A Glutathione Conjugate of 2,4-Dichloro-1-nitrobenzene: Its Detection as a Metabolic Intermediate and Further Metabolism in Mucor javanicus*

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(Received March 4, 1987)

Formation of a 2,4-dichloro-1-nitrobenzene glutathione conjugate \([S-(5\text{-chloro-2-nitrophenyl})\text{glutathione}, \text{2}]\) was confirmed in the cell-free system of Mucor javanicus which metabolizes 2,3- and 2,4-dichloro-1-nitrobenzenes into the corresponding chloro-methylthio-nitrobenzenes or chloro-methylthiobenzenamines. Further metabolisms of 2, the corresponding cysteine conjugate \((3)\) and 5-chloro-2-nitrobenzenethiol \((4)\) were investigated. Oxidation products \((S\text{-oxides and } S\text{-dioxides)}\) of the formerly identified methylthio-containing metabolites were isolated from the growing cultures of the fungus administered 2. \(S\text{-}(5\text{-chloro-2-nitrophenyl})\text{cysteine (3) and } N\text{-acetyl-}(5\text{-chloro-2-nitrophenyl})\text{cysteine (7)}\) were newly identified as metabolites of 2 in the resting cell system of the fungus.

INTRODUCTION

During the course of our previous studies,\(^1,2\) Mucor javanicus was found to metabolize 2,3- and 2,4-dichloro-1-nitrobenzenes (DCNB) into the corresponding benzenamines, 3- or 4-chloro-2-methylthio-1-nitrobenzene and 3- or 4-chloro-2-methylthiobenzenamine. On the basis of the metabolite structure, two reactions, namely, reduction of the nitro group and/or substitution of the ortho chlorine atom by a methylthio group were responsible for the formation of these metabolites. However, no information was obtained about a metabolic pathway or a source of the sulfur atom in methylthio-containing metabolites. Lamoureux and Rusness\(^3\) have recently reported on the pentachloronitrobenzene (PCNB) metabolism in onions, proposing a mechanism of pentachloro-methylthiobenzene (PCMTB) formation. Their investigation shows that \(S\text{-}(pentachlorophenyl)cysteine, which is suggested to be an intermediate derived from } S\text{-}(pentachlorophenyl)\text{glutathione, is metabolized to pentachlorobenzenethiol, followed by methylation to PCMTB. Their results suggest the formation of methylthio-containing metabolites from DCNB by } M. javanicus via the similar pathway to that yielding PCMTB from PCNB in onions.

This paper is to present some lines of evidence for the presence of a metabolic pathway in the fungus to afford methylthio-containing metabolites \((5,6)\) from 2,4-DCNB \((1)\) via its glutathione conjugate \([S\text{-}(5\text{-chloro-2-nitrophenyl})\text{glutathione, } \text{2}])\). For example, first, glutathione S-transferase (GST) activity was detected in the cell-free extract of M. javanicus, and glutathione conjugate 2 derived from 2,4-DCNB \((1)\) was successfully isolated from the enzymatic reaction mixture. Second, further metabolism of 2, a cysteine conjugate \([S\text{-}(5\text{-chloro-2-nitrophenyl})\text{cysteine, } \text{3}])\), or 5-chloro-2-nitrobenzenethiol \((4)\) resulted in the formation
of methylthio-containing metabolites (5, 6). In the course of the present study, the formation of an N-acetylcysteine conjugate (7) from 2 was unexpectedly observed in the resting cell suspension of *M. javanicus*.

**MATERIALS AND METHODS**

1. **General Methods**

   Melting points (mp) were determined by the micro hot-plate method and uncorrected. *Instrumentation.* Electron impact ionization mass spectra (EI-MS) were obtained on a JEOL JMS-D300 instrument (direct inlet system, 70 eV ionization potential), or a JMS-D300 combined with a gas chromatograph (JEOL JGC-20K equipped with 5% Silicone SE-30 on Uniport HP or 5% PEG-20M on Celite 545 AW DMCS each in 2 mm i.d. × 1.0 m glass column, GC-MS system, 23 eV ionization potential). Field desorption mass spectra (FD-MS) were determined on a JEOL JMS-01SG 2 (12–16 mA emitter current). UV, IR and 1H-NMR spectra were recorded on a Hitachi Model EPS-3T, a Hitachi Model 285 and a JEOL FX-100, respectively. Specific rotations were recorded on a JASCO DIP-4 Digital Polarimeter. GLC: Shimadzu GC-4CM (FID) equipped with 5% PEG-20M on Uniport W (AW) or 5% Silicone SE-30 on Uniport HP/4 mm i.d. × 1.0 m glass column, was used for quantitative or qualitative analyses. HPLC: JASCO 10G 15H equipped with a UV detector and a Cosmosil 5C18, 4.6 mm i.d. × 15 cm column. TLC: Silica Gel 60 F254 pre-coated glass plates (0.25 mm thickness) were used for analytical or preparative TLC, and aromatic compounds were visualized under UV254nm light. Solvent systems for development or elution were: Solvent A, CHCl3-MeOH-AcOH-H2O =10:7:1:2; Solvent B, n-BuOH–AcOH–H2O=12:3:5; Solvent C, MeOH–benzene–conc. ammonia water = 90:7:3; Solvent D, hexane–benzene–ether =1:1:1.

2. **Chemicals**

   See our previous papers. Glutathione and cysteine conjugates of 2,4-DCNB were prepared by the method of Lamoureux and Rusness. S-(5-Chloro-2-nitrophenyl) glutathione (2): Glutathione conjugate 2 was prepared from 2,4-DCNB (1, 1.24 g) and glutathione (1 g) in 100 ml of H2O–MeOH–acetic acid (43:40:17) containing 2.24 g of K2CO3 with stirring at room temperature for 9 hr. The reaction mixture was then adjusted to pH 7.0 and washed with ether (×3). The water layer was concentrated to ca. 40 ml, acidified to pH 3.0 and kept in a refrigerator overnight. The resulting precipitates were collected and recrystallized from 50% EtOH in pale yellow needles, mp 204–205°C. [α]D +28° (c=1, 1 N HCl). FD-MS m/z (%): 465 (M++3, 4l), 464 (M++2, 26), 463 (M++1, 100). IR νmax cm−1: 3270, 3060, 1640, 1580, 1500, 1420, 1320, 1230, 1120, 860, 820, 780, 750, 670, 550. 1H-NMR δDCl-D2O (100 MHz) ppm: 2.17 (2 H, br. q, J=6.5 Hz, He), 2.52 (2 H, t, J=6.5 Hz, He), 3.37 (1 H, dd, J = 8.8 and 14.3 Hz, He), 3.65 (1 H, dd, J=6.7 and 14.3 Hz, Hf), 3.96 (2 H, s, Hg), 4.08 (1 H, t, J = 6.5 Hz, Hf), 4.72 (1 H, dd, J = 6.7 and 8.8 Hz, Hg), 7.41 (1 H, d, J= 2.0 Hz, Hf), 7.69 (1 H, d, J =2.0 Hz, Hg), 8.20 (1 H, d, J =8.8 Hz, Hz). UV λmax nm (ε): 253 (10, 300), 285 sh (4200), 375 (1900).

S-(5-Chloro-2-nitrophenyl)cysteine (3): 2,4-DCNB (1, 1.91 g in 35 ml of acetone), L-cysteine (1.21 g in 50 ml of MeOH) and NaOH (2 g in 15 ml of H2O) were mixed and stirred at 40°C for 2.5 hr. Then the reaction mixture was neutralized (pH 7.0), concentrated to ca. 50 ml and washed with CHCl3 (×3). The water layer was kept in a refrigerator overnight to give precipitates, which were recrystallized from 50% EtOH in pale yellow needles, mp 160–161°C. [α]D +34° (c=1, 1 N HCl). FD-MS m/z (%): 279 (M++3, 4l), 278 (M++2, 32), 277 (M++1, 100), 276 (M+, 47), 203 (34), 189 (51), 74 (33). IR νmax cm−1: 3095, 3000, 2905, 1815, 1590, 1560, 1520, 1450, 1425, 1395, 1330, 1290, 1265, 1195, 1150, 1120, 860, 840, 780, 750, 670, 550. 1H-NMR δDCl-D2O (100 MHz) ppm: 3.62 (1 H, dd, J = 7.2 and 14.9 Hz, Hz), 3.83 (1 H, dd, J = 4.7 and 14.9 Hz, Hz), 4.42 (1 H, dd, J = 4.7 and 7.2 Hz, Hz), 7.50 (1 H, dd, J =2.1 and 8.8 Hz, Hz), 7.71 (1 H, d, J =8.8 Hz, Hz). UV λmax nm (ε): 252 (12, 000), 287 sh (4300), 377 (2800).

5-Chloro-2-nitrobenzenethiol (4): The method of Battistoni et al. was applied to prepare 4. To 2,4-DCNB (1, 2 g) in 30 ml of dimethyl
sulfoxide was added 2.4 g of Na₂S·8H₂O and the mixture was stirred at room temperature for 1 hr. The resulting deep-red solution was poured into 30 ml of H₂O and extracted with 30 ml of toluene (×3). The aqueous layer was then acidified to pH 3.0 and extracted with 30 ml of ether (×3). The combined ether extracts were concentrated to dryness and the residue was crystallized from benzene-petroleum ether in yellow needles (841 mg). Mp 96-97°C. EI-MS m/z (%): 191 (M++2, 1), 189 (M++, 3), 174 (5), 172 (34), 127 (17), 125 (100), 108 (18), 69 (16), 63 (25). IR vKBrmax cm⁻¹: 3085, 2545, 1590, 1555, 1495, 1450, 1130, 1055, 990, 860, 820, 790, 750, 670, 550. 1H-NMR δHMS013 (100 MHz) ppm: 4.07 (1 H, s, SH), 7.24 (1 H, dd, J=2.2 and 8.8 Hz, Hb), 7.44 (1 H, d, J=2.2 Hz, Ha), 8.21 (1 H, d, J=8.8 Hz, Hg). A minor product, bis(5-chloro-2-nitrophenyl) disulfide was obtained from the former toluene extract.

N-Acetyl-S-(5-chloro-2-nitrophenyl)cysteine (7): According to the method of Zbarsky and Young, a mixture of 3 with acetic anhydride and NaOH. Pale yellow needles from aq. EtOH, mp 177-178°C, [α]D+56° (c=0.5, EtOH), lit., [α]D+56° (c=0.5, EtOH). FD-MS m/z (%): 321 (M++3, 46), 320 (M++2, 55), 319 (M+, 100), 172 (24), 85 (55). IR vKBrmax cm⁻¹: 3380, 3090, 2920, 1725, 1615, 1585, 1500, 1420, 1325, 1290, 1215, 1120, 860, 815, 745, 665, 550. 1H-NMR δHMS013 (100 MHz) ppm: 1.93 (3 H, s, Ha), 3.47 (1 H, dd, J=7.1 and 13.8 Hz, H2), 3.78 (1 H, dd, J=5.1 and 13.8 Hz, H3), 4.85 (1 H, dd, J=5.1 and 7.1 Hz, H4), 7.44 (1 H, dd, J=1.9 and 8.8 Hz, H1), 7.81 (1 H, d, J=1.9 Hz, H2), 8.21 (1 H, d, J=8.8 Hz, H3). UV λmax nm (e): 253 (12,700), 285 sh (5200), 375 (3200), lit.7) UV λmax nm (e): 253 (12,700), 285 sh, 375 (3200).

3. Fungus and Its Cultivation

Mucor javanicus AHU 6010 was used in the present study. The fungus was cultured in a liquid medium consisting of 5% glucose, 1% peptone and 0.1% yeast extract (basal medium) at 25°C with shaking (90 rpm, 100 ml medium/500-ml flask) for a set time.

4. Metabolic Experiments Using the Growing Cells or the Resting Cells

Metabolism in the growing medium: Metabolic experiments using the growing cultures were carried out according to the procedure described previously7) with some modifications mentioned in the text. Quantitative and qualitative analyses of volatile metabolites were conducted as shown in our previous papers.1,2) Metabolism by the resting cells: The fungus grown for 4 days in the basal medium was harvested by filtration and sucked dry on a Buchner funnel (75-76% moisture). The mycelia were suspended in a reaction medium consisting of 67 mM phosphate buffer (pH 7.0) and a substrate. The suspension was shaked for a set time.

5. Experiments Using the Cell-free System

A part of mycelia (96 g) in the metabolic experiment using the resting cells was wetted with a phosphate buffer solution (67 mM, pH 6.6) and ground with silicaceous sand. The
resulting slurry diluted with the buffer solution (50 ml) was centrifuged at 4000 rpm for 5 min (−5°C) to give a supernatant. The precipitate was resuspended in the buffer solution (70 ml) and centrifuged similarly. The combined supernatants were further centrifuged at 17,000 rpm for 20 min to give a cell-free extract, which was diluted to 200 ml with the buffer solution. The amount of protein (28 mg/ml) was determined by the microbiuret method.9 Glutathione S-transferase activity was determined by the method of Habig et al.10 2,4-Dinitrochlorobenzene was used as a standard substrate to determine the specific activity of the crude enzyme preparation.

RESULTS AND DISCUSSION

1. Formation of a 2,4-DCNB Glutathione Conjugate (2) in the Cell-free System of Mucor javanicus

Glutathione S-transferase (GST, EC 2.5.1.18) with wide substrate-specificity which has been implicated in detoxification of xenobiotics by mammals, plants and insects11,12 has been recently found in microorganisms as well, such as Escherichia coli,13 Cunninghamamella elegans14 and Fusarium oxysporum.15

The results of our previous studies, in which some of DCNB were found to be converted into methylthio-containing metabolites by Mucor javanicus and other fungi,1,2 suggested that GST might play an initial role to introduce a sulfur atom into the chlorinated nitrobenzene substrate. Although the crude extract of M. javanicus showed GST activity [Specific activity of the crude protein precipitated in 80% saturation of (NH4)2SO4 was calculated to be 229 nmol/min/mg protein by the assay described by Habig et al.10 using 2,4-dinitro-1-chlorobenzene as a substrate.], its catalytic activity to conjugate 2,4-DCNB (1) with glutathione (GSH) was negligibly small. To confirm the occurrence of the expected reaction, a preliminary experiment was carried out using 14C-2,4-DCNB.16

As shown in Table 1, radioactivity of the substrate was partly but significantly transferred into its water-soluble fraction. A reaction product in the water phase was isolated from the large-scale experiment (×40) using cold 2,4-DCNB (1) as the substrate and confirmed to be a corresponding glutathione conjugate (2) in the following manner. A reaction mixture (200 ml) obtained similarly as described in Table 1 was washed with ether (200 ml × 3) and concentrated. The concentrate was subjected to column chromatography over Amberlite XAD-4 (3.5 cm i.d. × 20 cm). The charged column was washed with 800 ml of H2O and the adsorbed constituents were eluted with MeOH. The MeOH eluate (1600 ml), except for the initial 200 ml, was concentrated to give a spot comparable to that of authentic 2 on TLC plates at Rf 0.42 and 0.32 respectively developed in Solvent A and Solvent B. The spot on F254 TLC plates developed in Solvent A was eluted with Solvent C and the concentrated eluate was diluted with 20 ml of H2O prior to the treatment with active charcoal (100 mg). The collected charcoal was washed with 200 ml of H2O, followed by elution with 200 ml of

| Table 1 Distribution of radioactivity after enzymatic conjugation reaction. |
|-----------------------------|-----------------|-----------------|
| Cell-free extract           | Ether extract   | Water layer     |
| Intact                      | 86.3            | 13.7            |
| Boiled                      | 97.8            | 2.2             |

A reaction mixture consisting of the cell-free extract of M. javanicus (0.5 ml, protein 14 mg), GSH (1.5 mg in 0.5 ml of H2O), 14C-2,4-DCNB (0.5 mg in 0.5 ml EtOH; SA 80 mCi/ mol) and a phosphate buffer solution (3.5 ml, 67 mM, pH 7.0) was incubated at 25°C for 5 hr with gentle shaking. A complete assay mixture containing the boiled cell-free extract was used as a control. After reaction, the mixture was acidified to pH 1.0 and extracted with ether (5 ml × 3). Radioactivity of the combined ether extracts and the water layer was determined by a liquid scintillation spectrometer using Scintisol 500.
Solvent C. The eluate was concentrated to dryness and the residue (2.76 mg) was crystallized from 50% EtOH in pale yellow needles, mp 205–206°C. FD-MS m/z (%): 465 (M+3+, 30), 464 (M+2+, 35), 463 (M+1+, 100) and UV λ_{max} (nm): 253, 285 sh. Thus the isolate and authentic 2 were identical in physicochemical properties.

The formation of 2 from 2,4-DCNB (1) and GSH in the cell-free extract of M. javanicus was thus confirmed. Together with the result shown in Table 1, this fact fully supports the view that 1 was enzymatically (by GST) converted into a glutathione conjugate. It is, therefore, quite likely that an enzyme functions in the living cells of the fungus to yield glutathione conjugates of xenobiotics such as 1.

2. Formation of Methylthio-containing Metabolites from a 2,4-DCNB Glutathione Conjugate (2) and Its Related Compounds

Synthetic substrates, glutathione and cysteine conjugates of 2,4-DCNB (2 and 3) and 5-chloro-2-nitrobenzenethiol (4), which could be intermediates when 2,4-DCNB metabolizes to methylthio-containing products (5 and 6), were fed to the growing cultures of M. javanicus. To the fungus grown for 4 days was added each substrate, and the resulting methylthio-containing metabolites were analyzed by GLC and GC-MS after 24 hr of incubation.

As shown in Table 2, two conjugates (2, 3) and thiol (4) as well as 2,4-DCNB (1) were all converted into 4-chloro-2-methylthio-1-nitrobenzene (5) and 4-chloro-2-methylthiobenzamide (6) but in quite different quantities. Such results indicate that the metabolic pathway of 1 consists of glutathione conjugation and subsequent transformation into methylthio-containing products. However, if so, 3 should be a more effective substrate than 2 in producing end products (5, 6). The inconsistency in the metabolite yields may be partly explained by further metabolism of these metabolites (5, 6) and/or a side-path in the metabolism of 3, which will be discussed later.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Remaining substrate (%)</th>
<th>DCBAa) (%)</th>
<th>CMTNBb) (%)</th>
<th>CMTEAb) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-(5-Chloro-2-nitrophenyl) glutathione (2)</td>
<td>NDc)</td>
<td>-e)</td>
<td>5.7</td>
<td>0.6</td>
</tr>
<tr>
<td>S-(5-Chloro-2-nitrophenyl) cysteine (3)</td>
<td>ND</td>
<td>-</td>
<td>4.0</td>
<td>0.3</td>
</tr>
<tr>
<td>5-Chloro-2-nitrobenzenethiol (4)</td>
<td>ND</td>
<td>-</td>
<td>30.9</td>
<td>2.2</td>
</tr>
<tr>
<td>2,4-DCNB (1)</td>
<td>19.7</td>
<td>19.9</td>
<td>7.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Each substrate (16 μmol/100 ml medium) was added to cultures grown for 3 days and the cultivation continued for another 24 hr. The products were extracted and determined as described in the previous papers.1)2) Yields (%) in duplicates are averaged.
a) 2,4-Dichlorobenzammine (12).
b) 4-Chloro-2-methylthio-1-nitrobenzene (5).
c) 4-Chloro-2-methylthiobenzamide (6).
d) No determined.
e) No peak corresponding to the metabolite on the gas chromatograms.
and shaken twice with 300 ml of ether and once with EtOAc. The combined organic layers were successively washed with 5% NaHCO₃ solution and brine, dried over anhydrous Na₂SO₄ and concentrated to dryness. Four metabolites were found in the residue by TLC. These constituents were separated from each other on silica gel PTLC plates developed in Solvent D. Each band on the plate at RF 0.07, 0.27, 0.38 or 0.61 was scraped off and eluted from the silica gel with benzene.

A metabolite at RF 0.61: A benzene eluate containing a metabolite at RF 0.61 was analyzed by GLC and GC-MS. The GLC tR 4.63 min (SE-30 column, 170°C), and GC-MS m/z 235 (M⁺, base peak) and other fragments of the metabolite were identical to those of authentic 4-chloro-2-methylsulfonyl-1-nitrobenzene (9). The first metabolite was thus confirmed to be 9.

A metabolite at RF 0.38: The second metabolite at RF 0.38 was isolated from a benzene eluate in colorless plates 1.2 mg in a 4.2% yield, mp 130-131°C and MS m/z 219 (M⁺, base peak). The metabolite was identified to be 4-chloro-2-methylsulfinyl-1-nitrobenzene (8) by direct comparison with authentic 8 in RF, tR (4.16 min on a SE-30 column at 170°C) and MS.

A metabolite at RF 0.27: A benzene eluate containing a metabolite at RF 0.27 was concentrated to dryness and the residue was crystallized from hexane in pale yellow needles 1.18 mg in a 4.4% yield, mp 115.5-116°C. The metabolite was confirmed to be 4-chloro-2-methylsulfinylbenzenamine (11) by direct comparison with authentic 11 in GLC (tR 4.33 min on a SE-30 column at 170°C), TLC and MS (m/z 205; M⁻, base peak).

A metabolite at RF 0.07: A metabolite at RF 0.07 was similarly obtained in pale brown plates (4.8 mg in 20% yield), mp 61-62°C, MS m/z (%): 189 (M⁺, 32), 176 (31), 174 (100). RF and tR (3.35 min on a SE-30 column at 170°C) and MS of the isolate were in agreement with those of authentic 4-chloro-2-methylsulfinylbenzenamine (10). The final metabolite was thus confirmed to be 10.

The above results indicate that the fungus can transform 2,4-DCNB (1) into methylthio-containing metabolites via a glutathione conjugate (2) and further oxidize them into sulfoxides (8, 10) and sulfones (9, 11) with increased polarity. Thus a sulfur atom in the methylthio-containing metabolites of 1 is suggested to originate from GSH. Okazaki⁷ reports that pentachloromethylthiobenzene (PCMTB) is oxidized to the corresponding sulfinyl and sulfonyl derivatives by F. oxysporum f. lycopersici. Those oxidized derivatives were also found in soil and plants treated with PCNB or PCMTB.¹⁸,¹⁹

4. Further Metabolism of the Glutathione Conjugate (2) by the Resting Cells of M. javanicus: A Survey of Water-soluble Metabolites

Next, water-soluble metabolic intermediates were examined using the resting cells of M. javanicus and the 2,4-DCNB glutathione conjugate (2) as a substrate. The reaction conditions and the procedure for metabolite isolation are shown in Fig. 1. Each metabolite band on silica gel PTLC plates was scraped off and eluted from the gel with Solvent C.

A metabolite at RF 0.73: An eluate containing a metabolite at RF 0.73 (Fig. 1) was concentrated to dryness, and the residue was crystallized from 50% EtOH in pale yellow needles (2.01 mg), mp 160-161°C. The isolate gave a spot at RF 0.54 on TLC plates developed in Solvent B and reacted with ninhydrin to yield a blue color.

In FD-MS prominent peaks were at m/z 277 (base peak) and 276 (22%). Since M⁺+1 ion is detected more often than M⁺ in the FD-MS, the molecular weight of the isolate was estimated to be 276 corresponding to that of 2,4-DCNB cysteine conjugate (3). The direct comparison of the isolated metabolite with 3 indicated that their chromatographic (TLC) and spectroscopic (MS and IR) properties were reasonably identical. The first metabolite was thus confirmed to have the same structure as 3, a hydrolyzate of glutathione conjugate (2).

A metabolite at RF 0.86: The second metabolite at RF 0.86 (Fig. 1) was separated in the same manner as the first one and crystallized from 30% EtOH in yellow needles (2.34 mg), mp 177-178°C. Its FD-MS base peak at m/z 319 [277 (M⁺+1 of 3)+42 mass units] fitted that of the acetate of 3 in molecular weight. The direct comparison of the isolated metabolite with authentic N-acetyl-S-(5-chloro-2-
Fig. 1 An isolation procedure for the water-soluble metabolites produced from 2,4-DCNB glutathione conjugate (2) by the resting cells of Mucor javanicus.

*A reaction mixture consisting of 67 mM phosphate buffer solution (pH 7.0, 18 ml), 10 mg of 2,4-DCNB glutathione conjugate (2) in 2 ml of 50% EtOH and wet mycelia of M. javanicus (3 g) was incubated at 25°C for 25 hr with gentle shaking.

Fig. 2 A summarized scheme indicating relations between substrates and metabolites.

Abbreviations: GC, growing cells; RC, resting cells; CF, cell-free system. Metabolites 5, 6 and 12: previously identified. Metabolites 2, 3, 7 and 8-11: identified in the present study.

* Metabolites 8-11 were isolated from the growing cultures of M. javanicus administered the glutathione conjugate (2), but the precise routes resulting in these metabolites are not clear.
nitrophenyl)cysteine (7) in physico-chemical properties (mp, TLC, IR and MS) revealed that they were indeed identical. In contrast with the cysteine conjugate (3), 7 shows a remarkable absorption band at $\nu_{\max} = 1725 \text{ cm}^{-1}$ attributable to the acetamide carbonyl group. The second metabolite was thus confirmed to be mercapturic acid 7. Fungal conversion of the 2,4-DCNB glutathione conjugate (2) into new metabolites 3 and 7 was thus confirmed. Fungal formation of such a mercapturic acid (7) has not yet been reported.

Metabolites and metabolic pathways confirmed in the present study are summarized in Fig. 2. Time-course changes of these metabolic intermediates and details about the fungal metabolism of chlorinated nitrobenzenes will be discussed in the following paper.  

ACKNOWLEDGMENTS

We wish to thank Miss R. Kato for determining $^1$H-NMR spectra, and Miss Y. Atsuta and Mr. K. Watanabe for EI- and FD-MS analyses. The fungal strain used in the present study was kindly supplied by Professor S. Takao of our Department. Financial support (to S. T.) from the Sapporo Bioscience Foundation is also gratefully acknowledged.

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要約

2,4-ジクロロ-1-ニトロベンゼンのグルタチオン抱合体：Mucor javanicusにおける中間代謝物およびその代謝*  

 Jordansch, Zainuddin Hafsah, 水谷純也  

*Mucor javanicus は 2,4-dichloro-1-nitrobenzene(1) を 4-chloro-2-methylthio-1-nitrobenzene (5) や 4-chloro-2-methylthio-benzanemine (6) に変換する。本菌の無細胞抽出液中で、グルタチオンを 1 から抱合体 S-(5-chloro-2-nitrophenyl)glutathione (2) の生成が確認された。2 や対応するシステイン抱合体 (3), 5-chloro-2-nitrobenzenethiol (4) からメチルチオ置換代謝産物 (5, 6) の生成が明らかにされ、2 をこの菌の増殖培地に添加すると、さらに 5, 6 のスルホキシドやスルホン誘導体が検出された。また休止菌を用いて 2 を代謝させると、未変性成分として 3 および 3 の N'-アセチル化物 (7) が見いただされた。これらの事実より、1 から 5, 6 やその酸化物の生成に、1 のグルタチオン抱合体が代謝中間体として係わっていると判断した。

*塩素化ニトロベンゼン類の微生物代謝（第 3 報）