61. Biochemistry of the Wood-rotting Fungi

3. The Production of Methyl Mercaptan by *Schizophyllum commune* Fr.

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During cultural tests to determine the most suitable source of nitrogen for various wood-rotting fungi it was observed that a highly objectionable odour, akin to that of decaying cabbage, was produced by *Schizophyllum commune* Fr. when this fungus was grown on a synthetic medium containing ammonium and magnesium sulphates, although in the absence of sulphate no such odour was apparent. This suggested the possibility that the fungus was converting the sulphate present in the medium into volatile sulphides and since the reduction of sulphates to sulphides by fungi does not seem to have been recorded, it appeared worth while to investigate the nature of the volatile substance produced by *S. commune*.

The fungus was grown on a synthetic medium containing sulphates, and a current of sterile air, drawn over the surface of the culture to remove gaseous products, was bubbled through mercuric cyanide solution. The volatile organic product thus isolated as the mercaptide was found to be methyl hydrogen sulphide. A slight blackening of the reagent in the earlier stages of the experiment indicated the probable presence of traces of hydrogen sulphide. A second bubbler containing mercuric chloride solution collected only a small amount of precipitate which proved to be the compound CH_3SHgCl, which had evidently arisen from traces of methyl mercaptan not absorbed by the first bubbler. Thus no evidence of any other volatile sulphides was obtained. Quite apart from the indication afforded by the odour, which is absent when the medium does not contain sulphates, it is established on a quantitative basis that the methyl mercaptan produced must have arisen from sulphate.

Two features of this reaction are of interest, (*a*) the reduction of sulphate to sulphide and (*b*) the methylation with the production of methyl mercaptan, although it is not intended to imply that complete or even partial reduction necessarily precedes methylation. The reduction of sulphates to sulphides is a well-known and characteristic reaction of certain bacteria and has been stated to occur in the case of yeasts [cf. Tanner, 1918] although it is doubtful whether the H_2S which is undoubtedly formed arises from the sulphate present in the medium. Certain of the higher plants are able to reduce sulphates, thus Nightingale, Schermerhorn & Robbins [1932] found that sulphate is reduced to sulphite and apparently to sulphydryl in the comparatively alkaline phloem region of roots and tops, and reduction of sulphate to disulphide was found by Hammett & Reynolds [1937] to occur in extracts of *Phaseolus vulgaris* root tips. Direct evidence of the reduction of sulphates by fungi is lacking, although it is to be inferred from the fact that many fungi can grow and therefore assimilate sulphur on synthetic media containing sulphate as sole source of sulphur. From a survey of the ability of *Aspergillus niger* to assimilate inorganic sulphur compounds Steinberg [1941] concluded that sulphur is reduced to sulphoxylate before conversion into organic sulphur. Sulphide and disulphide were not assimilated. We believe that the present example is the first recorded instance of the reduction of sulphate to sulphide by fungi.

Methylation is a widespread biological phenomenon and numerous instances of it have been reported amongst the fungi. It is often regarded as a method of detoxication of substances harmful to the organism. Birkinshaw & Findlay [1940] showed that the wood-destroying fungus *Lentinus lepideus* produces methyl methoxycinnamate and suggested that this ability to produce methylated compounds might account for the high resistance of this fungus to antiseptics such as creosote. *L. lepideus*, when grown on wood preserved with zinc meta-arsenite gives off a strong smell of garlic [van den Berge, 1934], which on the strength of the investigation of Challenger, Higginbottom & Ellis [1933; see below] on *Penicillium brevicaule* has been attributed to trimethylarsine, although no identification of the gaseous products was undertaken. It is hoped to examine this aspect of methylation by *Lentinus lepideus* in a future investigation since it has not only theoretical interest but also obvious practical importance as indicating the possible danger of using in buildings timber preserved with arsenic compounds.

The methylation of inorganic sulphur by fungi has not been previously noted, but the observation of Haas [1935] that the seaweeds *Polysiphonia fastigiata* and *P. nigrescens* evolve dimethyl sulphide very shortly after being gathered is of interest in this connexion. The closest parallel to the present case is to be found in the observations of Challenger and his collaborators on the mould *Penicillium brevicaule* (*Scopulariopsis brevicaulis*). This organism, when grown on bread cultures containing arsenious oxide, sodium methyl arsonate or sodium cacodylate affords trimethyl arsine [Challenger *et al.* 1933]; in presence of sodium selenate or selenite, dimethyl selenide is obtained [Challenger & North, 1934]. On the other hand, no dimethyl sulphide could be obtained from sulphur, sodium sulphite, sodium thiosulphate, thiourea, sodium ethyl sulphonate, 'rongalite' or thiodiglycollic acid. Diethyl disulphide is converted into ethyl mercaptan and methyl ethyl sulphide and di-*n*-propyl disulphide yields *n*-propyl mercaptan and methyl-*n*-propyl sulphide [Challenger & Rawlings, 1937]. These last examples involving methylation of organically linked S are in a somewhat different category from the direct methylation of inorganic sulphur which is accomplished by *Schizophyllum commune*.

It thus appears that although the fungi *Penicillium brevicaule* and *Schizophyllum commune* may have somewhat similar methylating actions, *Penicillium brevicaule* lacks the power shown by *Schizophyllum commune* of reducing oxygen acids of sulphur to sulphide. It is unlikely that any of the moulds which have been cultivated in the laboratory on synthetic media are able to convert sulphates into volatile sulphides, since sulphates are usual constituents of synthetic media and any volatile sulphides formed would be readily detected by their odour.

Experimental

Organism. The strain of *Schizophyllum commune* Fr. employed was originally isolated from kiln-dried African mahogany and had been identified by the production in culture of typical sporophores. The culture was tested and found to be free from bacterial contamination.

Method of culture

The fungus was grown in a London tap-water medium containing the following added constituents: glucose 3 %, $(NH_4)_2SO_4$ 0·3 %, KH_2PO_4 0·25 %, $MgSO_4,7H_2O$ 0·1 %, Marmite (as source of aneurin) 0·01 %, traces of iron (as $FeCl_3$), copper, manganese and zinc (as sulphates) and boron (as borax). The medium was distributed in 12 one-litre conical flasks (350 ml. per flask) and sterilized by autoclaving. Each flask was inoculated with a small portion of mycelium from an agar slope, the inoculum being carefully floated on the surface of the culture solution. The cotton wool plugs were then replaced by sterilized rubber bungs carrying inlet and outlet tubes, the former reaching almost to the surface of the liquid. The flasks were connected in series by means of sterilized rubber tubing and

arranged so that a current of sterile air could be drawn through the system. The cultures were incubated at 24°.

After 8 days' incubation all the flasks were showing good growth. From this time 20 litres of air were drawn through the flasks daily on 5 days a week, the air being led from the last flask of the series through a bubbler containing 5% aqueous mercuric cyanide. From time to time as precipitate accumulated in the bubbler the latter was removed and replaced by another bubbler containing fresh solution. During the greater part of the experiment another bubbler containing mercuric chloride solution was inserted after the mercuric cyanide bubbler. The amount of precipitate formed in this bubbler was only small in amount.

Examination of the precipitates

(*a*) *Mercuric cyanide bubbler.* The early precipitate formed in the bubbler was greyish white in colour with blackening around the point where the aspirated air first came into contact with the solution. This was probably due to the production by the fungus of traces of hydrogen sulphide. This blackening was absent in the later stages of the experiment, the precipitate being almost pure white. Each lot of precipitate was collected, washed and dried, the weights obtained during the successive periods being: 8th to 17th day, 0·39 g. (M.P. 165°, decomp.); 17th to 28th day, 0·65 g.; 28th to 55th day, 1·39 g.; 55th to 105th day, 1·40 g. (M.P. 184°). After 3½ months the experiment was terminated but the precipitate was still being formed, although the rate of production had slackened.

The precipitate crystallized from ethyl alcohol in the form of shining plates although the solubility was low even in the boiling solvent. After this treatment the M.P. of all the fractions reached a steady value of 184° (decomp.). (Found: C, 8·74, 8·59; H, 2·23, 2·12; S, 21·4, 21·5%. $Hg(SCH_3)_2$ requires C, 8·15; H, 2·05; S, 21·75%.) It is evident that the precipitate consists of mercury methyl mercaptide, although the M.P. of this substance is given by Klason [1887] as 175°. Further evidence of its identity was obtained by preparing pure methyl mercaptan by the method of Arndt [1921] and allowing it to react with mercuric cyanide solution. The precipitate so obtained crystallized in plates from ethyl alcohol, had M.P. 185° (decomp.) and showed no depression when mixed with the product derived from the fungus.

The yield of crude methyl mercaptide (3·84 g.) obtained during the run of the experiment would contain 0·83 g. of sulphur. The only form of sulphur in the medium other than sulphates would occur in the Marmite (if we neglect the tap water). The total Marmite present was only 0·42 g. which obviously cannot account for the volatile sulphur obtained. Hence the sulphur of the methyl mercaptan formed must have been derived from sulphate.

(*b*) *Mercuric chloride bubbler.* The small amount of precipitate in this bubbler (0·54 g.) was collected, washed and dried. It did not melt below 360° and was shown by analysis to have the composition CH_3SHgCl. (Found: C, 4·47; H, 1·34; S, 12·2; Cl, 13·1%. CH_3SHgCl requires C, 4·24; H, 1·07; S, 11·3; Cl, 12·5%.)

As RSHgCl is one of the products of interaction of alkyl mercaptan with mercuric chloride [cf. Challenger & Rawlings, 1937] the product isolated is assumed to have been formed from small amounts of methyl mercaptan which escaped absorption in the mercuric cyanide bubbler. There is thus no evidence of the production of any volatile sulphur compound other than methyl mercaptan (apart from traces of H_2S) although it is recognized that only compounds forming insoluble products with the reagents employed would be detected.

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SUMMARY

The fungus *Schizophyllum commune* Fr. when grown on synthetic media containing inorganic sulphates produces methyl mercaptan and (probably) traces of hydrogen sulphide.

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