A New Antifungal Substance of Fungal Origin

FROM a mould filtrate we have extracted a new substance with strong antifungal properties. The mould itself, which we were unable to classify completely, belongs to the species *Monosporium bonorden* and has been isolated from a sample of soil from the Belgian Congo. It develops white, almost sterile mycelia on Czapec-agar, with the reverse of the colonies varying from straw-yellow to brown with age.

The best production of active substance is obtained on Czapec–Dox medium after six days at 27° C. Extraction must be carried out by cold chloroform (5 per cent by volume of the filtrate) because the substance loses its activity rapidly when boiled in organic solvents during continuous extraction.

The residue of the chloroform extraction may be recrystallized from chloroform, alcohol or benzene, yielding about 250 mgm./litre of colourless crystals melting at 193·5° C.

By incorporating the active substance into Czapec-agar medium, growth of many moulds was completely inhibited at the following concentrations: at 25 p.p.m., *Penicillium brevi compactum, Aspergillus itaconitus* and *Ascochyta pisi*; at 50 p.p.m., *Botrytis cinerea*; and at 100 p.p.m., *Alternaria solani*. The substance shows a pronounced selectivity towards the mould species, for a concentration of 500 p.p.m. was not enough to inhibit the growth of *Penicillium rubrum* Stoll and *Fusarium solani phaseoli*.

By application of the 'slide germination method'[1] spore germination of *Penicillium cyclopium* Westling and *Penicillium stoloniferum* Thom was completely prevented by a concentration as low as 5 p.p.m.

At *p*H 7 the activity is not affected by sterilization for 5 min. at 105° C.

Against bacteria, concentrations of 200, 200 and 1,000 p.p.m. were required to inhibit the growth of, respectively, *Bacillus mycoides, T.B.C.* and *Staphylococcus aureus* during 24 hr. at 37° C. Seven other species of bacteria were not affected by concentrations up to 4,000 p.p.m. The substance is optically active, $[\alpha]_D^{20} = +203°$ in chloroform, and contains only carbon, hydrogen and oxygen. Molecular weight determinations gave, by different methods: 323, 332 and 337. Semimicro analyses gave: C, 60·40 and 60·41 per cent; and H, 4·81 and 4·75 per cent, leading to the empirical formula $C_{17}H_{16}O_7$.

The presence has been detected of two phenolic hydroxyl groups, one active hydrogen on an aromatic ring, one double link in a side-chain and a free carboxyl group. Ferric chloride produces a violet colour. The substance is almost insoluble in water but dissolves readily in dilute alkalis giving a yellow solution.

Acetylation and benzoylation give, respectively, a diacetate of melting point 189° C. and a dibenzoate of melting point 215° C., neither of which showed antifungal properties. Linking the substance with diazosulphanilic acid gives a brick-red pigment of melting point 123° C. with the same antifungal properties as the original substance. Oxidation with alkaline potassium permanganate breaks up the molecule, and one of the products isolated has been identified as succinic acid.

The analyses and chemical properties of the new antifungal substance we have isolated indicate that its molecular structure may be closely related to the structure proposed for mycophenolic acid[2], an antibiotic extracted from *Penicillium brevi compactum*[3]. Further experiments are being carried out to confirm this relationship.

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[1] *Phytopathologia*, **33**, 624, 633 (1943).
[2] *Biochem. J.*, **43**, 216 (1948).
[3] *Biochem. J.*, **39**, 398 (1945).

Ribose and the Maillard Reaction in Fish Muscle

THE occurrence and control of the Maillard reaction has been studied fairly thoroughly in dehydrated milk, eggs, fruits and vegetables; but little information is available concerning its prevalence in flesh foods. It has been shown in previous work that moist fish flesh usually becomes brown during exposure to high temperatures (for example, 120° C. for 1 hr.), and that this alteration is due to reactions of the Maillard type[1]. The nature of the sugar or substance contributing the aldehyde groups essential for these reactions was not ascertained. The present investigation indicates that ribose is responsible.

It was found convenient to determine the relative intensity of browning in heated muscle of white-fleshed fish by use of a Beckman model *DU* spectrophotometer equipped with a reflectance accessory attachment. As an arbitrary standard a 'white' surface made by blending 4 per cent high-viscosity carboxymethylcellulose and 2·5 per cent magnesium carbonate with water was used. The percentage of light reflected from the surface of heated fish samples was referred to this standard set as 100 per cent, a wave-length of 500 mμ being used.

It was found initially that the muscle of the sea fish examined had a total pentose content which often exceeded 1,500 μgm. per gm. of wet weight. This is probably associated with, or derived from, the nucleic acid of fish muscle nucleotropomyosin[2]. Since tests[3] of aqueous extracts of the muscle for deoxyribonucleic acid were negative, it was reasonably certain that deoxyribose was absent, thus verifying other work[2].

For comparative studies, muscle of halibut (*Hippoglossus stenolepis*), which browns very slightly on heating at 120° C. for 1 hr., was compared with that of lingcod (*Ophiodon elongatus*), which, in common with local species of flounders and *Sebastodes*, browns markedly on heating. With lingcod muscle, total pentose, as determined using a trichloracetic acid extract[2], decreased considerably after heating for 1 hr. at 120° C.; but there was no appreciable further loss after an additional 2-hr. heating. On the other hand, with halibut there was very little measurable loss of total pentose even after heating for 3 hr. When 1,000 μgm. of D-ribose was added per gm. of either halibut or lingcod muscle, it could no longer be recovered from the heated flesh. These findings indicated that only free and not combined pentose was inactivated in heated muscle. In addition, it was found that the ribosides guanosine, uridine and adenosine did not occasion an increase in browning of heated muscle as did ribose itself.

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