found to yield identifiable end products with the expected chromogenic properties[7]. These end products proved to be derivatives of desoxyribose. In the case of the *p*-NPH method a single derivative of desoxyribose was detected, namely, the phenylhydrazone derivative. In the case of the DPA method, however, free desoxyribose, as well as a second unidentified diphenylamine reactive substance, was found in the incubation system. These findings will be published in the near future.

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# REDUCTION OF CERTAIN AROMATIC ACIDS TO ALDEHYDES AND ALCOHOLS BY *POLYSTICTUS VERSICOLOR*

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SUMMARY

1. *Polystictus versicolor* reduced the following acids to the corresponding aldehydes and alcohols: *m*-, *p*-methoxybenzoic, 3:4-dimethoxybenzoic, β-naphthoic. Only the alcohols were detected from *o*-methoxybenzoic and benzoic acids. *o*- and *p*-Hydroxybenzaldehydes were reduced to alcohols. The following acids were not reduced: 2:4-dimethoxybenzoic, phenylacetic and α-naphthoic.

2. The rate of conversion varied with the position and nature of ring substituents.

3. There was a small amount of demethoxylation of the following with production of the corresponding hydroxy acids: *o*-, *m*- and *p*-methoxybenzoic and 2:4-dimethoxybenzoic acids. The following were hydroxylates in the *para* position: cinnamic, benzoic, and phenylacetic acids, the last two in only small amounts. Some breakdown of the aromatic structure occurred during metabolism of cinnamic, β-naphthoic, *p*- and *o*-hydroxybenzoic acids.

4. The fungus produced an extracellular alcohol dehydrogenase.

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## INTRODUCTION

Following an investigation into the metabolism of methoxylated aromatic compounds by micro-fungi[1] a similar study was made with the wood-rotting basidiomycete *Polystictus versicolor*. Using preformed mats of this fungus a type of metabolism was obtained which differed markedly from that previously found. Whereas micro-fungi converted methoxybenzoic acids to the corresponding hydroxybenzoic acids which were then further metabolised through cleavage of the ring, the predominant system in *P. versicolor* was one of reduction of these acids to their corresponding aldehydes and/or alcohols. Several other aromatic acids and aldehydes were found to be reduced by this fungus. The presence of an extracellular enzyme system was also detected which could dehydrogenate the aromatic alcohols formed to regenerate the aldehydes.

## METHODS

### *Organism*

*Polystictus versicolor* was isolated from fructifications collected from a tree-stump.

### *Cultural conditions*

Mats of the fungus were obtained by the technique used previously for the micro-fungi[1]. In the present work the inocula were discs cut from the periphery of cultures growing on potato dextrose agar plates, four discs being added to each flask. After 12 days incubation, mats were obtained which completely covered the surface of the culture medium. The medium beneath the mat was then replaced by the substrate as described by KLUYVER AND VAN ZIJP[2].

### *Preparation of substrates*

Substrates were made up at a concentration of 0.004 *M*, unless stated otherwise, for the solutions analysed spectrochemically, and at 0.01 *M* for those analysed chromatographically, and were adjusted to pH 6.5 before autoclaving at 15 lb./sq. in. pressure for 20 min. Since the aldehydes were volatile, weighed amounts were autoclaved at 22.5 lb./sq. in. in small bottles with tightly screwed caps. After autoclaving, the contents were added to sterile water and aliquots then taken aseptically from each solution to determine the concentration as described below.

### *Spectrochemical analysis*

The course of metabolism was followed by studying the u.v. absorption of suitably diluted aliquots from the culture solution, and by examining the infrared spectra of the ether-soluble products. In estimating the concentration of substituted benzoic acids, use was made of the change in absorption on going from acidic to alkaline solution (0.05 *N* in $H_2SO_4$ or NaOH), corresponding to a change from the undissociated acid to the anion. As the absorption of other components of the culture solutions was generally insensitive to pH, their overlapping absorption did not interfere. The wavelengths at which $\Delta e$, the change in molar absorption coefficient, were measured are given in Table I for the various acids studied. Metabolic products from *o*-hydroxybenzoic acid were sensitive to pH and this method of estimation could not be applied. Estimations of this acid were based on measurements at 297 m$\mu$ in alkaline solution after an approximate correction for background absorption.

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TABLE I

THE DIFFERENCE IN MOLAR ABSORPTION COEFFICIENT, $\Delta\varepsilon$, BETWEEN ACID AND ALKALINE SOLUTION AT WAVELENGTH $\lambda$ (m$\mu$) FOR VARIOUS AROMATIC ACIDS

| *Compound* | $\lambda$ | $\Delta\varepsilon$ |
|---|---|---|
| *p*-Methoxybenzoic acid | 265 | 7,800 |
| *m*-Methoxybenzoic acid | 300 | 1,330 |
| | 245 | 2,790 |
| *o*-Methoxybenzoic acid | 300 | 2,700 |
| | 240 | 3,900 |
| 3:4-Dimethoxybenzoic acid | 300 | 3,700 |
| | 265 | 5,900 |
| Benzoic acid | 235 | 4,700 |
| $\beta$-Naphthoic acid | 240 | 18,240 |
| *p*-Hydroxybenzoic acid | 285 | 15,625 |
| Cinnamic acid | 250 | 4,360 |
| *p*-Hydroxycinnamic acid | 340 | 17,400 |

TABLE II

THE MOLAR ABSORPTION COEFFICIENT, $\varepsilon$, AT WAVELENGTH $\lambda$ (m$\mu$) FOR VARIOUS AROMATIC ALDEHYDES AND ALCOHOLS

| *Compound* | $\lambda$ (m$\mu$) | $\varepsilon$ |
|---|---|---|
| *p*-Methoxybenzaldehyde | 290 | 15,000 |
| *m*-Methoxybenzaldehyde | 310 | 2,550 |
| *o*-Methoxybenzaldehyde | 320 | 4,000 |
| *o*-Methoxybenzyl alcohol | 230 | 2,380 |
| *p*-Hydroxybenzaldehyde | 330* | 26,400 |
| *p*-Hydroxybenzyl alcohol | 245* | 11,800 |
| *o*-Hydroxybenzaldehyde | 375* | 5,750 |
| *o*-Hydroxybenzyl alcohol | 290* | 3,500 |
| $\beta$-Naphthaldehyde | 250 | 33,800 |
| $\beta$-Naphthyl carbinol | 223.5 | 90,000 |

* $\varepsilon$ values at these wavelengths refer to alkaline solution.

Aldehydes and alcohols in culture solutions were estimated at wavelengths where their absorption was high relative to other constituents whose absorption was corrected for where necessary. The selected wavelengths, together with approximate molar absorption data, are given in Table II. Total amounts of *p*-methoxybenzaldehyde plus *p*-methoxybenzyl alcohol were estimated at 225 m$\mu$, where their absorption is equal and $\varepsilon = 10{,}000$.

*Paper chromatographic analysis*

For this, 0.01 *M* solutions of the substrates were used. Analyses of the culture solutions were made at intervals of a few days, using the method previously described[1].

## RESULTS

*Reduction of mono-methoxybenzoic acids by* P. versicolor

The general pattern of metabolism is well-illustrated by the 3 monomethoxybenzoic acids. The concentration of all three acids fell as shown in Table III. The

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TABLE III

METABOLISM OF MONO-METHOXYBENZOIC ACIDS BY *P. versicolor*

| Days | *Residual acid as percentage of original concentration (0.004 M)* | | |
|---|---|---|---|
| | *p-Methoxybenzoic* | *m-Methoxybenzoic* | *o-Methoxybenzoic* |
| 1 | 60 | 62 | 84 |
| 2 | 32 | 32 | — |
| 3 | 14 | 17 | 73 |
| 4 | — | 5 | — |
| 5 | 4 | — | 67 |
| 7 | — | 3 | — |
| 8 | 4 | — | — |
| 9 | — | — | 48 |
| 15 | — | — | 20 |
| 21 | — | — | 10 |

— = No analysis

*para* and *meta* acids disappeared at a similar rate, but the *ortho* acid was metabolised much more slowly. During the disappearance of *p*-methoxybenzoic acid the absorption spectrum of *p*-methoxybenzaldehyde appeared in the culture medium (Table IV). The concentration of aldehyde varied erratically, ranging from 21 to 46%, over 8 days. The culture solution remaining on the 8th day was extracted with ether. The infrared spectra of the ether-soluble material showed it to be a simple mixture of *p*-methoxybenzyl alcohol and *p*-methoxybenzaldehyde in a ratio of about 1.75:1. This material gave a 2:4-dinitrophenylhydrazone whose infrared spectrum was identical with that of the dinitrophenylhydrazone of *p*-methoxybenzaldehyde.

TABLE IV

FORMATION OF *p*-METHOXYBENZALDEHYDE FROM *p*-METHOXYBENZOIC ACID BY *P. versicolor*

| *Days* | *Percentage of p-Methoxybenzaldehyde* |
|---|---|
| 1 | 21 |
| 2 | 31 |
| 3 | 46 |
| 5 | 45 |
| 8 | 33 |

In 4- and 7-day samples from *m*-methoxybenzoic acid cultures a new maximum insensitive to pH appeared at 255 mμ, which corresponded to about 2% *m*-methoxybenzaldehyde. Its presence was confirmed, in a separate experiment, by the identification of its 2:4-dinitrophenylhydrazone by its infrared spectrum. An ether extract made after 7 days incubation contained a product whose infrared spectrum showed *m*-methoxybenzyl alcohol to be the chief constituent but an impurity (not the aldehyde) was present which absorbed at 9.04 μ.

The formation of *o*-methoxybenzaldehyde from *o*-methoxybenzoic acid could not be detected by ultraviolet analysis of the culture solution nor was any precipitate obtained with 2:4-dinitrophenylhydrazine. After 22 days incubation, an ether extract

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of the culture solution yielded an oil whose infrared spectrum was identical with that of *o*-methoxybenzyl alcohol.

TABLE V

METABOLISM OF MONO-METHOXYBENZALDEHYDES BY *P. Versicolor*

| Days | *Residual aldehyde as percentage of original concentration* | | |
|---|---|---|---|
| | *p-Methoxybenzaldehyde* $2.6 \cdot 10^{-3}$ *M** | *m-Methoxybenzaldehyde* $2.0 \cdot 10^{-3}$ *M** | *o-Methoxybenzaldehyde* $2.1 \cdot 10^{-3}$ *M** |
| 1 | 60 | 37 | 0 |
| 2 | 60 | — | — |
| 4 | 44 | — | — |
| 5 | 78 | 15 | 0 |
| 6 | 65 | 25 | — |
| 8 | 46 | 25 | — |
| 10 | 48 | — | — |
| 12 | 73 | 17 | — |
| 13 | — | 32 | — |
| 14 | 56 | 22 | — |

* Original concentration.

Starting with the aldehydes, at initial concentrations as given in Table V, it was found that only the *ortho* form disappeared (Table V). With the *meta* and *para* forms, after the concentration had fallen to a certain level, no further reduction took place. The *o*-methoxybenzaldehyde culture solution was extracted with ether on the 6th day and *o*-methoxybenzyl alcohol was identified in the extract by infrared analysis. The experiment with *o*-methoxybenzaldehyde was repeated and u.v. analyses of the culture solutions made at more frequent intervals. After 4, 8 and 24 h the concentration had been reduced to 70, 45 and 5 % respectively of the initial concentration of $2.4 \cdot 10^{-3}$ *M*, while *o*-methoxybenzyl alcohol accumulated in amounts which indicated that there was a quantitative conversion of aldehyde to alcohol. Further analyses after 6 and 10 days showed that there was no re-accumulation of the aldehyde. *o*-Methoxybenzyl alcohol was again identified on examining an ether extract of the culture solution prepared on the 13th day after inoculation. The experiment with *p*-methoxybenzaldehyde was also repeated and ether extracts prepared after 2 and 7 days. A mixture of *p*-methoxybenzyl alcohol and aldehyde was identified by infrared analysis in both extracts. The presence of a readily available substrate, 1 % glucose, was found to have no effect on the rate of reduction of *p*-methoxybenzaldehyde, or on the final equilibrium reached.

*Dehydrogenation of* p-*methoxybenzyl alcohol by* P. versicolor

Examination of Table V shows that the concentrations of aldehyde tended to fluctuate in successive samples. It was found that this could be attributed to an increase in aldehyde concentration during the time that a sample was stored in the refrigerator before analysis. For example the 5- and 12-day samples, in which the aldehyde concentration was particularly high, were stored for 2 days before analysis. To confirm this, a mat of *P. versicolor* was incubated over *p*-methoxybenzoic acid for 5 days, when the acid was completely reduced, and 37.5 % of *p*-methoxybenzaldehyde had been formed. On storing a cell-free sample from this culture in the refriger-

ator for 2 days, the proportion of aldehyde increased to 53 % at the expense of the alcohol, but no increase occurred in a sample autoclaved at 15 lb./sq. in. pressure for 20 min. These results indicate that an extracellular alcohol dehydrogenase is produced by the fungus.

Confirmation of the ability of *P. versicolor* to dehydrogenate *p*-methoxybenzyl alcohol was obtained by following the conversion of the alcohol to *p*-methoxybenzaldehyde under a fungal mat. The alcohol solution (0.004 *M*) was sterilised by filtration. Analyses over 8 days showed that conversion of alcohol to aldehyde varied between 24 and 35 %. There was no overall loss of the methoxyphenyl group and no acid was formed.

*Formation of acetals*

Ether extracts from *p*-methoxybenzoic acid and *p*-methoxybenzaldehyde cultures generally gave a mixture of the alcohol and aldehyde. Sometimes, however, the acetal formed by reaction between the components of the mixture was obtained, *i.e.* $R.CHO + 2\,R.CH_2OH \longrightarrow R.CH(OCH_2R)_2 + H_2O$. It is presumed that some accidental contaminant catalysed the formation of the acetal in these extracts. Synthetic mixtures of *p*-methoxybenzyl alcohol and *p*-methoxybenzaldehyde did not react at room temperature, even on long standing, but the acetal was obtained when the reagents were heated together with $CaCl_2$ and $NH_4Cl$ crystals[3]. It was then isolated as waxy crystals, melting point 34–35° on crystallising from light petroleum. The impurity associated with *m*-methoxybenzyl alcohol obtained from cultures of *P. versicolor* on the corresponding acid may also have been an acetal, but efforts to synthesise this material failed.

*Reduction of dimethoxybenzoic acids by* P. versicolor

The metabolism of 3:4- and 2:4-dimethoxybenzoic acids was followed by the same method as was that of the mono-methoxybenzoic acids. The former was metabolised in a similar way to the monomethoxy acids, only 5 % remaining after 8 days. A 2:4-dinitrophenylhydrazone prepared from a carbon tetrachloride extract made on the 9th day was identical with that obtained from 3:4-dimethoxybenzaldehyde. An ether extract made after extracting the culture solution with carbon tetrachloride was shown by infrared analysis to contain 3:4-dimethoxybenzyl alcohol together with some aldehyde.

2:4-Dimethoxybenzoic acid was metabolised very slowly. An intermediate product, which reached a maximum after 2 days, was formed. This product had a strong absorption at 270 m$\mu$ in alkaline solution probably due to a phenolic substance. Its presence prevented estimation of residual acid, but chromatography showed that considerable amounts remained unchanged after 8 days. Chromatographic analysis (see below) also showed the formation of phenolic products. Carbon tetrachloride and ether extracts of the neutral solution, which would have extracted any 2:4-dimethoxy-benzaldehyde or -benzyl alcohol formed, yielded no products. Similarly, on incubation for 3 weeks no products could be obtained.

*Reduction of other aromatic acids and aldehydes by* P. versicolor

*Benzoic acid:* The concentration fell steadily and only 5 % remained after 7 days. On the 8th day the culture solution was extracted with carbon tetrachloride and

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ether. Benzyl alcohol was identified in the ether extract. Benzaldehyde was not detected although its characteristic smell was clearly evident during the course of the experiment.

*Phenylacetic acid:* This acid did not appear to be attacked by the fungus. No marked change in the u.v. spectrum occurred up to 7 days. No neutral products were obtained from either carbon tetrachloride or ether extractions, indicating that this acid was not reduced. Chromatography confirmed that much of the acid remained unattacked.

*Cinnamic acid:* Only 12 % of this acid remained after 11 days under the mat. The absorption of *p*-hydroxycinnamic acid, which appeared as a transient intermediate, reached a maximum corresponding to 18 % of the original acid on the fourth day, but fell off to only 2.5 % after 11 days. No other aromatic product was detectable. In a separate experiment the neutralised culture solution was extracted with ether when the concentration of cinnamic acid had fallen to 44 %. This extract yielded very little material, indicating that no significant amounts of cinnamyl alcohol or aldehyde were present.

*α- and β-Naphthoic acids*: 0.0025 *M* solutions of these acids were used. α-Naphthoic acid was not attacked. β-Naphthoic acid was converted until 49 % remained after 4 days, after which it was not changed further (Table VI). At this time 27 % β-naphthaldehyde was present. On the 8th day the culture solution was extracted with ether. A 2:4-dinitrophenylhydrazone prepared from the extract had an infrared spectrum which was identical with that of the 2:4-dinitrophenylhydrazone of β-naphthaldehyde. In the culture solution a new band at 223 mμ rose to its maximum on the 2nd day. Although β-naphthaldehyde has a maximum at 223 mμ, this does not account for the maximum at this wavelength from 2 days onwards. β-Naphthyl carbinol was synthesised from its aldehyde by reduction with $LiAlH_4$ and was found to have a very strong absorption band at 223.5 mμ. On the basis of its u.v. absorption at this wavelength, the percentage of the carbinol present in the samples was estimated (Table VI).

TABLE VI

METABOLISM OF β-NAPHTHOIC ACID BY *P. versicolor*

| *Days* | *Percentage residual acid* | *Percentage aldehyde formed* | *Percentage carbinol formed* |
|---|---|---|---|
| 1 | 71 | 6.5 | 4 |
| 2 | 52 | 11.5 | 19 |
| 4 | 49 | 27 | 4 |
| 5 | 42 | 30 | 8 |
| 7 | 48 | 30 | 4 |
| 8 | 47 | 30 | 5 |

The failure to reduce the acid completely in this experiment is probably due to the formation of toxic products in the culture solution. A lower initial concentration of acid (0.0013 *M*) was completely reduced in 4 days, with the formation of 47 % aldehyde and 20 % β-naphthyl carbinol. The low yield of products indicates that there was some attack on the naphthyl nucleus. This was confirmed when the fungus was incubated over 0.0004 *M* β-naphthyl carbinol. After 6 days 35 % had been oxidised

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to aldehyde, but only 5 % of the carbinol remained. No β-naphthoic acid was formed.

p-*Hydroxybenzaldehyde:* This compound was metabolised steadily and fairly rapidly, only 6 % remaining after 4 days. *p*-Hydroxybenzyl alcohol was produced and estimation of the amounts of aldehyde and alcohol present in the culture solution showed that there was a quantitative conversion. The alcohol was extracted with ether at pH 6.5 since it polymerises in acid conditions. It was purified by recrystallisation and gave an infrared spectrum identical with that of *p*-hydroxybenzyl alcohol synthesised by reduction of *p*-hydroxybenzaldehyde by $LiAlH_4$.

p-*Hydroxybenzoic acid:* This acid was metabolised very slowly, 10 % remaining after 14 days. U.v. analysis showed that no significant conversion to *p*-hydroxybenzyl alcohol occurred. On the 16th day the culture solution was neutralised with bicarbonate, extracted with ether and the ether extracts washed with bicarbonate solution to remove any acid present. The resulting product was too small for analysis.

o-*Hydroxybenzaldehyde:* Table VII shows the course of metabolism of *o*-hydroxybenzaldehyde, starting with an initial concentration of 0.002 *M*. The aldehyde was rapidly converted to the alcohol which was further metabolised. In order to extract the alcohol for identification by infrared analysis four flasks each containing 100 ml of $3.5 \cdot 10^{-3}$ *M* *o*-hydroxybenzaldehyde were incubated for 1 day, extracted at pH 7 with carbon tetrachloride to remove aldehyde, then with ether to extract the alcohol. The identity of the alcohol was confirmed from its infrared spectrum, melting point and mixed melting point.

TABLE VII

METABOLISM OF *o*-HYDROXYBENZALDEHYDE BY *P. versicolor*

| *Days* | *Residual aldehyde as percentage of initial concentration* | *o-Hydroxybenzyl alcohol* |
|---|---|---|
| 1 | 24 | 50 |
| 2 | 6 | 35 |
| 4 | 0 | 7 |

o-*Hydroxybenzoic acid:* At an initial concentration of 0.004 *M*, this acid was metabolised very slowly. The concentration dropped to 86 % of the original after 1 day, but after 10 days 63 % still remained. The acid was probably toxic at this level and when its initial concentration was lowered to 0.001 *M* it was metabolised slowly and steadily, less than 13 % remaining after 11 days. The corresponding aldehyde and alcohol could not be detected in the culture solution. The u.v. spectrum indicated that a complex phenolic product had been formed.

*Effect of aeration on the reduction of* p-*methoxybenzoic acid by* P. versicolor

The experimental results point to the existence of a strong reducing system in *P. versicolor*. It was considered possible that the conditions in the culture flasks might have been conducive to an anaerobic type of metabolism. However, even when a steady stream of air was bubbled through a solution of *p*-methoxybenzoic acid under a mat of the fungus, *p*-methoxybenzaldehyde was formed. This indicates that lack of oxygen was not a governing factor in the reduction of the acid. In another

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experiment the metabolism of *p*-methoxybenzoic acid under air and nitrogen was compared. After 7 days under air the concentration of acid fell to 3 %, while under nitrogen it fell to 60 % of the initial concentration. *p*-Methoxybenzaldehyde was formed in both flasks, 53 % being present in the air flask and 11 % in the nitrogen flask after 7 days. These results indicate that anaerobic conditions were not responsible for the reducing reactions which were taking place, and actually slowed down the rate of reduction considerably. *p*-Methoxybenzaldehyde was extracted from both cultures and its identity was confirmed by examining the infrared spectra of the 2:4-dinitrophenylhydrazones.

*Analysis of culture solutions by paper chromatography*

Results obtained by paper chromatography indicated that demethoxylating and hydroxylating systems were present in *P. versicolor*. Thus the formation of *p*-, *m*- and *o*-hydroxybenzoic acids from the corresponding methoxybenzoic acids was indicated. 2:4-dihydroxybenzoic acid was detected in the extract from a culture solution containing 2:4-dimethoxybenzoic acid. In addition a strong pink-orange spot, considerably larger than the spot of 2:4-dihydroxybenzoic acid, was obtained with diazotised sulphanilic acid spray. When the solvent was *n*-propanol–ammonia–water (80/5/15 vol.) this unidentified phenolic compound had an $R_F$ value of 0.536. There was no indication of demethoxylation or hydroxylation of 3:4-dimethoxybenzoic acid. Benzoic acid was hydroxylated to form *p*-hydroxybenzoic acid and phenylacetic acid to form *p*-hydroxyphenylacetic acid. Extracts from β-naphthoic acid cultures yielded only traces of coloured compounds when sprayed with diazotised sulphanilic acid. Spectrochemical analysis revealed that the naphthalene nucleus was being attacked, but these results show that the main attack did not lead to naphthol formation. Similarly, traces of coloured products were obtained with α-naphthoic acid cultures. Cinnamic acid was partly hydroxylated to *p*-hydroxycinnamic acid and an additional unidentified phenolic compound of $R_F$ 0.813 with butanol–ammonia–water (80/5/15 vol.) was detected as a yellow spot with diazotised sulphanilic acid.

These results show that several different enzyme systems were acting in the mycelium under the conditions operating in the culture flasks. U.v. absorption analysis indicated that the hydroxy acids were present only in very small quantities except in the case of 2:4-dimethoxybenzoic and cinnamic acids.

## DISCUSSION

A survey of the results reveals that there is a general pattern of reduction involving the conversion of aromatic acids and aldehydes to alcohols by *P. versicolor*. The reduction of aldehydes to alcohols is a common metabolic step, and has been reported for several aromatic aldehydes[4,5] but the reduction of acids does not appear to have been reported previously in biological systems. The rates of conversion vary with the position and nature of ring substituents. In particular there is evidence that the steric effect of *ortho* substituents may interfere with the reduction of the aromatic acids. Thus *m*- and *p*-methoxybenzoic acids are much more rapidly reduced than the *ortho* isomer. Again β-naphthoic acid is readily reduced, but α-naphthoic acid is not attacked and 3:4-dimethoxybenzoic acid is reduced but the corresponding 2:4 isomer is not. A marked reduction in metabolic rates when *ortho* substituents are present

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has been reported in the β-oxidation of various aryloxy-propionic and butyric acids[6], in the demethoxylation of mono-methoxybenzoic acids and in the decomposition of hydroxybenzoic acids[1]. Steric factors, however, do not appear to inhibit reduction of the methoxybenzaldehydes, since the *ortho* isomer is rapidly reduced to the alcohol.

Apart from the above instances where steric effects may operate, reduction products were not obtained from four of the acids, namely, *o*- and *p*-hydroxybenzoic, phenylacetic and cinnamic acids. The first two are of interest in that the corresponding aldehydes are readily reduced. The aromatic rings of both hydroxy acids are attacked by the fungus: nevertheless it can be concluded that no significant reduction of *p*-hydroxybenzoic acid could have occurred, since this would have led to *p*-hydroxybenzyl alcohol, which is not attacked. It is uncertain, however, whether *o*-hydroxybenzoic acid is reduced, since the expected reduction products are also attacked by the fungus.

Phenylacetic and cinnamic acids differ from the other acids examined in that their carboxylic acid groups are not directly attached to the ring, although in cinnamic acid this group is conjugated with the ring through the C = C bond of the side chain. No significant reduction of phenylacetic acid could have occurred, as much of this acid remained unmetabolised in the experiment. Cinnamic acid, however, was rapidly metabolised, so that reduction of this acid, with subsequent attack on the reduction products, cannot be excluded.

In several instances only partial reduction of aldehyde to alcohol occurred, the final ratio of the two varying with the different compounds. This is clearly seen for *m*- and *p*-methoxybenzaldehydes in Table VI. Other aldehydes not completely reduced were 3-4-dimethoxybenzaldehyde and β-naphthaldehyde. The persistence of aldehydes in the culture solution can probably be ascribed to the action of the extracellular dehydrogenase found there, and further investigations on it are being carried out. In contrast to the above, *o*-methoxybenzaldehyde, *p*-hydroxybenzaldehyde and, presumably, benzaldehyde itself were quantitatively reduced to alcohols. *o*-Hydroxybenzaldehyde was also reduced to the alcohol, but the alcohol was further metabolised.

Paper chromatography revealed that the ability possessed by micro-fungi to demethoxylate aromatic acids, with formation of hydroxylated compounds, was by comparison only weakly developed in *P. versicolor*.

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