, which represented the highest possible effective dose of benztropolone, lay in the range 50–100 mgm./litre; γ-thujaplicin was more soluble.

Spore germination test. Spores of *Penicillium digitatum* were suspended in a series of concentrations of the substances in 'spore germination medium'. The percentages of spores which germinated after 18 hr. at 25° were estimated. Benztropolone allowed 50 per cent germination at 80 mgm./litre concentration and γ-thujaplicin at 60 mgm./litre. In a parallel experiment, trichothecin[5] completely inhibited germination at 1·25 mgm./litre, and 50 per cent germination took place at 0·4 mgm./litre.

Fungistatic activity. A series of concentrations of the substances in the range 0·6–80 mgm./litre was incorporated in nutrient medium on which the test organisms were sown. Growth was estimated visually after five days incubation at 25°.

Benztropolone gave complete inhibition of all the test organisms at 80 mgm./litre and partial inhibition of growth at 16 mgm./litre. γ-Thujaplicin was somewhat less active and caused only partial inhibition of growth of *A. niger*, *M. erectus*, *P. digitatum* and *Trichoderma viride* at 80 mgm./litre. In parallel experiments, sodium pentachlorophenate and tetramethylthiuram disulphide caused complete inhibition of *P. digitatum* and *F. graminearum* at 16 mgm./litre and partial inhibition at 3·2 mgm./litre.

Fungicidal activity. Spores of six test fungi and cells of *S. carlsbergensis* were suspended in solutions of benztropolone and γ-thujaplicin (50 mgm./litre) and incubated at 25° for 53 hr. Loopfuls of the suspension were taken at intervals and plated on to beer-wort agar medium. After five days incubation at 25°, the colonies were counted. Both benztropolone and γ-thujaplicin were fungicidal to *Pullularia pullulans* (De Bary and Low) Berkhout (laboratory collection No. 273); in a solution of the former, approximately 12 per cent of the spores survived after 24 hr., 8 per cent after 33 hr., and none after 53 hr.; the corresponding results for γ-thujaplicin were 12 per cent survivors after 24 hr., 6 per cent after 33 hr. and none after 53 hr. Similar results were obtained with *Trichothecium roseum* spores, 30 per cent of which survived 24 hr. in benztropolone solution, 10 per cent survived 33 hr., and none was viable after 53 hr. In γ-thujaplicin solution, 50 per cent of *T. roseum* spores were viable after 7 hr., 25 per cent after 24 hr., and none after 33 hr. Exposure to solutions containing 50 mgm./litre of benztropolone or γ-thujaplicin for 53 hr. had no effect on the survival of spores of *Aspergillus niger*, *Penicillium digitatum*, *P. nigricans-janczewskii* series, *Trichoderma viride* or cells of *Saccharomyces carlsbergensis*.

FUNGISTATIC ACTIVITY OF BENZTROPOLONE AND γ-THUJAPLICIN: PLATE TEST

Laboratory collection No.	Organism	Relative growth at benztropolone concentrations (mgm./litre)				Relative growth at γ-thujaplicin concentrations (mgm./litre)				Control
		80	16	3·2	0·6	80	16	3·2	0·6	
*F*160	*Aspergillus niger* van Tiegh.	0	3	4	4	2	3	4	4	4
*F*197	*Fusarium graminearum* Schwabe	0	2	4	4	–	–	–	–	4
*F*266	*Mucor erectus* Bain.	0	3	4	4	2	4	4	4	4
*F*196	*Penicillium digitatum* Sacc.	0	2	4	4	1	3	4	4	4
*F*211	*P. nigricans-janczewskii* series	0	3	4	4	0	3	4	4	4
*B*55	*Saccharomyces carlsbergensis* Hansen	0	3	4	4	0	3	4	4	4
*F*5	*Trichoderma viride* Pers. ex Fries	0	2	4	4	1	3	4	4	4
*F*292	*Trichothecium roseum* Link	0	3	4	4	0	3	4	4	4

Key: No growth, 0; trace of growth, 1; slight growth, 2; good growth, 3; abundant growth as control, 4; not tested, –.

Bactericidal activity. *Staphylococcus aureus* survived 3 hr. in benztropolone solution (50 mgm./litre) at 25°, but not 7 hr. *Bact. coli* was viable after 7 hr. under these conditions, but not after 24 hr. *Aerobacter aerogenes* was not killed after 53 hr. exposure. γ-Thujaplicin had no bactericidal effect at 50 mgm./litre concentration on *Staph. aureus*, *Bact. coli* or *Aerobacter aerogenes* after 53 hr.

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[1] Dewar, M. J. S., *Nature*, **155**, 50 (1945).
[2] See Corbett, R. E., Hassall, C. H., Johnson, A. W., and Todd, A. R., *Chem. and Indust.*, 626 (1949).
[3] Erdtman, H., and Gripenberg, J., *Nature*, **161**, 719 (1948). Erdtman, H., Anderson, A. B., and Gripenberg, J., *Acta Chem. Scand.*, **2**, 625, 639, 644 (1948).
[4] Cook, J. W., and Somerville, A. R., *Nature*, **163**, 410 (1949).
[5] Freeman, G. G., and Morrison, R. I., *Nature*, **162**, 30 (1948).