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# Microbial Transformation of Precocene II: Oxidative Reactions by *Streptomyces griseus*

F. SIMA SARIASLANI,* LAWRENCE R. McGEE, AND DERICK W. OVENALL

*Central Research and Development Department, Experimental Station (E228/338), E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898*

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**Various species of "*Streptomyces*," "*Aspergillus*," "*Rhodotorula*," "*Brevilegnia*," "*Syncephalastrum*," and "*Stysanus*" were found to transform precocene II to three major metabolites. These major biotransformation products were isolated from a preparative-scale incubation of precocene II with *Streptomyces griseus* and were conclusively identified as (−)*cis*- and (+)*trans*-precocene II-3,4-dihydrodiols and (+)-3-chromenol. $^{18}O_2$ incorporation studies indicated the involvement of a monooxygenase enzyme system in precocene II transformation by *S. griseus*. A mechanism is proposed for the formation of (+)-3-chromenol.**

Precocenes I and II are naturally occurring chromenes known for their insecticidal properties (4, 5, 16). Metabolism of precocenes in insects and rats has been studied before (6, 7, 11, 12), and parallels have been drawn between the formation of carcinogenic bay region diol epoxides from polycyclic aromatic hydrocarbons in mammals and the bioactivation of precocenes by insects and rats (10, 16). Precocene II (6,7-dimethoxy-2,2′-dimethyl-2H-benzo[b]pyran), which is the more potent analog, selectively destroys the corpora allata of insects, terminating production of the juvenile hormone (2).

Precocenes are now widely used as tools in experimental arthropod endocrinology and have been considered prototypes of "fourth-generation" pesticides (5, 7). Microbial metabolism of these natural products has not been reported before. The present communication describes the examination of selected soil fungi and streptomycetes for their ability to transform precocene II and proposes a mechanism for biotransformation of precocene II by *Streptomyces griseus* (ATCC 13273).

## MATERIALS AND METHODS

**Microorganisms, growth, and maintenance.** Microorganisms used in this study were maintained on either sporulation agar (ATCC medium 5) or Sabouraud maltose agar slants and stored in sealed screw-capped tubes at 4°C. Microorganisms were routinely grown in a medium composed of soybean flour (5 g), glycerol (20 g), yeast extract (5 g), NaCl (5 g), $K_2HPO_4$ (5 g), and distilled water (1.0 liter); pH was adjusted to 7.0 with 5 N HCl.

Microorganisms were grown by a previously described two-stage fermentation procedure (3). Precocene II (10 mg in 0.1 ml of methanol) was added to 25 ml of 24-h-old stage II cultures, and samples (3 ml) were withdrawn at various time intervals for analysis. These were extracted with ethyl acetate (1 ml), and approximately 30 μl of the extracts was examined by thin-layer chromatography (TLC). Controls consisted of fermentations without substrate and solutions of substrate in soybean flour medium without cells. These were incubated with shaking at 27°C for 72 h.

**Chromatographic procedures.** TLC was performed on 0.25-mm-thick layers of silica gel 254 (Redi-plate; Fisher Scientific Co., Pittsburgh, Pa.). The solvent systems used for TLC were as follows: solvent system A, chloroform-ethanol (65:1, vol/vol); solvent system B, chloroform-methanol (120:3); solvent system C, chloroform-methylene chloride-methanol-ethyl acetate (10:10:1:1); and solvent system D, methylene chloride-methanol (120:5). Chromatograms were visualized by viewing under short-wave (254 nm) UV light and spraying with *p*-anisaldehyde (*p*-anisaldehyde–60% $HClO_4$–acetone–$H_2O$, 0.5:5:10:40, vol/vol), followed by heating. Under these conditions precocene II and its metabolites produced light to dark blue spots. Column chromatography was performed on silica gel (60–200 mesh; J. T. Baker Chemical Co., Philipsburg, N.J.). Columns were usually slurry packed in the developing solvent, and fractions were collected in a Haakebuchler LC 100 automatic fraction collector. The solvent systems used for column chromatography were: solvent system E, ethyl acetate-hexane (1:5, vol/vol); solvent system F, methylene chloride-methanol (120:1); solvent system G, chloroform-ethanol (9:1), and solvent system H, ethyl acetate-hexane (2:5).

**Other analytical methods.** UV spectra were recorded on a Perkin Elmer lambda 5 spectrophotometer. $^1H$ nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ or $CD_3OD$ with either a Nicolet NT 360 WB or a Bruker WM 400 instrument, with tetramethylsilane as the internal standard. High-resolution mass spectra (HRMS) were obtained with a VG 7070 H mass spectrometer from the same company. Gas chromatography-high-resolution mass spectrometry (GC-HRMS) was performed on the VG Micromass spectrometer coupled to a Varian 3700 gas chromatograph equipped with a 30-m DB-1 (methylsilicone; Megabore 0.53-mm inner diameter) column programmed at a temperature gradient of 100 to 275°C (10°C/min). Infrared (IR) spectra were obtained with a Nicolet 5ZDX FT-IR spectrometer. The circular dichroism (CD) spectrum was recorded on a Jasco J-500C spectropolarimeter.

**Oxygen-18 enrichment studies.** Stage I cultures of *S. griseus* (50 ml) were harvested by centrifugation (18,000 × *g*, 20 min). The cell paste (8.7 g) and precocene II (38 mg in 0.2 ml of methanol) were suspended in 50 ml of soybean glycerol medium in a DeLong flask (250-ml capacity) fitted with a center well. The center well contained 2 ml of 1 N KOH and a piece of filter paper. The flask was purged with nitrogen and then connected to a break-seal container of $^{18}O_2$ (98

* Corresponding author.

atom% $^{18}O_2$, 500 ml) while under reduced pressure. The culture was then incubated at 27°C on a rotary shaker for 6 h, prior to extraction (three times) with ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous $Na_2SO_4$ and concentrated to dryness under reduced pressure.

The residue was chromatographed on a silica gel TLC plate, and metabolites formed were separated with solvent system C. The bands having $R_f$ of 0.31 and 0.47, corresponding to *trans*- and *cis*-precocene II-3,4-dihydrodiols, respectively, were scraped off the plate and eluted from the silica gel by methanol. The diol isomers thus obtained were further analyzed by GC-HRMS.

**Chemical synthesis of *5*.** To a solution of *4* (33 mg) in 30 ml of ethyl acetate was added 165 ml of 5 N HCl. The mixture was stirred, heated, and refluxed for 15 min under an argon atmosphere. The mixture was then extracted with methylene chloride, and the organic solution was dried over magnesium sulfate, filtered, and concentrated to give a colorless oil (25 mg).

**Chemical synthesis of *6*.** Synthesis of *6* was achieved by refluxing a mixture of *4* (20 mg in 40 ml of MeOH) and HCl (100 ml, 5 N) under a stream of nitrogen for 10 min. Methanol was then removed under reduced pressure, and the aqueous solution was extracted three times with equal volumes of methylene chloride. The organic layer was evaporated to dryness, and the residue was dissolved in methanol (40 ml), followed by the addition of a few crystals of $NaBH_4$. The mixture was left stirring for 1 h before the methanol was removed under reduced pressure. The residue was then dissolved in ethyl acetate and extracted three times with equal volumes of water. The organic layer was then dried with $Na_2SO_4$ before removal of ethyl acetate under reduced pressure. The residue obtained (15 mg) was then further analyzed by GC-HRMS and $^1H$ NMR.

**Production of metabolites from precocene II.** *S. griseus* ATCC 13273 was grown on soybean glycerol medium as mentioned above. Precocene II (5.1 g) was dissolved in 10 ml of methanol and distributed evenly among 10 liters of 24-h-old stage II cultures. Incubations were monitored by TLC to determine the rate and extent of conversions and were terminated 120 h after substrate addition. At this stage almost 70% of the substrate had been converted into metabolites (TLC estimate), and the cultures were harvested. The pooled supernatants were extracted exhaustively with ethyl acetate, and the organic phases were combined, dried over anhydrous $Na_2SO_4$, and evaporated under vacuum to yield a brownish oil (5.64 g).

The oil was adsorbed on silica gel and applied as a dry powder to a column (39 by 3 cm) containing 114 g of 60-200 mesh silica gel. The column was flushed with solvent F, and 500-ml fractions were collected. Precocene II was recovered in fraction 1 (1.34 g), which also contained some of the higher-$R_f$ metabolites. Complete separation and purification of metabolites required several passages through silica gel columns with solvent systems E, F, G, and H.

**Transformation of *5* by *S. griseus*.** Precocene II-3-ketone(*5*) (24 mg) was added to 50 ml of 24-h-old stage II cultures of *S. griseus*. The culture was extracted with ethyl acetate after 24 h of incubation, and a small sample taken for GC-HRMS analysis while the rest was applied to a 0.25-mm-thick layer of silica gel. The plate was developed with solvent system C, and the 3-chromenol(*6*) was eluted from the silica by methanol. An analytically pure sample of *6* (3.5 mg) was obtained after the TLC eluant was passed through a silica gel column eluted with solvent E.

**Chemicals.** Precocene II(*1*) ($R_f$ 0.9 on TLC in solvent system C) was purchased from Aldrich Chemical Co. Milwaukee, Wis., and had the following properties: $\lambda_{max}$ (ethanol [EtOH]), 276 ($\log_\varepsilon$ 3.4) and 321 nm ($\log_\varepsilon$ 3.7); HRMS *m/e* 220.1095 (22%, calculated 220.1099 for $C_{13}H_{16}O_3$), *m/e* 205 (100%, $C_{12}H_{13}O_3$), *m/e* 189 (1.5%, $C_{12}H_{13}O_2$), *m/e* 161 (7%, $C_{10}H_9O_2$); $^1H$ NMR in $CDCl_3$, ppm 1.39 (*s*, 6H, 2×$CH_3$), 3.81 (*s*, 6H, 2×OMe), 5.45 (*d*, $J$ = 9.7, 1H, aromatic), 6.22 (*d*, $J$ = 9.7, 1H, aromatic), 6.51 (*s*, 1H, H-5), 6.40 (*s*, 1H, H-8). Soybean flour routinely used in this study was obtained from Natural Sales Co., Pittsburgh, Pa.

## RESULTS

A total of 52 microorganisms known to transform a wide range of structurally diverse xenobiotics were screened for their ability to transform precocene II. Included in these tests were members of the genera "*Streptomyces*" (17 species), "*Aspergillus*" (6 species), "*Rhodotorula*," "*Stysanus*," "*Syncephalastrum*," "*Helicostylum*," and "*Brevilegnia*." The following microorganisms were capable of metabolizing precocene II: *Aspergillus nidulans* ATCC 24528, "*Aspergillus alliaceus*," UI 315, *Aspergillus flavus* ATCC 24741, "*Brevilegnia*" sp. strain UI MR BREV, "*Rhodotorula rubra*" UI 36994, *S. griseus* ATCC 10137, 13273, and 13968 and NRRL B8090, "*S. griseus*" UI 1158 and UI L103, *Streptomyces canus* ATCC 12646, *Streptomyces ostreogriseus* NRRL 2558, *Streptomyces flocculus* ATCC 25453, *Streptomyces atroolivaceus* ATCC 27627, "*Streptomyces*" sp. strains CA10, WH110, ND95, and ND2, "*Streptomyces rimosus*" UI 2234, *Streptomyces achromogenes* ATCC 12767, "*Stysanus stemonites*" UI 2831, and "*Syncephalastrum*" sp. strain UI MR 2600. (Abbreviations: UI, University of Iowa culture collection; WH, Woods Hole Oceanographic Institute soil isolate; CA, California soil isolate; ND, North Dakota soil isolate.) Metabolites *3*, *4*, and *6* were produced by all the organisms that were capable of transforming precocene II. Streptomycetes in general were highly active in transforming precocene II, but *S. griseus* ATCC 13273 consistently produced the highest yields of metabolites and was therefore selected for further studies.

**Biotransformation of precocene II.** TLC of *S. griseus* cultures oxidizing precocene II revealed three major metabolites, *3*, *4*, and *6*. These were isolated as described above.

**Properties of metabolite *3*.** An analytical sample of *3* ($R_f$ 0.4 on TLC in solvent system C) was isolated as a solid residue (148 mg) and had the following properties: $\lambda_{max}$ (EtOH), 213 ($\log_\varepsilon$ 3.4) and 286 nm ($\log_\varepsilon$ 3.2); optical rotation $[\alpha]^D_{25}$ (−)2.2 ± 0.8 (0.99 g/100 ml of EtOH); HRMS *m/e* 254.1173 (17.8%, calculated 254.1154 for $C_{13}H_{18}O_5$), *m/e* 236 (53%, $C_{13}H_{16}O_4$), *m/e* 182 (38% $C_9H_{10}O_4$); $^1H$ NMR in $CD_3OD$, ppm 1.24 (*s*, 3H, $CH_3$), 1.39 (*s*, 3H, $CH_3$), 3.62 (*d*, $J_{3,4}$ = 4.3 Hz, H-3), 3.76 (*s*, 6H, 2×OMe), 4.71 (*d*, $J_{3,4}$ = 4.3 Hz, H-4), 6.36 (*s*, 1H, H-8), and 6.99 (*s*, 1H, H-5); IR (neat) 3,439.6, 1,667.4, and 1,506.1 $cm^{-1}$. The $^1H$ NMR data were identical to those reported for *cis*-3,4-dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2′-dimethyl-2H-benzo[b]-pyran (*cis*-precocene-3,4-dihydrodiol) (12, 15, 20).

**Properties of metabolite *4*.** The analytical sample of *4* ($R_f$ 0.31 on TLC in solvent system C) was obtained as creamy white crystals (347 mg) which had the following physical properties: $\lambda_{max}$ (EtOH), 287 ($\log_\varepsilon$ 3.2) and 214 nm ($\log_\varepsilon$ 3.4); HRMS *m/e* 254.1213 (55%, calculated 254.1154 for $C_{13}H_{18}O_5$), *m/e* 236 (45%, $C_{13}H_{16}O_4$), *m/e* 182 (100%, $C_9H_{10}O_4$); optical rotation $[\alpha]^D_{25}$ (+)11.0 ± 0.8 (1.06 g/100 ml of EtOH); $^1H$ NMR in $CDCl_3$, ppm 1.44 (*s*, 3H, $CH_3$), 1.19 (*s*,

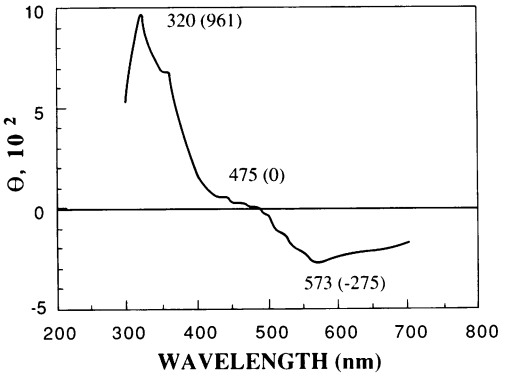


FIG. 1. CD spectrum (Jasco J-500C spectropolarimeter) of 1.598 mg of (+)*4* in 0.385 ml of ethanol and 0.63 ml of 0.01 M Cupra A (10).

3H, $CH_3$), 3.56 (*d*, $J_{3,4}$ = 8.3 Hz, H-3), 3.81 (*s*, 6H, 2×OMe), 4.48 (*d*, $J_{4,3}$ = 8.3 Hz, H-4), 6.33 (*s*, 1H, H-8), and 6.88 (*s*, 1H, H-5); CD spectrum (Fig. 1); IR (neat) 2,361.5, 2,338.6, 1,667.4, and 1,507.5 $cm^{-1}$. The physiochemical data obtained for metabolite *4* were identical to those reported for *trans*-3,4-dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2′-dimethyl-2H-benzo[b]pyran (*trans*-precocene-3,4-dihydrodiol) (12, 15, 20).

**Properties of compound *5*.** The analytical sample of the ketone (compound *5*) ($R_f$ 0.9 on TLC in solvent system C) had the following properties: $\lambda_{max}$ (EtOH), 216 ($\log_\varepsilon$ 3.8) and 290 nm ($\log_\varepsilon$ 3.6); HRMS *m/e* 236.1047 (90%, calculated 236.1049 for $C_{13}H_{16}O_4$), *m/e* 208 (20%, $C_{12}H_{16}O_3$), *m/e* 193 (85%, $C_{11}H_{13}O_3$), and *m/e* 167 (100%, $C_9H_{11}O_3$); $^1H$ NMR in $CDCl_3$, ppm 1.38 (*s*, 6H, 2×$CH_3$), 3.51 (*s*, 2H, H-4), 3.84 (*s*, 3H, $OCH_3$), 3.88 (*s*, 3H, $OCH_3$), and 6.57 (*s*, 2H, H-5 and H-8); IR (neat) 1,726.5, 1,515.3, and 1,229.5 $cm^{-1}$.

**Properties of 3-chromenol (compound *6*).** Compound *6* was obtained by three different methods; chemical synthesis from *5*, metabolism of precocene II, and metabolism of *5* by *S. griseus*. Except for the optical rotation, these samples had identical properties. The analytical sample of *6* from metabolism of precocene II was obtained as a solid residue (25 mg) and had the following properties: $\lambda_{max}$ (EtOH), 289 ($\log_\varepsilon$ 3.5) and 212 nm ($\log_\varepsilon$ 3.6); HRMS *m/e* 238.1196 (50%, calculated 238.1205 for $C_{13}H_{18}O_4$), *m/e* 220 (5%, $C_{13}H_{16}O_3$), and *m/e* 167 (100%, $C_9H_{11}O_3$); $^1H$ NMR in $CDCl_3$, ppm 1.29 (*s*, 3H, $CH_3$), 1.36 (*s*, 3H, $CH_3$), 1.85 (*bs*, 1H, $C_3$-OH), 2.69 (*ddd*, 1H, $J_{gem}$ = 16.6 Hz, $J_{4b,3}$ = 4.9 Hz, $J_{4b,5}$ = 1.0 Hz, H-4b), 2.99 (*ddd*, 1H, $J_{gem}$ = 16.6 Hz, $J_{4a,3}$ 5.1 Hz, $J_{4a,5}$ = 0.7 Hz, H-4a), 3.77 (*t*, 1H, $J_{3,4a}$ = 5 Hz, $J_{3,4b}$ = 5 Hz, H-3), 3.81 (*s*, 6H, 2×OMe, H-6, H-7), 6.41 (*s*, 1H, H-8), 6.54 (*t*, 1H, $J_{5,4a}$ = 1.0 Hz, $J_{5,4b}$ = 1.0 Hz, H-5) (Fig. 2). The optical rotation of the 3-chromenol obtained from *S. griseus* cultures metabolizing precocene II was $[\alpha]^D_{25}$ (+)27.5 ± 0.8 (0.39 g/100 ml of EtOH). Similar optical rotation properties (i.e., $[\alpha]^D_{25}$ (+)21.3 ± 5.3, 1.00 g/100 ml of EtOH) were also observed for the metabolite isolated from cultures tranforming *5*, while synthetic *6* was a racemic mixture.

**MS properties of *3* and *4* formed under $^{18}O_2$-saturated atmosphere.** Trace amounts of *3* and *4* were isolated from incubations of *S. griseus* with precocene II in an $^{18}O_2$-saturated atmosphere. GC-HRMS analysis of *4* gave a molecular ion peak at *m/e* 256 (23%), *m/e* 254 (10%), *m/e* 238 (100%), and *m/e* 236 (38%). Similar results were obtained when *3* was analyzed by MS. Figure 3 illustrates the MS properties of *4* containing oxygen-18 versus the nontagged sample of this metabolite.

**GC-HRMS analysis of *S. griseus* cultures transforming precocene II.** Precocene II (10 mg) was dissolved in ethyl acetate and added to 25 ml of the 24-h-old stage II cultures. Samples (5 ml) were withdrawn from these cultures after 24 h, extracted with ethyl acetate (2 ml), and analyzed by GC-HRMS. The data obtained indicated the presence of *3*, *4*, *5*, and *6* in these incubations.

**GC-HRMS analysis of *S. griseus* cultures metabolizing *5*.** The major component formed in these cultures was *6*; however, two other compounds with *m/e* 250 for $C_{13}H_{14}O_5$ and *m/e* 252 for $C_{13}H_{16}O_5$ were also detected. Based on their elemental compositions, these compounds were tentatively assigned the structures of precocene II-3,4-diketone and 3-keto-4-hydroxy-precocene II, respectively. These latter derivatives were formed nonenzymatically by air oxidation of *5*.

## DISCUSSION

**Metabolites of precocene II.** Metabolites *3* and *4* produced by *S. griseus* exhibited properties identical to those reported previously for (−)-*cis*- and (+)-*trans*-precocene II-3,4-dihydrodiols produced by insects and rats during oxidation of precocene II (12, 15, 20). Although the absolute stereochemistry of these metabolites has not been determined, Halpin et al. (10) assigned the 3S, 4R configuration for the related (+)-*trans* diol produced by rat liver microsomes during oxidation of precocene I. The structural difference between the diols described by Halpin et al. and *3* and *4* produced by *S. griseus* is the absence of one methoxyl group on C-6 in precocene I. The CD spectrum of the Cupra A complex of (+)-diol *4* (Fig. 1) is qualitatively identical to that reported by Halpin et al. for the same complex of (+)-*trans*-precocene I-3,4-dihydrodiol. These clearly possess the same absolute stereochemistry which, on the basis of that report (10) is assigned the 3S, 4R configuration.

Metabolite *6* exhibited a molecular ion peak at *m/e* 238.1196 for $C_{13}H_{18}O_4$, consistent with the addition of one oxygen and two hydrogen atoms to prococene II. As in metabolites *3* and *4*, the $^1H$ NMR spectrum of *6* was similar to that of precocene II except for the signals for positions 2, 3, and 4 (Fig. 2). The signals for the methyl protons at position 2 appeared as two distinct singlets resonating at 1.29 and 1.36 ppm, each integrating for three protons. This implicated the addition of the hydroxyl group to either position 3 or 4 of the precocene II structure. The signal for the hydroxyl group was seen as a characteristic broad singlet at 1.85 ppm. The proton for H-3 resonated as a triplet at 3.77 ppm which was coupled to H-4a ($J$ = 5 Hz) and H-4b ($J$ = 5 Hz). Further proof for the structure of *4* was obtained by Nuclear Overhauser Effect (NOE) studies. When proton 5 was irradiated, small but detectable NOEs were observed at H-4. The chemical shift values of H-4a and H-4b (2.69 and 2.99 ppm) indicated that these two protons are adjacent to an aromatic ring, providing evidence that the hydroxyl group is probably located on H-3. Conclusive evidence for the assignment of the hydroxyl group to H-3 of metabolite *6* was obtained through resolution enhancement studies. After resolution enhancement, additional splittings were observed in the signals for H-4, which result from the coupling between H-4 protons and the proton at position 5 ($J_{4a,5}$ = 0.7 Hz and $J_{ab,5}$ = 1.0 Hz). Moreover, after resolution enhancement, the aromatic proton at H-5 appeared as a short distorted triplet resonating at 6.54 ppm, while the resonance for H-8 at 6.41 ppm remained a sharp singlet (see Fig. 2 inset). These results clearly indicate that the hydroxyl group

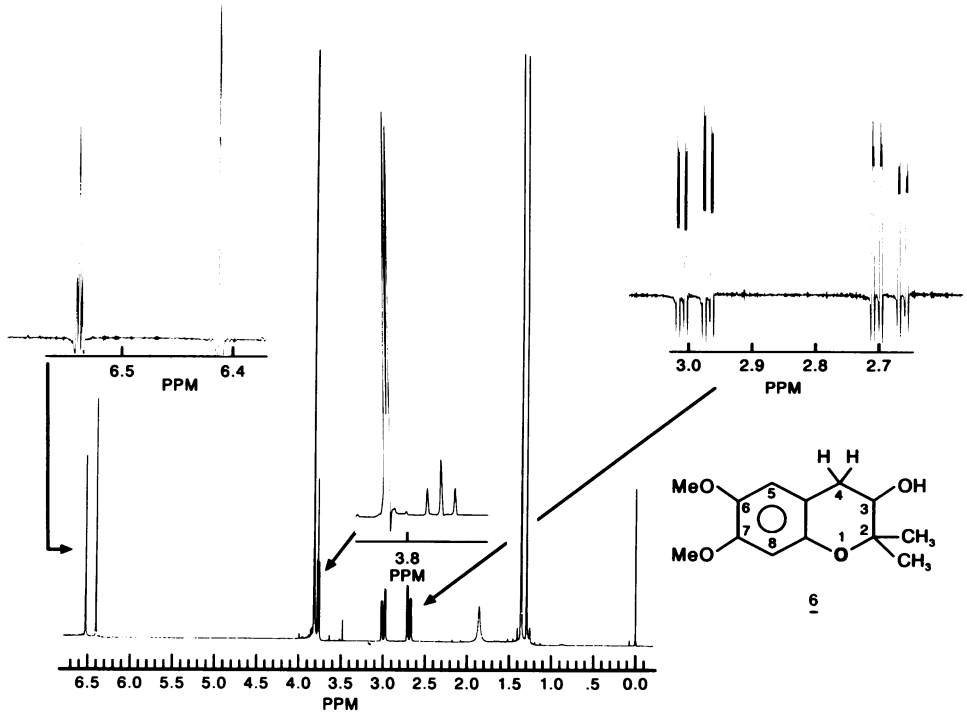


FIG. 2. $^1H$ NMR of 3-chromenol in $CDCl_3$ at 400 MHz. Insets show the resolution enhancement of respective regions.

in metabolite *6* is located on position 3 of the molecule, and *6* was therefore assigned the structure of (+)-3-chromenol. While the presence of a similar metabolite has been reported during the biotransformation of precocene II by insects (15, 20), no mechanism has been postulated for its formation.

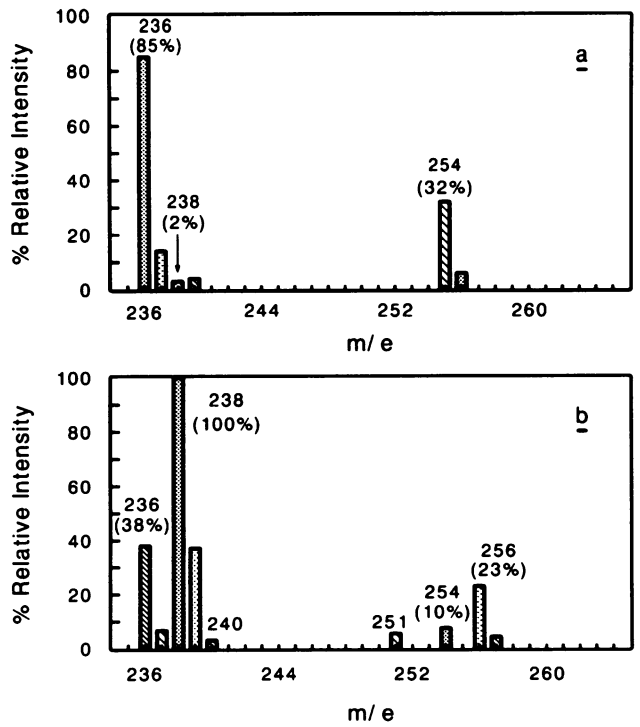


FIG. 3. MS analyses of samples of *4* containing (a) oxygen-16 and (b) oxygen-18.

It is well established that mammalian and fungal systems employ a monooxygenase pathway for activation of the benzene nucleus (13, 14), while procaryotic organisms use a dioxygenase mechanism when catalyzing the same reactions (8, 9). The nature of the initial reaction(s) employed by *S. griseus* for precocene II oxidation was established through $^{18}O_2$ studies. MS analysis of *3* and *4* containing $^{18}O_2$ gave molecular ion peaks at *m/e* 256 and base peaks at *m/e* 238 for both metabolites. These results indicated that only one of the oxygens in the structures of *3* and *4* had been derived from the atmosphere, providing conclusive evidence for the involvement of a monooxygenase enzyme system in precocene II oxidation by *S. griseus*. Monooxygenation of precocene II generates a highly reactive precocene-3,4-epoxide, and nonenzymatic hydrolysis of the epoxide can account for the observed *cis-* and *trans*-3,4-dihydrodiols (Fig. 4). The reactive precocene-3,4-epoxide has also been reported as the initial intermediate in precocene II metabolism by insects and rat liver microsomes (1, 2, 12, 20). This reactive epoxide is thought to either bind to cellular macromolecules, causing cell damage, or undergo spontaneous hydrolysis to generate *cis-* and *trans*-3,4-dihydrodiols in water (10). Such a reactive epoxide could also easily rearrange to generate the ketone (compound *5*). Evidence for the presence of *5* in *S. griseus* cultures oxidizing precocene II was obtained through GC-HRMS analysis. GC-HRMS analysis also indicated that *5* is air-oxidized to two compounds tentatively identified as precocene II-3,4-diketone and 3-keto-4-hydroxy-precocene II. Formation of the latter compound has also been reported by Aizawa et al. (1) as a degradation product of *2*; however, they have not postulated a mechanism for the formation of this product. When *5* was used as substrate for *S. griseus*, the optically active 3-chromenol (compound *6*) was obtained, while nonenzymatic reduction of *5* resulted in the formation of the racemic mixture of *6*.

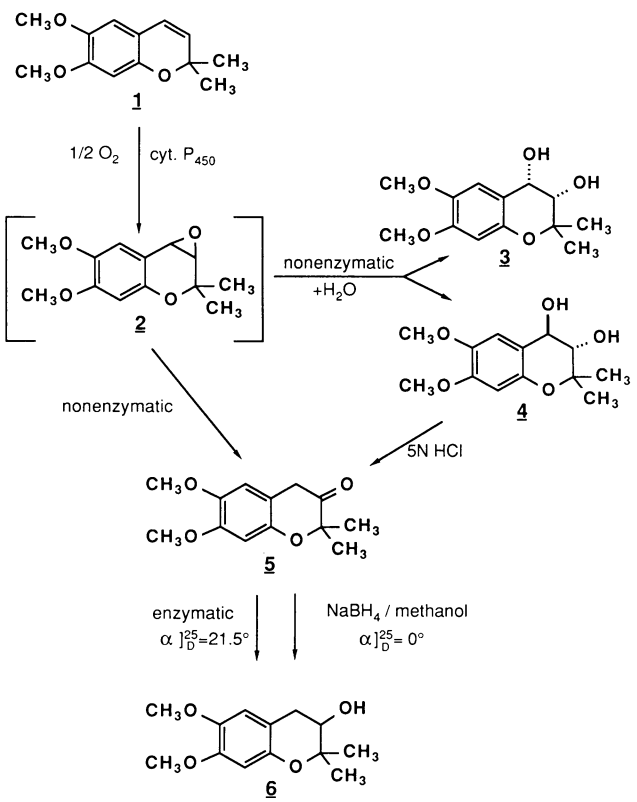


FIG. 4. Proposed route for precocene II biotransformation by *S. griseus*.

The results obtained provide concrete evidence for the role of precocene II-3-ketone(*5*) as the immediate precursor of the enzymatically generated (+)*6* as illustrated in Fig. 4.

This is the first report on microbial metabolism of precocene II. The results presented in this study clearly demonstrate the ability of *S. griseus* to mimic eucaryotic systems when oxidizing precocene II and adds to the already impressive list of the reactions catalyzed by this organism (17–19). Conclusive information on the involvement of an inducible cytochrome P-450 enzyme system in precocene II biotransformation by *S. griseus* will be presented in another communication.

## ACKNOWLEDGMENTS

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