purpose. This fungus did not grow on a medium containing Sabouraud glucose agar, 'Difco' and 10 per cent serum with 0·002 per cent 2,9-dimethyl-*o*-phenanthroline. Growth was strongly inhibited by this substance in concentrations ranging from 0·001 to 0·0005 per cent. Normal growth occurred if the medium contained less than 0·0005 per cent of the substance.

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Effect of Oxidized *d*-Limonene on Micro-organisms

DURING our studies on microbiological problems in the citrus industry, we found it necessary to study the effect of essential oils on micro-organisms. As a first step in this study, we investigated in a synthetic medium the influence of *d*-limonene, which constitutes more than 90 per cent of the orange essential oil, on micro-organisms normally present on the surface of oranges.

It was shown in this way that *d*-limonene, freshly distilled over sodium, has no inhibitive action on the micro-organisms tested (a strain of *Sacar. elipsoideus*, *Wilia hansenula*, *Oidium lactis*) when added to the synthetic medium. However, the vapours of this limonene exhibit a bacteriostatic effect.

When exposed to air, *d*-limonene reveals inhibitive properties, which increase with the duration of exposure. Also, when samples of the oxidized limonene are tested for the presence of peroxides by the potassium iodide method, iodine is liberated and by titration an increase of peroxide formation may be shown.

Quantitative estimation of the inhibition by Hinshelwood's method[1] has revealed that this action is marked only by shifting the time of the lag phase, without any influence on the logarithmic growth phase and the number of the total population. When plotting the lag time against the increased doses of an oxidized *d*-limonene, an adsorption isotherm curve is obtained, similar to those obtained by Hinshelwood. Samples of *d*-limonene, taken at different times of exposure to air, give a set of such curves with the point of lethal dose decreasing with increasing time of oxidation.

The oxidized *d*-limonene, in the aqueous nutrient medium, however, loses its inhibitive properties on keeping. This decrease of inhibition is enhanced by the presence of *l*-ascorbic acid in the medium.

Steam distillation of oxidized *d*-limonene in presence of sodium hydroxide also destroys its inhibitive properties. Neither the distillate nor the resinous viscous residue added in adequate doses to the synthetic medium shows any inhibition of the micro-organisms tested.

Recent advances in organic chemistry[2] show that the fixation of an oxygen molecule from air in *d*-limonene will take place by forming a peroxide bond. This oxidation can be considered as an 'initiation reaction', which will catalyse a second reaction, namely, a polymerization of the activated terpen radicals. Hence, we may assume that the first reaction is responsible for the appearance of the inhibitive properties of limonene exposed to air. This accords with the generally known toxic properties of peroxides or epoxides. (However, the possibility that the second reaction may influence the specific toxicity of any peroxide formed cannot be excluded.)

There is a lack of precise chemical–physical data as to the mechanisms of the two reactions. When in our experiments the oxidation of *d*-limonene was carried out in different physical conditions, quantitative estimation of the reaction showed a change in the value of the parameters of the adsorption isotherms. A more detailed account of this work will be published elsewhere.

The fact that the oxidation is catalysed by light and chlorophyll[3] suggests that the reaction is possibly of biological importance, and experiments in this direction are under way.

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Isolation of Tetrahydroharman from *Petalostyles labicheoides*

IN the course of a comprehensive study of the alkaloids in Australian Leguminosæ, the examination of *Petalostyles labicheoides* was undertaken. This erect and bushy shrub grows to a height of several feet, and is found mainly in northern New South Wales and in Queensland. Alkaloids were detected in this plant in the course of the extensive field tests carried out by Mr. L. J. Webb[1], who kindly arranged for a quantity of the plant to be supplied for more intensive investigation.

The finely ground and dried plant was first intracted with chloroform to remove various colouring matters, after which the alkaloid was extracted with methanol. Concentration of the extract gave a thick syrup which was taken up in 1 per cent hydrochloric acid. The acid solution was basified, and the precipitated alkaloid extracted with ether. (The ether-insoluble material probably contained another (unstable) alkaloid, which, however, has so far resisted all attempts at purification.) The crude crystalline material obtained on evaporation was dissolved in anhydrous ether and purified by chromatography on alumina. The pure alkaloid formed very pale yellow prisms or needles, of melting point 178–180° (found: C, 77·5; H, 7·7; calc. for $C_{12}H_{14}N_2$; C, 77·4; H, 7·6 per cent). The melting point, analysis and ultra-violet absorption spectrum, the last kindly determined by Mr. R. S. Pearce (maxima at 2255 A., log ε, 4·57; 2830 A., log ε, 3·90; and 2910 A., log ε, 3·82), indicated that the alkaloid was tetrahydroharman (II, melting point in the literature, 179–180° [2]). The benzoyl derivative first separated from ether as colourless crystals, melting point 165–167° (in the literature, 168–169°), but on recrystallization from 50 per cent alcohol it formed long needles of melting point 194–195° (found: C, 78·55; H, 6·0; calc. for $C_{18}H_{16}N_2O$; C, 78·25; H, 5·8 per cent). This was evidently a dimorphic form. The identity of the alkaloid was confirmed by the preparation of the hydrochloride, melting point