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Novel Biotransformations of 7-Ethoxycoumarin by *Streptomyces griseus*

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Biotransformation of 7-ethoxycoumarin by *Streptomyces griseus* resulted in the accumulation of two metabolites which were isolated and identified as 7-hydroxycoumarin and 7-hydroxy-6-methoxycoumarin. A novel series of biotransformation reactions is implicated in the conversion of the ethoxycoumarin substrate to these products, including *O*-deethylation, 6-hydroxylation to form a 6,7-dihydroxycoumarin catechol, and subsequent *O*-methylation. Either 7-hydroxycoumarin or 6,7-dihydroxycoumarin was biotransformed to 7-hydroxy-6-methoxycoumarin by *S. griseus*. Trace amounts of the isomeric 6-hydroxy-7-methoxycoumarin were detected when 6,7-dihydroxycoumarin was used as the substrate. Efforts to obtain a cell-free catechol-*O*-methyltransferase enzyme system from *S. griseus* were unsuccessful. However, [*methyl*-^{14}C]methionine was used with cultures of *S. griseus* to form 7-hydroxy-6-[^{14}C]methoxycoumarin.

Streptomyces griseus (UI 1158, NRRL 8090) catalyzes a wide array of oxidative biotransformation reactions with simple aromatic compounds (23), alkaloids (7, 8, 16, 17, 20, 21), and terpene substrates (5). Reactions common for *S. griseus* include *O*- and *N*-dealkylations, aromatic hydroxylation, epoxidation, and carbon-carbon bond fission. These kinds of reactions are usually catalyzed by monooxygenases or dioxygenases in other organisms. However, at present only a single literature source documents this type of cell-free enzymatic activity, and that at extremely low levels, in another streptomycete (18).

As a prelude to identifying the oxidative enzymes of *S. griseus*, 7-ethoxycoumarin was selected as a simple and sensitive analytical probe for detecting low levels of cell-free monooxygenase activity (25). The product of *O*-dealkylation, 7-hydroxycoumarin, possesses intense fluorescent properties, enabling its detection in the nanomolar range. Experiments were conducted with *S. griseus* cells to verify that the substrate would undergo *O*-dealkylation with the organism and that no side products would form to complicate the analysis of reaction mixtures when 7-ethoxycoumarin was used as the substrate.

This report describes a novel and unexpected series of oxidative and conjugative metabolic transformations of 7-ethoxycoumarin carried out by *S. griseus*. Reactions which have been confirmed include *O*-deethylation of 7-ethoxycoumarin to form 7-hydroxycoumarin; hydroxylation of 7-hydroxycoumarin to form 6,7-dihydroxycoumarin; and methylation of 6,7-dihydroxycoumarin to form the monomethoxy derivative, 7-hydroxy-6-methoxycoumarin. Our findings demonstrate for the first time that procaryotic organisms such as *S. griseus* possess the biosynthetic machinery necessary to assemble coumarin derivatives heretofore found almost exclusively in eucaryotic systems.

MATERIALS AND METHODS

Microorganisms, growth and maintenance. *S. griseus* (UI 1158, NRRL 8090) used in this study is maintained in the University of Iowa, College of Pharmacy (Iowa City, Iowa) culture collection and is stored in sealed screw-capped tubes at 4°C. Organisms were grown in a medium composed of soybean meal (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. Media were sterilized in an autoclave at 121°C for 15 min.

Microorganisms were grown according to a previously described two-stage fermentation procedure (3). Substrates (10 mg in 0.1 ml of dimethylformamide) were added to 24-h-old stage II cultures, and samples (4 ml) were withdrawn at various time intervals for analysis. These were extracted with 1 ml of ethyl acetate, 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 0.1 M sodium phosphate buffer (pH 7.00) or soybean meal glycerol medium. These were incubated with shaking for 72 h.

Chromatographic procedures. TLC was performed on 0.25- or 0.5-mm-thick layers of Silica Gel GF254 (E. Merck AG, Darmstadt, Federal Republic of Germany)

prepared on glass plates with a Quickfit Industries spreader (Quickfit Industries, London, United Kingdom). The solvent system used for TLC was benzene-ethanol (45:5), and chromatograms were visualized by viewing under long-wave (366 nm) UV light and spraying with Pauly reagent (0.5% sulfanilic acid in 2% HCl plus 0.5% $NaNO_2$ [1:1] followed by 5% NaOH in 50% ethanol). Under these visualizing conditions, 7-ethoxycoumarin appears light orange, 7-hydroxycoumarin (umbelliferone) appears dark orange, 6,7-dihydroxycoumarin (esculetin) appears yellowish green, and 7-hydroxy-6-methoxycoumarin (scopoletin) and 6-hydroxy-7-methoxycoumarin (isoscopoletin) appear as rust-colored spots.

Column chromatography was performed on silica gel (Baker 3404, 60 to 200 mesh, Baker Analyzed Reagents; J. T. Baker Chemical Co., Phillipsburg, N.J.). Columns were usually slurry packed in the developing solvent, and fractions were collected in a Fractomette 200 instrument. Solvent systems used for column chromatography were: solvent A, benzene-ethanol (70:1); solvent B, benzene-ethanol (40:1); solvent C, chloroform (100%); solvent D, chloroform-methanol (100:1); solvent E, benzene-ethanol-formic acid (50:1:0.1); solvent F, benzene-ethanol (9:1).

High-performance liquid chromatography (HPLC) was performed with a Waters Associates ALC/GPC 202 instrument equipped with an M6000 solvent delivery system, U6K Universal injector, and a 254-nm differential UV detector. A reversed-phase μ-Bondapak-C_{18} column (Waters, 3.9 by 30 cm) was eluted with a solvent system of methanol-water-acetic acid (25:75:0.1) at a flow rate of 2 ml min^{-1} and an operating pressure of 2,500 lb in^{-2}. Under these conditions, the retention volumes of 6,7-dihydroxycoumarin, 7-hydroxycoumarin, 7-hydroxy-6-methoxycoumarin, and 6-hydroxy-7-methoxycoumarin were 16, 31, 40.5, and 35 ml, respectively (Fig. 1).

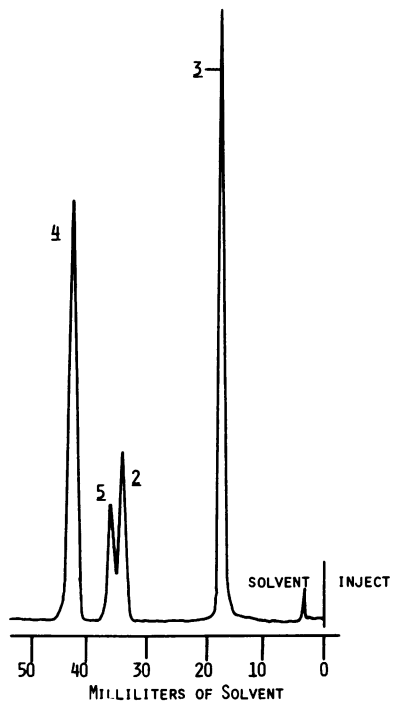

FIG. 1. HPLC elution profile of an extract of *S. griseus* containing 6,7-dihydroxycoumarin (*3*), 7-hydroxycoumarin (*2*), 7-hydroxy-6-methoxycoumarin (*4*), and 6-hydroxy-7-methoxycoumarin (*5*). Chromatographic conditions are described in the text.

Other analytical methods. Melting points were determined in open-ended capillary tubes in a Thomas-Hoover capillary melting-point apparatus and are uncorrected. UV spectra were recorded on a Beckman ratio-recording spectrophotometer; infrared spectra were obtained on a Beckman model 4240 spectrophotometer with KBr disks; ^1H nuclear magnetic resonance (NMR) spectra were obtained in chloroform-d with a Bruker 90-MHz or a Varian T-60 spectrometer with tetramethylsilane as the internal standard ^{13}C NMR spectra were obtained in dimethyl sulfoxide-(DMSO)-d_6 with a Bruker HX90E spectrometer at 22.63 Hz incorporating a time-shared internal deuterium lock, a Bruker SXP high-power radiofrequency amplifier, a Nicolet BNC-12 computer, a model 293 I/O controller for signal averaging, and Fourier transformation of the free induction decay. Low-resolution mass spectra were obtained with a Finnigan model 3200 spectrometer, and high-resolution mass spectra were obtained with Kratos-AEI MS-50 system through the mass spectral services of the Department of Chemistry, University of Nebraska, Lincoln, Nebr.

Chemicals. 7-Ethoxycoumarin was purchased from Sigma Chemical Co., St. Louis, Mo., and had the following properties: melting point, 86 to 89°C UV (ethanol; nanometers) (log ε), 321 (4.2), 250 (3.1), and 241 (3.3); low-resolution mass spectrometry *m/e* (percentage relative abundance), 190 (45), 162 (25), 134 (100), 105 (14); ^1H NMR, DMSO-d_6, parts per million (ppm), 1.32 (t, 3 H, CH_2-CH_3), 4.16 (q, 2 H, CH_2-CH_3), 6.26 (d, 1 H, J = 10 Hz, H-3), 6.86 (m, 2 H, H-6 and H-8), 7.63 (d, 1 H, J = 10 Hz, H-5), and 8.0 (d, 1 H, J = 10 Hz, H-4); infrared (KBr disk) cm^{-1}, 3,400, 2,970, 1,725, and 1,610. The R_f on TLC was 0.62 (solvent system F).

The following other compounds were obtained from commercial sources and were all fully characterized as to melting point, ^1H NMR, and mass spectrum before use: 7-hydroxy-6-methoxycoumarin and 6,7-dihydroxycoumarin from Sigma; and 7-hydroxycoumarin from Aldrich Chemical Co., Milwaukee, Wis.

Production of metabolites from 7-ethoxycoumarin. *S. griseus* was grown as described, and 1 g of 7-ethoxycoumarin dissolved in 20 ml of *N,N*-dimethylformamide was distributed evenly to 24-h-old stage II culture flasks holding 2.27 liters of culture. Incubations were monitored by TLC to determine the extent of conversion, and incubations were harvested 72 h after substrate addition. At this stage, the extent of the formation of 7-hydroxycoumarin and 7-hydroxy-6-methoxycoumarin formed in the cultures were 30 and 10% (TLC estimate), respectively. The pooled cultures were then extracted exhaustively with ethyl acetate. The organic phases were combined and dried over anhydrous sodium sulfate and evaporated to dryness to yield a brownish oil (1.6 g).

Isolation of 7-hydroxycoumarin. The brownish oil (1.6 g) was adsorbed on silica gel and was applied as a dry powder to a column (39 by 3.5 cm) containing 90 g

of silica gel. The column was washed with 110 ml of chloroform before fraction collection and then was washed with chloroform-methanol (100:1) at a flow rate of 2 ml min^{-1} while 15-ml fractions were collected. Complete purification of 7-hydroxycoumarin required several passages through silica gel columns using solvent systems A, B, C, D, and E.

Production of metabolites from 7-hydroxycoumarin. 7-Hydroxycoumarin (5 mg) was used as substrate for 25 ml of 24-h-old stage II culture of *S. griseus*. Metabolite production was monitored by TLC. 7-Hydroxy-6-methoxycoumarin was the only metabolite formed in approximately 30% yield (TLC) in 48 h. The culture was then extracted as before, and the identity of the metabolite was verified by TLC and HPLC comparison with authentic 7-hydroxy-6-methoxycoumarin.

Production of metabolites from 6,7-dihydroxycoumarin. *S. griseus* was grown as described, and 6,7-dihydroxycoumarin (100 mg) (dissolved in 1.0 ml of *N,N*-dimethylformamide) was distributed evenly among 250 ml of 24-h-old stage II culture. 7-Hydroxy-6-methoxycoumarin and 6-hydroxy-7-methoxycoumarin were formed in estimated 30 and 10% yields (TLC), respectively, 1 week after the addition of the substrate. Neither of these compounds could be detected in control incubations. Incubations were harvested, and the pooled cultures were extracted and dried as above to yield a brownish oil (277 mg).

Isolation of 6-hydroxy-7-methoxycoumarin and 7-hydroxy-6-methoxycoumarin. The brownish oil was adsorbed on silica gel and was applied as a dry powder to a column (35 by 2.5 cm) containing 30 g of silica gel. The column was eluted with solvent system A at a flow rate of 1.8 ml min^{-1} while 8-ml fractions were collected. 6-Hydroxy-7-methoxycoumarin was recovered in fractions 11 through 13, and 7-hydroxy-6-methoxycoumarin was recovered in fractions 17 through 46 of the same column.

Formation of ^{14}C-labeled 7-hydroxy-6-methoxycoumarin from 6,7-dihydroxycoumarin. A sample of [^{14}C]methionine (10 μCi, 54 μCi/μmol) and 10 mg of unlabeled methionine were divided between two 125-ml Delong flasks each containing 25 ml of 24-h-old stage II cultures. Flasks were then shaken for 5 min, after which 10 mg of 6,7-dihydroxycoumarin was added to each flask. Substrate-containing cultures were incubated on the shaker for 72 h and then were extracted as described above. The extract was evaporated under a stream of nitrogen, and the dried crude extract (81.2 mg) was dissolved in 0.5 ml of methanol. A 30-μl sample of this solution was examined by TLC, and the developed plate was scanned with a two-dimensional radiochromatogram scanner equipped with an integrator and recorder (Packard models 7230, 7240, and 7231). The concentration of 7-hydroxy-6-methoxycoumarin in this extract was determined by HPLC, and the amount of radioactivity was determined by adding 5 μl of the extract to 10 ml of scintillation fluid (Hydrofluor; National Diagnostic, Somerville, N.J.). Radioactivity was measured in a Beckman LS-100G liquid scintillation counter.

^{14}C-labeled 7-hydroxy-6-methoxycoumarin was purified from the extract by preparative TLC with solvent system F. The ^{14}C-labeled 7-hydroxy-6-methoxycoumarin obtained from the preparative TLC layer was dissolved in 1 ml of methanol. The purity of this compound was ascertained by HPLC, and its concentration was accurately determined by UV spectrometry by using the molar extinction value of 12,096 at 344 nm (1). The specific activity of chromatographically pure ^{14}C-labeled 7-hydroxy-6-methoxycoumarin was then determined as before and is noted in Table 1.

RESULTS

Metabolism of 7-ethoxycoumarin by *S. griseus*. When 7-ethoxycoumarin was used as the substrate with *S. griseus*, two biotransformation products were detected. These were isolated as described above. The analytical sample of 7-hydroxycoumarin (5 mg) obtained from the incubation (R_f, 0.4 by TLC) was recrystallized from hot water to provide the following physical properties: melting point, 227 to 230°C; UV λ maximum (nanometers) (log ε) in ethanol, 322 (4.1), 240 (3.8), and 202 (4.4); high-resolution mass spectrum, *m/e* (percentage relative abundance), 161.9499 (86, calculated 162.0421 for $C_9O_3H_6$), 134.0368 (100, calculated for $C_8H_6O_2$ 134.0368), 105 (20, C_7H_5O); ^{1}H NMR in DMSO-d_6, ppm 6.25 (d, 1 H, J = 10 Hz, H-4), 6.8 (s, 1 H, H-8), 6.86 (d, 1 H, J = 9 Hz, H-6), 7.59 (d, 1 H, J = 9 Hz, H-5), and 8.0 (d, 1 H, J = 10 Hz, H-3). All of the physical data obtained for the metabolite were identical to those obtained with an authentic sample of 7-hydroxycoumarin and are consistent with values reported in the literature (22). The second biotransformation product was obtained in small amounts, but preliminary chromatographic and spectral data suggested a metabolite structure like 7-hydroxy-6-methoxycoumarin. This compound was completely identified as a metabolite formed when 6,7-dihydroxycoumarin was biotransformed by *S. griseus* cells.

TABLE 1. Distribution of radioactivity in *S. griseus* cultures containing [^{14}C]methionine and 6,7-dihydroxycoumarin as substrates

Sample	Total dpm (duplicates)	7-Hydroxy-6-methoxycoumarin recovered (mg)	Sp act (μCi/μmol)[a]
Aqueous culture after extraction	26,946,080		
Crude ethyl acetate extract	1,687,100	0.94	
Purified (preparative TLC) 7-hydroxy-6-methoxycoumarin	497,912	0.327	0.133

[a] The total amount of methionine added was 10.028 mg with a specific activity of 0.148 μCi/μmol.

Biotransformation of 6,7-dihydroxycoumarin by *S. griseus*. Two metabolites were obtained when 6,7-dihydroxycoumarin was used as the substrate with *S. griseus*. These metabolites were isolated as described above and were identified as follows.

The analytical sample (5 mg) of 6-hydroxy-7-methoxycoumarin (R_f, 0.33 by TLC) was obtained as a noncrystalline solid to give UV (ethanol) λ maximum (nanometers) (log ε) 344 (3.6), 296 (3.5), 260 (3.5), and 228 (3.8); in 0.1 N ethanolic NaOH, 400 (3.5), 308 (3.5), 277 (3.6), and 250 (3.9); high-resolution mass spectrometry, *m/e* (percentage relative abundance), 192.0411 (100, calculated at 192.0561 for $C_{10}H_8O_4$), 177 (12.52, $C_9H_5O_4$), 164 (42.72, $C_9H_8O_3$), 149 (72.91, $C_8H_5O_3$), and 121 (17.71, $C_7H_5O_4$); ^{1}H NMR, DMSO-d_6, ppm, 3.86 (s, 3 H, CH_3O), 6.25 (d, 1 H, J = 10 Hz, H-3), 7.03 (s, 2 H, H-5 and H-8), 7.9 (d, 1 H, J = 10 Hz, H-4).

The analytical sample of 7-hydroxy-6-methoxycoumarin (9.1 mg) (R_f, 0.27 by TLC) was obtained as white feathery crystals from hot ethanol; melting point, 203 to 204°C; UV, λ maximum (log ε) (nanometers) (ethanol), 344 (4.0), 296 (3.6), 260 (3.5), 252 (3.5), and 228 (4.1); in 0.1 N ethanolic NaOH, 395 (4.4), 275 (3.8), 240 (4.1); high-resolution mass spectrometry, *m/e* (percentage relative abundance), 192.0411 (100, calculated at 192.0561 for $C_{10}H_8O_4$), 177 (57, $C_9H_5O_4$), 164 (23, $C_9H_8O_3$), 149 (38, $C_8H_5O_3$), and 121 (16, $C_7H_5O_2$); ^{1}H NMR, DMSO-d_6, ppm 3.86 (s, 3 H, OCH_3), 6.25 (d, 1 H, J = 10 Hz, H-3), 6.8 (s, 1 H, H-5), 7.25 (s, 1 H, H-8), and 7.9 (d, 1 H, J = 10 Hz, H-4); ^{13}C NMR, DMSO-d_6, ppm 58 (q, CH_3O), 103.2 (d, C-8), 109.9 (d, C-5), 110.9 (s, C-10), 111.7 (d, C-3), 144.9 (d, C-4), 149.8 (s, C-6), 151.5 (s, C-9), 161.2 (s, C-7). The data were all identical to authentic 7-hydroxy-6-methoxycoumarin and were consistent with published values (1).

Biotransformation of 6,7-dihydroxycoumarin by *S. griseus* with [*methyl*-^{14}C]methionine. Extracts of cultures grown with [*methyl*-^{14}C]methionine plus 6,7-dihydroxycoumarin as the substrate revealed the presence of a spot with chromatographic mobility identical to that of 7-hydroxy-6-methoxycoumarin. Radiochromatogram scanning of the developed TLC plate indicated that 85.3% of all radioactivity in the crude extract was associated with the 7-hydroxy-6-methoxycoumarin peak. A relatively small amount of radioactivity occurred at the origin of the TLC plate (11.67%), and this was associated with unknown extractable substances.

DISCUSSION

S. griseus (UI 1158, NRRL 8090) has repeatedly demonstrated an unusually high propensity to achieve oxidative transformations of xenobiotics. Observed enzymatic transformations include *O*- and *N*-dealkylation and aromatic hydroxylations. Preliminary experiments with intact cells suggested that all of these oxidative transformation reactions are likely catalyzed by the same enzyme system of *S. griseus* (unpublished observations). We selected 7-ethoxycoumarin as a substrate to detect enzymatic *O*-dealkylating activity in cell-free extracts of the microorganism.

When 7-ethoxycoumarin was used with this culture, more than one fluorescent metabolite was obtained. The metabolites were isolated from preparative scale incubations with *S. griseus* and were identified by chemical and spectral techniques as 7-hydroxycoumarin and 7-hydroxy-6-methoxycoumarin. Pathways operating in the conversions of 7-ethoxycoumarin to 7-hydroxycoumarin and 7-hydroxy-6-methoxycoumarin by *S. griseus* were confirmed by incubating putative intermediates with the microorganism, and these are outlined in Fig. 2.

The first and major product formed from 7-ethoxycoumarin is 7-hydroxycoumarin. The formation of this phenolic coumarin derivative indicates the presence of an *O*-deethylating enzyme system in *S. griseus*. In growing cultures, 7-hydroxycoumarin was further hydroxylated to form 6,7-dihydroxycoumarin, a compound which was never actually isolated from cultures containing either 7-ethoxycoumarin or 7-hydroxycoumarin as the substrate. The catechol 6,7-dihydroxycoumarin is a logical intermediate in the biotransformation of these substrates to 7-hydroxy-6-methoxycoumarin. The intermediacy of 6,7-dihydroxycoumarin was established by incubating *S. griseus* with the catechol as substrate to accumulate 7-hydroxy-6-methoxycoumarin and an isomeric compound, 6-hydroxy-7-methoxycoumarin. The identities of the

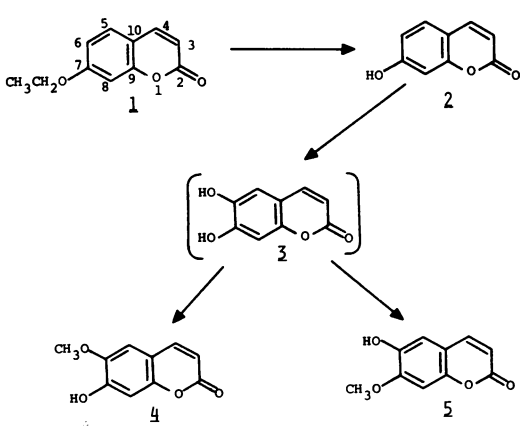

FIG. 2. Proposed pathways for the biotransformation of 7-ethoxycoumarin by *S. griseus*.

metabolites 7-hydroxy-6-methoxycoumarin and 6-hydroxy-7-methoxycoumarin were clearly established by NMR and UV spectral analyses and by comparison of these compounds with authentic 7-hydroxy-6-methoxycoumarin. The ^1H NMR spectra of these two metabolites are shown together in Fig. 3. The spectrum of 7-hydroxy-6-methoxycoumarin shows two singlets for protons at positions 5 and 8 at 6.8 and 7.25 ppm, respectively, whereas the same two protons in 6-hydroxy-7-methoxycoumarin overlap and appear as a two-proton singlet at 7.03 ppm. The UV spectra of the two isomers are also different, particularly in alkaline solvents (1, 9, 10). The isomers give identical UV spectra in neutral solvents (Fig. 4A), whereas in alkali, 6-hydroxy-7-methoxycoumarin absorbs intensely at 308 nm and 7-hydroxy-6-methoxycoumarin absorbs maximally at 240 nm (Fig. 4B). The isomeric coumarin metabolites were readily separated from one another by TLC and HPLC.

The formation of 7-hydroxy-6-methoxycoumarin and 6-hydroxy-7-methoxycoumarin from 6,7-dihydroxycoumarin suggested the presence of a catechol-*O*-methyltransferase enzyme system in *S. griseus*. Attempts were made to demonstrate catechol-*O*-methyltransferase enzyme activity directly in cell-free extracts of *S. griseus* by standard methods (4). However, these efforts were unsuccessful.

Indirect evidence of the presence of a catechol-*O*-methyltransferase system was obtained by conducting bioconversions of 6,7-dihydroxycoumarin with cultures containing [*methyl*-^{14}C]methionine. Incubations were conducted for shorter time periods than those used in previous preparative scale incubations with the catechol 6,7-dihydroxycoumarin as substrate. HPLC and TLC revealed the presence of only 7-hydroxy-6-methoxycoumarin in incubation mixtures. The isolated 7-hydroxy-6-methoxycoumarin contained nearly all of the radioactivity found in ethyl acetate extracts (Fig. 5). None of the isomeric compound 6-hydroxy-7-methoxycoumarin was observed in this incubation, and the reasons for this are unclear. However, based on earlier analytical experiments, it appears that the methylation of the catechol 6,7-dihydroxycoumarin by the enzyme system of *S. griseus* is a kinetically controlled process, and that 6-hydroxy-7-methoxycoumarin only appears upon prolonged incubation.

The presence of an *S*-adenosylmethionine transferase system in this strain of *S. griseus* could be implied by an examination of the structure of the antibiotic cycloheptamycin, which it produces (11). The antibiotic contains two arylether moieties which presumably derive from such an enzyme system. Such methylating enzymes have been demonstrated in other strepto-

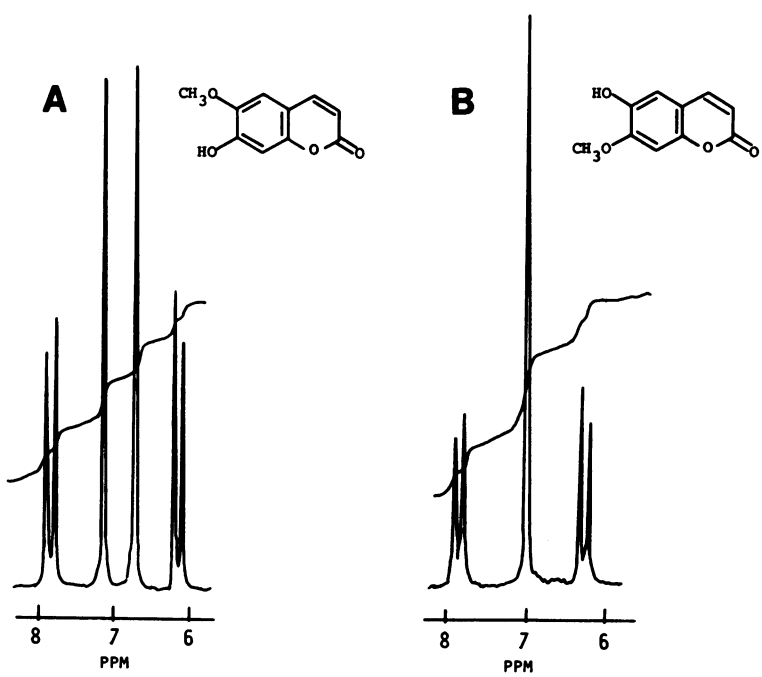

FIG. 3. ^1H NMR spectra of 7-hydroxy-6-methoxycoumarin (A) and 6-hydroxy-7-methoxycoumarin (B). Spectra were recorded in DMSO-d_6 with a Bruker 90-MHz instrument.

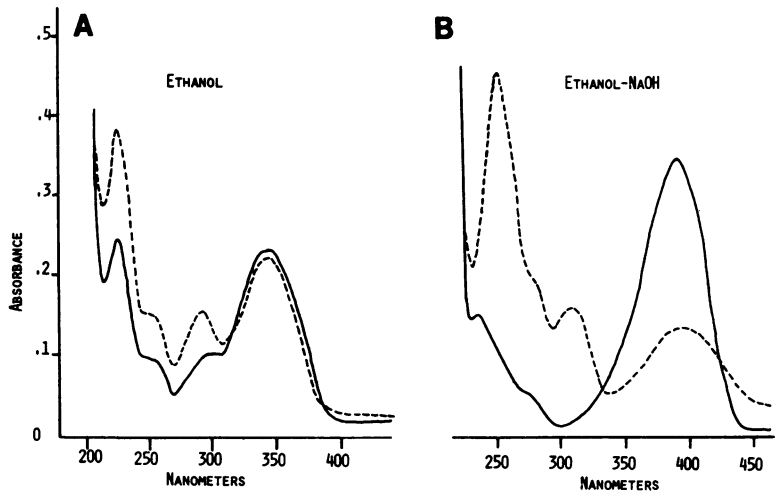

FIG. 4. Comparison of the UV spectra of 7-hydroxy-6-methoxycoumarin (solid line) and 6-hydroxy-7-methoxycoumarin (broken line) in ethanol (A) and ethanolic NaOH (B).

mycetes, but apparently not with catechols as substrates (19). For example, an enzyme system of *Streptomyces erythreus* methylates secondary alcohol functional groups in the biosynthesis of erythromycin (6). Involvement of an indolepyruvate 3-methyltransferase in the production of indolmycin (24) by *S. griseus* and the presence of a methyltransferase system in *Streptomyces fradiae* (2) have also been reported before. Catechol-*O*-methyltransferase enzyme systems have been specifically identified in the eucaryotic organism *Candida tropicalis* (26), and they have been well studied in mammals (4, 14).

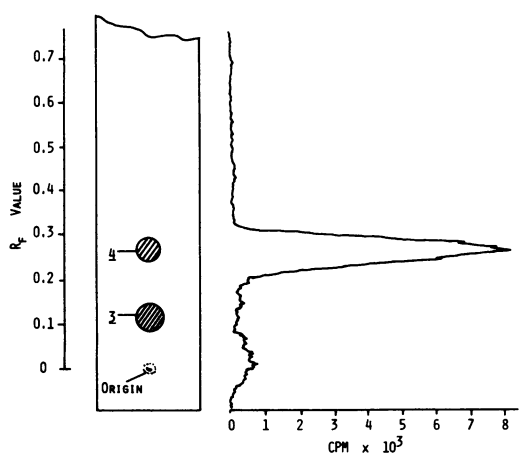

FIG. 5. Radiochromatogram of concentrated *S. griseus* extracts from incubations containing 6,7-dihydroxycoumarin (*3*) and [*methyl*-^{14}C]methionine as substrates. The inset shows the TLC chromatogram of the culture extract, and spots correspond to the substrate 6,7-dihydroxycoumarin (*3*) and the radiolabeled metabolite 7-hydroxy-6-methoxycoumarin (*4*).

The coumarins are among the largest groups of secondary natural products found frequently in higher plants and less commonly in microorganisms (15). The microbial metabolism of coumarins has been studied (12, 13, 18), mostly from a biodegradative perspective. For example, after reduction and hydrolysis, coumarin is known to yield the phenylpropanoid derivative melilotic acid (13). We previously demonstrated that *S. griseus* could form 7-hydroxycoumarin when coumarin was used as the substrate (23). Reactions observed in the present work are a novel and unexpected series of metabolic transformations catalyzed by this streptomycete. They add to the already impressive list of oxidative and conjugative transformations catalyzed by enzyme systems from this microorganism. The observed transformations demonstrate that when provided with the coumarin skeleton, a soil microorganism like *S. griseus* is capable of forming oxygenated coumarins like scopoletin and isoscopoletin. This is significant since compounds like 7-hydroxy-6-methoxycoumarin and 6-hydroxy-7-methoxycoumarin are among the most common coumarin derivatives known and they have been found almost exclusively in higher plants.

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