A New Trichothecin-like Antifungal Antibiotic

In a screening programme for antibiotic effects of Basidiomycetes we found a strain which inhibited *Candida albicans*. The antifungal substance was produced by this strain in surface and submerged culture in a medium containing peptone, glucose, inorganic salts and aneurin. The substance for which we provisionally propose the name 'antibiotic-*T*' was easily extracted from the fermentation liquor by most of the usual solvents. After extraction with benzene and evaporation of the solvent the residue became crystalline within a few days. Recrystallization twice from methyl alcohol gave pure crystals (prisms), melting point 126°C., $[\alpha]_D^{20}+13{\cdot}5°$, *c.* 1 in chloroform. No ultra-violet absorption characteristic of antifungal antibiotics of the polyene type was found. The antibiotic contains no halogen, sulphur or nitrogen. Micro-analyses: found (per cent): carbon, 68·51; hydrogen, 7·6; and oxygen, 25·0. The substance is very slightly soluble in hot water and gives a neutral solution; it is soluble in alcohols and sunflower oil and readily soluble in non-polar solvents. Its solution in water is stable for two months, is thermo-stable at its boiling point, but is inactivated at *p*H 12 within a few hours.

From the chemical and biological data available antibiotic-*T* seems to be very similar to the antifungal antibiotic trichothecin[1,2]. The two materials have however different R_F values in paper chromatography tests. A further difference between the two antibiotics is the negative 2:4-dinitriphenylhydrazine test of the new antibiotic. The infra-red absorption spectra of the two substances (Fig. 1) are very similar antibiotic-*T* however has no band at 1686 cm.$^{-1}$ and therefore presumably contains no ketone group.

Freeman *et al.*[1] found trichothecin to be an ester, the components of which are isocrotonic acid and a ketonic alcohol, trichothecolone. The structure of the latter was given by Freeman recently[3]. The new antibiotic was hydrolysed with a cold methanolic solution of potassium hydroxide. The acid component obtained seems to be identical with the acid component of trichothecin by the paper chromatography test.

The new alcohol component of hydrolysis has a m.p. 152°C. on recrystallization from a mixture of benzene and light petroleum. The hydroxyl content was 6·82 per cent, which corresponds to a molecular weight of 250, if one OH group is present per molecule.

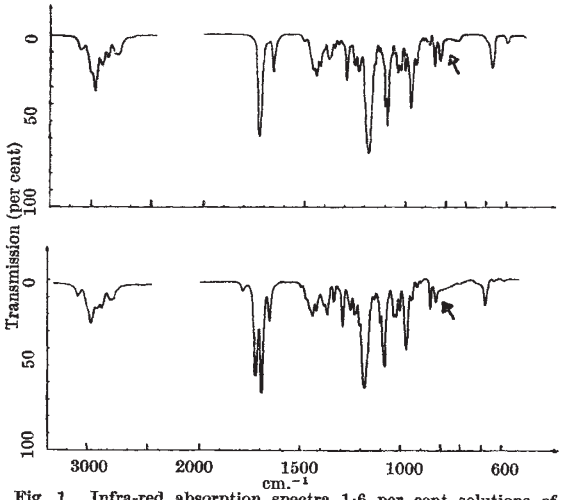

Fig. 1. Infra-red absorption spectra 1·6 per cent solutions of (top) antibiotic-*T* and (bottom) trichothecin in carbon tetrachloride. The region of solvent absorption is marked with an arrow.

Table 1.

	Days of incubation 1	2	5	8	14
	Inhibitory concentrations, μgm./ml.				
Candida albicans	4·0	11·0	45·0	90·0	
Saccharomyces cerevisiæ ...	5·0	10·0	20·0	37·0	
Cryptococcus neoformans ...		2·5	10·0	15·5	40·0
Aspergillus niger		100·0			
Trichophyton mentagrophytes		2·5	40·0	90·0	
Epidermophyton inguinale ...				4·0	20·0
Microsporon audouini ...			1·2	2·5	5·0

The fungi were seeded on the surface of agar slants containing various antibiotic concentrations. The tubes were incubated at 25°C. The figures indicate mean total inhibition on a given day of incubation.

The antifungal effects of antibiotic-*T* (Table 1) are similar to those of trichothecin[2], though somewhat weaker in the case of most of the fungi examined. The effect is fungistatic. Bacteria are not inhibited at a concentration of less than 500 μgm./ml. The LD_{50} in mice after intraperitoneal administration in a gum arabic suspension is 810 mgm./kgm. and after administration *per os* more than 1000 mgm./kgm. Doses smaller than the LD_{50} produce transient collapse, ataxia, paralysis of the hind legs and sometimes convulsions, symptoms which are analogous to those observed by Freeman with trichothecin[2]. No antibiotic was found in the blood after administration by various routes of 50–200 mgm./kgm. to mice and rats. The antibiotic is inactivated when incubated with blood at 37°C. for 24–48 hr. It is effective in reducing the yeast cells found in fæces of mice fed a standard diet containing terramycin, after the administration of 250 mgm./kgm. by mouth with a sonde. Reddening and irritation is caused when the antibiotics applied to the skin of guinea pigs, rabbits and human beings; the alcohol component of the new antibiotic does not have this effect.

The antibiotic isolated seems to differ from those mentioned in the literature but is very similar to trichothecin. The antifungal antibiotic cephalothecin[4], perhaps similar to trichothecin, contains carbon, hydrogen and oxygen and decomposes at 124–26°C. Antibiotic-*T* melts without decomposition at this temperature.

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Phenazine Di-N-Oxide as a Carcinostatic Agent

Phenazine di-*N*-oxide has been found to be a carcinostatic agent for the Ehrlich ascites tumour. More than 90 per cent of tumour-bearing animals treated with this compound intraperitoneally survived 30 days or more, and were then free of tumours as