BIOORGANIC CHEMISTRY 11, 338–349 (1982)

Aromatic Origin of Cyclopentenoid Metabolites[1]

L. O. ZAMIR[2,*,†,‡] AND C. C. CHIN*

Department of Chemistry, Department of Biology,† and Center for Somatic-cell Genetics and Biochemistry,‡ State University of New York at Binghamton, Binghamton, New York*

Received January 18, 1982

Oxidative cleavage of aromatic compounds is often part of a degradative process and is widely observed in nature. The immediate catabolic products can sometimes cyclize or rearrange to new secondary metabolites. The enzymatic contraction of a dehydroisocoumarin to yield cyclopentenoid metabolites in *Cryptosporiopsis* sp. is reported. The label distribution of (+) cryptosporiopsin, a chlorinated cyclopentenone, was determined by analysis of the [^{13}C]nmr of [1-^{13}C] and [2-^{13}C]acetate enriched-cryptosporiopsin. The putative aromatic precursor of cyclopentenoid metabolites, 2,3-dihydro-6,8-dihydroxy-2-methylisocoumarin (**6**), was isolated from *Aspergillus terreus*. This metabolite (**6**) was prepared doubly labeled (T/^{14}C). The aromatic origin of the *Cryptosporiopsis* chlorinated cyclopentenoid metabolites was rigorously proven from feeding experiments with doubly labeled compound **6**. A related but nonchlorinated metabolite, terrein, was isolated from *A. terreus* and was also shown to be derived from [T/^{14}C]-2,3-dihydro-6,8-dihydroxy-2-methylisocoumarin.

INTRODUCTION

In 1948, Woodward postulated (*1*) that one of the biosynthetic steps leading to strychnine involved cleavage of 3,4-dihydroxyphenylalanine (DOPA). This ingenious "Woodward fission" (*2*) postulate turned out to be relevant to an important step in the biosynthesis of natural products, albeit not of strychnine (*3*). Oxidative cleavage of aromatic compounds (*4–7*) provides an efficient biosynthesis of cyclopentenoid metabolites. The isolation as well as the structural and stereochemical elucidation of cryptosporiopsin (**1**, Fig. 1) was reported in 1969, the cyclopentenoid natural product being derived from cultures of *Cryptosporiopsis* sp. (*8–10*) and from cultures of *Sporormia affinis* (*11*). A different antipode of cryptosporiopsin was isolated from cultures of *Phialophora asteris* f. sp. helianti (*12*). A reduced homolog (**2**, Fig. 1) was found in *Periconia macrospinosa* (*13*). Recently, Zamir and Chin (*14*) proved the structures and stereochemistry of two additional cyclopentenoid metabolites in *Cryptosporiopsis* sp. (**3** and **4**, Fig. 1). A nonchlorinated derivative, terrein (**5**, Fig. 1), was isolated from *Aspergillus terreus* (*15*). Enrichments with [1-^{13}C], [2-^{13}C], and [(1,2)-^{13}C]acetate revealed the labeling distribution of terrein as well as the intact acetate unit incorporated (*22*) (Fig. 2). Garson *et al.* (*23*) studied the incorporation of [2-^{13}C,

[1] This study represents part of a thesis presented by C. C. Chin to the Graduate School of the State University of New York at Binghamton in partial fulfillment of the requirements for the Ph.D. degree, 1981.

[2] Present address: McGill University, 801 Sherbrooke St. West, Montreal, P. Q., H3H 2K6, Canada.

0045-2068/82/030338-12$02.00/0

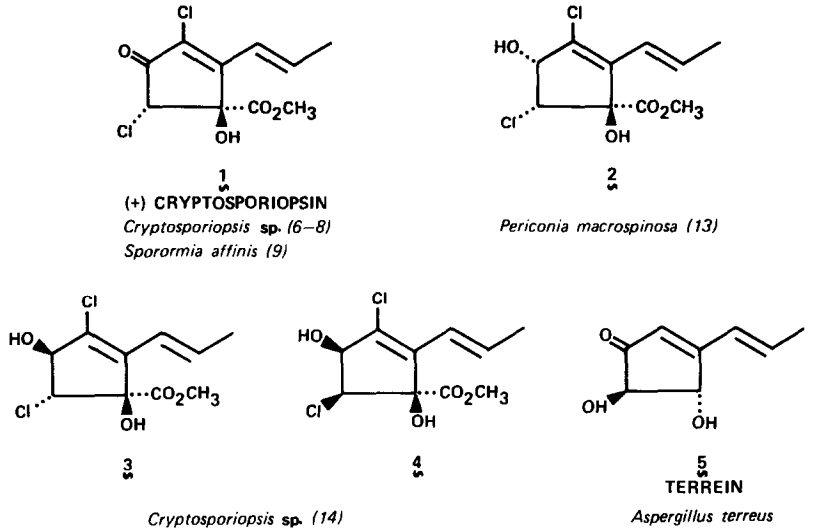


FIG. 1. Some natural cyclopentenoid metabolites.

$^2H_3$]acetate into terrein. The purpose of this study was to establish the integrity of the C—H bond throughout the discrete biochemical steps of biosynthesis. Deuterium atoms were retained in positions 1, 3, and 5 (Fig. 2). The dihydroisocoumarin (**6**) specifically labeled with ^{14}C at the C-2 position was incorporated into C-1 of terrein (Fig. 2) (*22*). A related cyclopentenoid metabolite (**2**) isolated from *Periconia macrospinosa* was found to be polyacetate derived. The labeling distributions, however, were very different in **5** and **2** (Fig. 2).

In this study, we have determined the labeling distribution of cryptosporiopsin

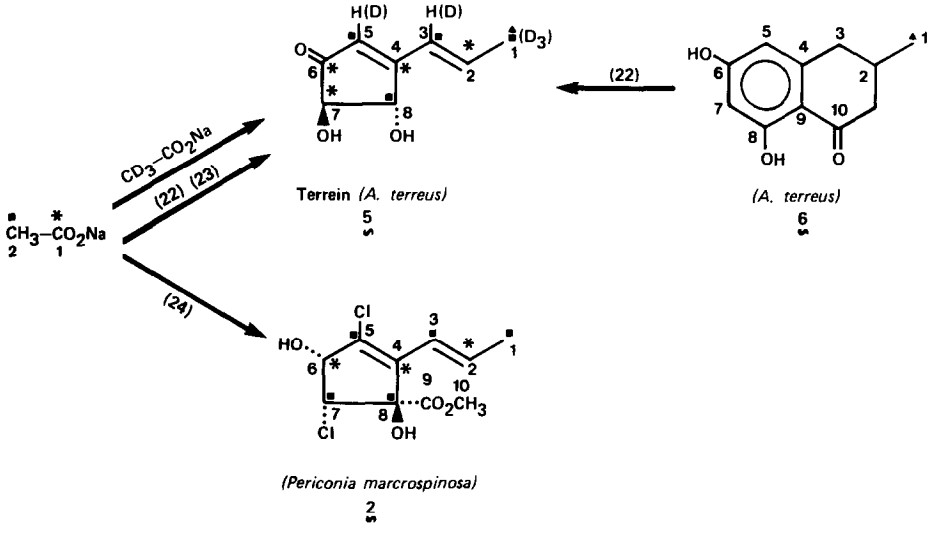


FIG. 2. Label distribution of terrein (**5**) and a chlorinated cyclopentenoid metabolite (**2**).

(1) isolated from *Cryptosporiopsis* sp. from [1-^{13}C] and [2-^{13}C]acetate incorporations. We have also demonstrated the biosynthetic aromatic origin of four cyclopentenoid natural metabolites (1, and 3–5).

EXPERIMENTAL PROCEDURES

Instrumentation

Mass spectra were obtained on a Finnegan 3300 mass spectrometer at 70 eV using a direct probe for introduction of samples (source temperature 110–130°C above ambient). High-resolution mass spectra were measured on an AEI Model MS-902 (CIS-2 source from Scientific Research Instruments Corp.) coupled to a VG Micromass 2040 data system and using perfluorokerosene as internal mass standard. Ultraviolet absorption data were recorded on a Cary 118 instrument, ir absorption spectra were recorded on a Nicolet 3600A FT-IR machine, and specific rotation values were measured on a Jasco Model ORD/UV/CD-5 instrument. [^{1}H]NMR spectra were taken on a Varian Model 360A instrument (60 MHz), a Varian FT80A instrument (80 MHz), and a Bruker WM-300 instrument (300 MHz), while [^{13}C]nmr spectra were recorded on a Varian FT80A instrument (20 MHz) and a Joel FX-90 instrument (22.5 MHz). All nmr spectra were recorded using either $CDCl_3$ or CD_3COCD_3 as solvent and tetramethylsilane as internal standard. Melting points were recorded on a Gallenkamp apparatus and are uncorrected. Radioactivity content and ratios were measured on a Tracor Analytic Delta 300 instrument and Beckman LS 7500 instrument, using Bray's solution as scintillation cocktail.

Chromatography

Two types of precoated thin-layered chromatography (TLC)[3] plates were used: Analtech Uniplate silica-gel GF (1000 μm thickness) which shall be referred to as preparative plates, and EM Reagents silica-gel 60 F-254 (250 μm thickness) which shall be referred to as analytical plates.

Strain and Cultivation

Cryptosporiopsis sp. (FSC 505) was kindly supplied by Dr. G. M. Strunz. The cultivation of this strain and isolation of its metabolites were done as previously described (*14*). Stock cultures of *A. terreus* (ATCC 12238) were maintained on 2% agar (Difco) slants of potato-dextrose media (*16*) containing 24 g of potato-dextrose broth powder (Difco) per liter. Two-liter Pivotsky still flasks containing 1 liter of potato-dextrose broth were inoculated using a spore suspension. Following 4 days of incubation at 29°C, the culture medium was gently removed by suction. Care was taken not to disturb the mycelial mat. The mycelium was refloated with 1 liter of Czapek Dox solution containing 50 g dextrose, 2 g sodium nitrate, 1 g

[3] Abbreviation used: TLC, thin-layered chromatography.

potassium phosphate monobasic, 5 g potassium chloride, 0.5 g magnesium sulfate heptahydrate, 0.01 g ferrous sulfate (Fisher Scientific Co.), and 1 g yeast extract (Difco) per liter. After 10 days incubation at 29°C the metabolites were isolated and purified as shown below.

*Isolation of 2,3-Dihydro-6,8-dihydroxy-2-methylisocoumarin (**6**) from A. Terreus*

The mycelium was discarded and the culture broth (850 ml) was concentrated under reduced pressure at 40°C to 170 ml, acidified to pH 2.5, and continuously extracted with diethyl ether for 18 hr. After evaporation of the ether, the resulting brown gum was chromatographed on a preparative TLC plate using benzene: ethyl acetate (3 : 1) (v/v) as a developer. The lightly fluorescent band (R_f 0.7) was eluted with acetone and chromatographed on an analytical TLC plate with the same developer. Elution of the fluorescent band (R_f 0.5) and evaporation of the acetone yielded a clear viscous oil which after repeated sublimations (145°C/0.03 mm) was obtained as colorless crystals (5 mg/liter fermentation) (**6**). The physical and spectroscopic properties of (**6**) match the literature values (*17, 18*) for 6-hydroxymellein obtained from the mutant strains of *A. terreus*.

*Isolation of Terrein (**5**) from A. terreus*

Unlabeled terrein was best isolated in natural media according to published procedures (*16*). Purification of terrein included chromatography on preparative TLC plates using benzene : ethyl acetate (1 : 4) as developer, and sublimation of the colorless crystal at 83°C/0.03 mm Hg. The physical and spectroscopic properties of terrein matched the published values (*19–22*).

*Isolation of Cryptosporiopsin (**1**) from Cryptosporiopsis* sp.

Cryptosporiopsin (**1**) was isolated according to published procedures (*14*). Its spectroscopic properties matched the literature (*15*). The [^{13}C]nmr spectra and the off-resonance multiplicity were the following: [^{13}C]nmr ($CDCl_3$): δ 20.29 (*q*, J = 129 Hz), 54.48 (*q*, J = 149 Hz), 65.26 (*d*, J = 156 Hz), 82.78 (s), 121.59 (*d*, J = 160 Hz), 130.33 (s), 143.74 (*d*, J = 158 Hz), 156.54 (s), 170.95 (s), 187.87 (s).

[1-^{13}C]Acetate-derived Cryptosporiopsin

Twenty-five 125-ml Erlenmeyer flasks, each containing 60 ml of 3% malt extract broth (0.075% KCl, pH 3.5), were inoculated with a spore suspension of *Cryptosporiopsis* sp. (FSC 505) and agitated on a culture shaker at 175 rpm and 22°C. At 168 hr after inoculation, 400 μl of a sodium [1-^{13}C]acetate solution (90 atom %, 1.0 g in 10 ml H_2O) (Prochem Isotopes, Summit, N.J.) was added to each culture. It was noted that the pH was raised from 3.4 to 4.4. At 335 hr, the cultures were harvested and the supernatant extracted with chloroform (3 × 250 ml), the organic extracts dried, and the solvent removed by distillation under reduced pressure. The resultant gum was then chromatographed on preparative TLC plates using chloroform : benzene : acetone (60 : 35 : 5) as developer. [1-^{13}C]Acetate-derived cryptosporiopsin was extracted and purified. The proton noise decoupled

[^{13}C]nmr of the [1-^{13}C]acetate-enriched cryptosporiopsin was then compared with that of the natural occuring analog. It was noted that the peaks at δ 143.74, 156.54, 170.95, and 187.87 ppm were enhanced.

[2-^{13}C]*Acetate-derived Cryptosporiopsin*

The above procedure was repeated using a sodium [2-^{13}C]acetate solution (90 atom %, 1 g in 10 ml H_2O) for the feedings. Comparison of the proton noise decoupled spectrum of this sample with that of the natural occurring metabolite shows enhancements for the signals at δ 20.29, 65.26, 82.78, and 121.59 ppm.

[*2,4,6,8,10-*^{14}*C*]-*2,3-Dihydro-6,8-dihydroxy-2-methyl-isocoumarin* (*6*)

Refloating conditions described above were used for the biosynthesis of labeled metabolites. At 120 hr after the refloating of the mycelium with Czapek Dox solution, 500 μl of a sodium [1-^{14}C]acetate solution (1 mCi/ml) ethanol, specific activity 54 mCi/mmol) was added to the culture medium. This was repeated at 168 hr. Harvest at 244 hr yielded ^{14}C-labeled isocoumarin (**6**) which was purified several times to constant radioactivity. The last three purifications showed (i) 74,576,669, (ii) 74,432,86, and (iii) 75,004,276 dpm/mg. Total radioactivity after the purifications was 8.44 × 10^6 dpm.

[(*1,1,1*)-*3,3-5,7-*^{3}*H*]-*2,3,-Dihydro-6,8-dihydroxy-2-methylisocoumarin* (*6*)

In an analogous experiment, the above procedure was repeated using 1.25 ml of a sodium [2-$^{3}H_3$]acetate solution (25 mCi/2.5 ml EtOH, specific activity 48 mCi/mmol) for each feeding. The product was then purified to constant radioactivity. The radioactivity of the last four purifications was (i) 68,696,146, (ii) 79,496,742, (iii) 66,313,037, and (iv) 67,389,240 dpm/mg; average 6.74 × 10^7 dpm/mg. Total radioactivity after the purifications was 9.36 × 10^7 dpm.

[*2,4,6,8,10-*^{14}*C*]-*2,3-Dihydro-8-hydroxy-2-methylisocoumarin* (*7*)

Cryptospriospsis sp. (FSC 505) was grown according to previous procedure (*14*). Five 125-ml Erlenmeyer flasks, each containing 0.075% KCl in 60 ml of 3% malt extract broth (pH 3.5), were inoculated with a spore suspension and placed on a culture shaker at 175 rpm and 22°C. At 117 hr, 200 μl of a sodium [1-^{14}C] acetate solution (1 mCi/ml EtOH, specific activity 54 mCi/mmole) was added to each culture. Harvest at 216 hr followed by extraction of the supernatant with chloroform (3 × 150 ml) yielded a gum which was chromatographed on preparative TLC plates using benzene : chloroform : acetone (60 : 35 : 5) as developer. The labeled isocoumarin (**7**) (R_f 0.85) was purified to constant radioactivity by subliming it several times at 46°C/0.3 mm Hg. The radioactivity of the last four purifications was (i) 4,157,847, (ii) 4,194,705, (iii) 4,220,496, and (iv) 4,204,832 dpm/mg; average 4.20 × 10^6 dpm/mg. Total radioactivity after the purifications was 3.05 × 10^6 dpm.

[(1,1,1)-3,3-5,7-^3H]-2,3-Dihydro-8-hydroxy-2-methylisocoumarin (7)

In a parallel experiment, ten 125-ml cultures were grown and 250 μl of a sodium [2-$^3H_3$]acetate solution (25 mCi/2.5 ml EtOH, specific activity 48 mCi/mmol) was added to each culture. Repeated purifications were carried out as for the preceding procedure. The radioactivity of the last four purifications was (i) 7,426,841, (ii) 7,298,290, (iii) 7,198,574, and (iv) 7,208,410 dpm/mg; average 7.22×10^6 dpm/mg. Total radioactivity after the purifications was 1.36×10^8 dpm.

Preparation of Doubly Labeled Isocoumarins

Doubly labeled putative precursors were prepared by combining predetermined amounts of each labeled compound and purifying them by TLC to a constant $^3H/^{14}C$ dpm ratio.

[$^3H/^{14}C$]-2,3-Dihydro-6,8-dihydroxy-2-methylisocoumarin (**6**) (Fig. 4): ^3H, 53,903,960 dpm; ^{14}C, 5,099,712 dpm; $^3H/^{14}C$, 10.57 : 1.0.

[$^3H/^{14}C$]-2,3-dihydro-8-hydroxy-2-methylisocoumarin (**7**) (Fig. 4): ^3H, 37,607,934, ^{14}C, 1,497,131; $^3H/^{14}C$, 25.12 : 1.0.

Feeding of Doubly Labeled [$^3H/^{14}C$]-2,3-Dihydro-6,8-dihydroxy-2-methylisocoumarin (**6**) *into Cryptosporiopsis* sp. *Metabolites*

Four 125-ml Erlenmeyer flasks, each containing 0.075% KCl in 60 ml of 3% malt extract broth (pH 3.5), were inoculated with a spore suspension of *Cryptosporiopsis* sp. (FSC 505) and placed on a culture shaker at 175 rpm and 22°C.

At 166 hr after inoculation, ($^3H/^{14}C$)-labeled (**6**) (^3H, 53,903,960 dpm; ^{14}C, 5,099,712 dpm; $^3H/^{14}C$, 10.57) was dissolved in ethanol (2 ml) and 500 μl of this solution was added to each culture. At 288 hr the cultures were harvested. The workup was as previously described and the crude extract chromatographed on preparative TLC plates using benzene : chloroform : acetone (60 : 35 : 5) as developer. The four major bands at R_f 0.85, 0.50, 0.40, and 0.28 corresponding to mellein (**7**), cryptosporiopsin (**1**, **3**, and **4**) were independently scraped and eluted with acetone. Mellein was further purified repeatedly by subliming at 46°C/0.3 mm Hg. Cryptosporiopsin and (**3**) were independently purified by repeated chromatography using the original developer. Compound (**4**) was chromatographed several times using hexane : ethyl acetate (1 : 1) as developer.

Mellein (**7**) was nonradioactive. Cryptosporiopsin was radioactive and was purified to constant radioactivity.

A representative purification value is ^3H, 2,957,115 dpm; ^{14}C, 384,450 dpm; $^3H/^{14}C$, 7.69. The average value obtained ($^3H/^{14}C$, 7.67) agrees well with the theoretical 7.55. The novel cyclopentenoid metabolites (**3**) and (**4**) isolated from *Cryptosporiopsis* sp. were also radioactive. A representative purification value for metabolite **3** is ^3H, 3,706,520 dpm; ^{14}C, 486,340 dpm; $^3H/^{14}C$, 7.62. The average value ($^3H/^{14}C$, 7.71) for metabolite **3** agrees well with the theoretical value of 7.55. A representative purification value for metabolite **4** is ^3H, 1,230,894 dpm; ^{14}C, 157,922 dpm; $^3H/^{14}C$, 7.79. The average value ($^3H/^{14}C$, 8.02) for metabolite **4** agrees well with the theoretical value of 7.55.

*Feeding of Doubly Labeled [$^3H/^{14}C$]-2,3-Dihydro-6,8-dihydroxy-2-methylisocoumarin (**6**) into Terrein in A. terreus*

Four 1-liter Pivotsky flasks, each containing 100 ml potato-dextrose broth were inoculated with a spore suspension of *A. terreus* (ATCC 12238). The culture medium was gently removed by suction after 3 days incubation at 29°C and replaced with 100 ml Czapek Dox solution. At 48 hr after replacement of the growth media, the ($^3H/^{14}C$) metabolite (**6**) (^3H, 3,013,517 dpm; ^{14}C, 186,135 dpm; $^3H/^{14}C$, 16.19) was dissolved in 2 ml ethanol, and 250 μl of this solution was added to each culture. This was repeated at 60 hr. Harvest at 108 hr followed by concentration of the supernatant and continuous extraction with ether for 18 hr yielded a gum which was chromatographed on preparative TLC plates using benzene : ethyl acetate (1 : 4) as developer. Elution of the band containing terrein (R_f 0.45) with acetone was followed by repeated chromatography to constant radioactivity.

A representative purification value for terrein is ^3H, 177,779 dpm; ^{14}C, 11,897 dpm; $^3H/^{14}C$, 14.94. The average value ($^3H/^{14}C$, 14.93) agrees well with the theoretical value of 14.49.

*Feeding of [$^3H/^{14}C$]-2,3-Dihydro-8-hydroxy-2-methyl-isocoumarin (**7**) to Metabolites of Cryptosporiopsis and A. terreus*

The $^3H/^{14}C$ metabolite (**7**) which was fed to *Cryptosporiopsis* sp. and *A. terreus* contained ^3H, 37,607,934 dpm; ^{14}C, 1,497,131 dpm; $^3H/^{14}C$, 25.12. The resulting metabolites in *Cryptosporiopsis* sp. (**1**, **3**, and **4**), and *A. terreus* (**5**) were found nonradioactive.

RESULTS AND DISCUSSION

*Labeling Distribution of Cryptosporiopsin (**1**)*

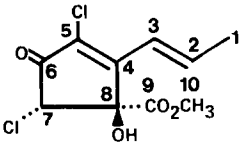
Assignments of the constituent carbons in cryptosporiopsin are shown in Table 1. These values agree with the published data on the related cyclopentenoid metabolites **2–5** (*14, 22, 24*). These chemical shifts have been confirmed by their signal multiplicities in the off-resonance proton noise decoupled [^{13}C]nmr spectrum, where the signals for C_1 and C_{10} are split into quartets, while those for C_2, C_3, and C_7 are split into doublets. [1-^{13}C]Acetate-derived cryptosporiopsin showed enhancement of the peaks at C-2, C-4, C-7, and C-9. [2-^{13}C]acetate-derived cryptosporiopsin confirmed the labeling distribution shown in Fig. 3. The pattern is therefore similar to the results of Holker and Young (*24*) for the *P. macrospinosa* chlorinated metabolite (*2*) (Fig. 2). On the other hand, terrein shows linkage of two [1-^{13}C]acetate-derived carbons at C_6 and C_7 (Fig. 2).

Aromatic Origin of Cyclopentenoid Metabolites

The labeling distributions of cryptosporiopsin (**1**) and terrein (**5**) (Figs. 2 and 3) are different. In **1**, carbons 7 and 8 both originate from the methyl group of acetate whereas in **5**, carbons 6 and 7 are derived from the carboxyl group. The structural similarities of **5** and **1** do, however, suggest a common origin. Terrein (**5**) was

TABLE 1

[^{13}C]NMR DATA FOR CRYPTOSPORIOPSIN



Carbons	Chemical shifts (ppm) (off-resonance multiplicity)
C_6	187.87 (s)
C_9	170.95 (s)
C_4	156.54 (s)
C_2	143.74 (*d*; J = 158 Hz)
C_5	130.33 (s)
C_3	121.59 (*d*; J = 160 Hz)
C_8	82.78 (s)
C_7	65.26 (*d*, J = 156 Hz)
C_{10}	54.48 (*q*, J = 149 Hz)
C_1	20.29 (*q*, J = 129 Hz)

shown by specific labeling to be derived from the dihydroisocoumarin (**6**). The dihydroisocoumarin **6** was reported to be isolated from a mutant of *A. terreus*. We have isolated metabolite **6** from wild type *A. terreus* using a different growth media. We could therefore prepare doubly labeled dihydroisocoumarin (T/^{14}C) biosynthetically by feeding alternatively [1-^{14}C] and [2-$^{3}H_3$]acetate to *A. terreus*. The resulting dihydroisocoumarin **6** had a T/^{14}C ratio of 10.57. Preliminary experiments with ^{14}C-labeled **6** showed that we could achieve a 15% incorporation into **1** upon incubation of 264 hr. Under these conditions, however, it was not possible to isolate detectable amounts of the other cyclopentenoid metabolites **3** and **4**. The optimum incubation period was found to be 288 hr. The doubly labeled compound **6**, based on the acetate feeding, must have 7 ^{3}H atoms and 5 ^{14}C atoms. In order for the dihydroisocoumarin **6** to be converted to cryptosporiopsin **1** with the observed labeled distribution (Fig. 4), the mode of aromatic ring contraction must ultimately connect C_7 and C_9 of the precursor **6**. If the chlorination occurs at the aromatic stage we would expect loss of two tritium atoms. Furthermore, genera-

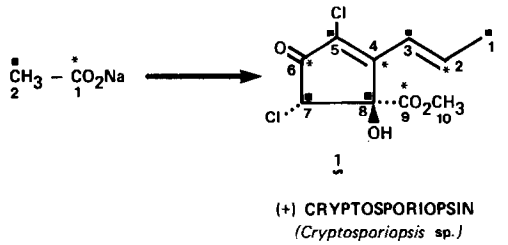


FIG. 3. Label distribution of cryptosporiopsin (**1**).

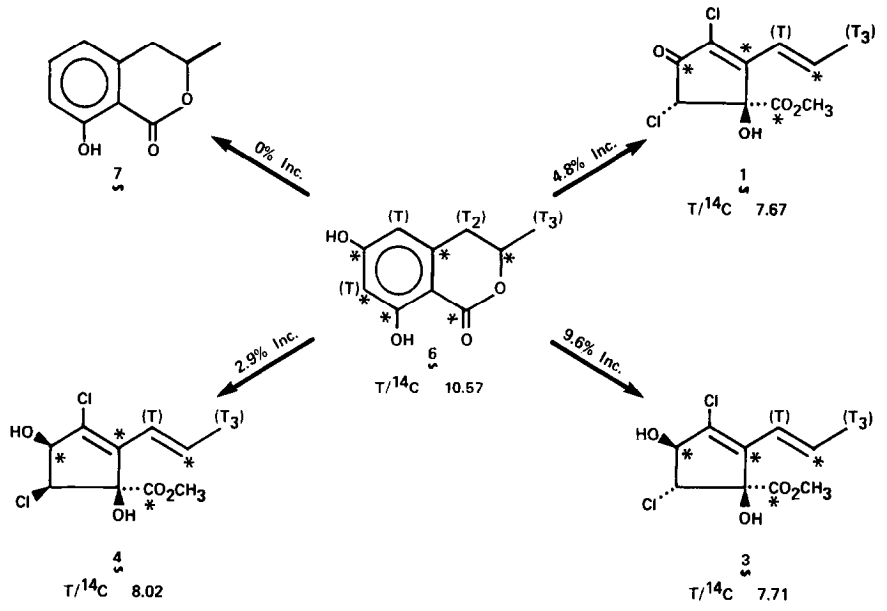


FIG. 4. Aromatic origin of *Cryptosporiopsis* cyclopentenoid metabolites.

tion of the double bond in the side chain of **1**, **3**, and **4** would require the elimination of another tritium atom. One carbon atom is lost in the conversion to the cyclopentenoid metabolites, possibly through decarboxylation of C_{10}. We therefore expect to retain 4 tritium and 4 ^{14}C atoms into **1**, **3**, and **4**, which amounts to a $^{3}H/^{14}C$ decrease of about 28%. The isolation of cyclopentenoid metabolites after extensive purifications showed the following $T/^{14}C$ ratios: cryptosporiopsin (**1**) $T/^{14}C$, 7.67; metabolite **3** $T/^{14}C$, 7.71; metabolite **4** $T/^{14}C$, 8.02. The theoretical value for 4 ^{3}H and 4 ^{14}C is 7.55. The agreement is very good; indeed, the biggest deviation which is shown in metabolite **4** $T/^{14}C$, 8.02 implies 1.06 ^{3}H per 1 ^{14}C (instead of the theoretical 1 ^{3}H per 1 ^{14}C). If the chlorination step had not occurred at the aromatic stage but at a later time the tritiums might have been retained. In that later case we would expect the cyclopentenoid metabolite to show a $T/^{14}C$ of 10.6 (7 ^{3}H atoms and 4 ^{14}C) or of $T/^{14}C$ 11.33 (6 ^{3}H atoms and 4 ^{14}C). The observed $T/^{14}C$ ratio of cryptoporiopsin (7.67), metabolite **3** (7.71), and **4** (8.02) refute that possibility. As a control, we isolated mellein (**7**) from *Cryptopsoriopsis* sp. cultures fed with the doubly labeled dihydroioscoumarin (**6**) ($T/^{14}C$, 10.57). Loss of a phenolic hydroxyl group is very unlikely, and indeed the dihydroisocoumarin **6** was not incorporated into **7**. All these results therefore establish the aromatic origin of cryptosporiopsin and the cyclopentenols **3** and **4** and prove that these compounds are derived from the dihydroisocoumarin **6**, which had been described as a precursor to terrein **5**. We confirmed that indeed terrein is derived from **6** by feeding doubly labeled ($T/^{14}C$) dihydroisocoumarin **6** ($T/^{14}C$: 16.19) to *A. terreus* cultures. The labeling distribution of terrein (Fig. 2) demands that the aromatic ring contraction connect carbons C_6 and C_8 of the dihydroisocoumarin **6**. Here again one ^{14}C atom is lost, probably C_{10} of **6** via decarboxylation. The side chain

double bond in terrein necessitates the loss of one tritium atom. Carbon 7 of **6** is probably eliminated in the conversion to terrein; therefore we can expect one more tritium atom lost. Since there is no chlorination step here, the resulting terrein should retain 5 ^{3}H atoms and 4 ^{14}C-labeled carbons. The calculated $T/^{14}C$ ratio of the resulting terrein (14.5) agrees favorably with the observed ratio of $T/^{14}C$, 14.9 (Fig. 5). A second feeding experiment with doubly labeled **6** with a different $T/^{14}C$ ratio ($T/^{14}C$, 16.19) to *Cryptopsoriopsis* sp. cultures confirmed again the precursor relationship to cryptosporiopsin (**1**) (Fig. 5). The experimental conditions this time enabled a 15% incorporation. Although mellein (**7**) has recently been found to be a metabolite of *Cryptopsoriopsis* sp. (*14*), it is not a precursor to the cyclopentenoid metabolites (Fig. 5).

Biosynthesis of Terrein and Chlorinated Cyclopentenoid Metabolites

In this study we have proven rigorously that the dihydroisocoumarin **6** is a precursor both to terrein of *A. terreus* and to cryptosporiopsin and two other cyclopentenoid structures in *Cryptosporiopsis* sp. The labeling distribution found in comparison of terrein and the *Cryptosporiopsis* sp. metabolites, however, was different. A proposed pathway to accommodate speculation about the biosynthesis of these compounds in view of these experimental results is shown in Fig. 6. The first enzyme step involves oxidative decarboxylation to form the putative metabolite **8**, which would then be the last metabolite common to *A. terreus* and *Cryptosporiopsis* sp. pathways. In *A. terreus* an oxygenase enzyme cleaves **8** (possibly at "site a") via a probable quinone intermediate. Decarboxylation and

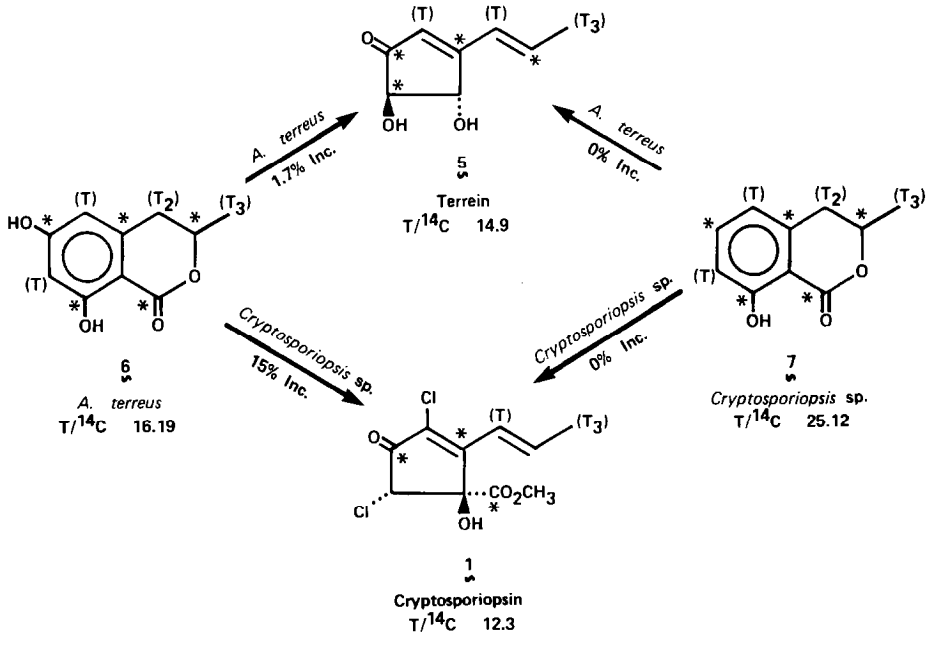


FIG. 5. Aromatic origin of terrein (**5**) and cryptosporiopsin (**1**).

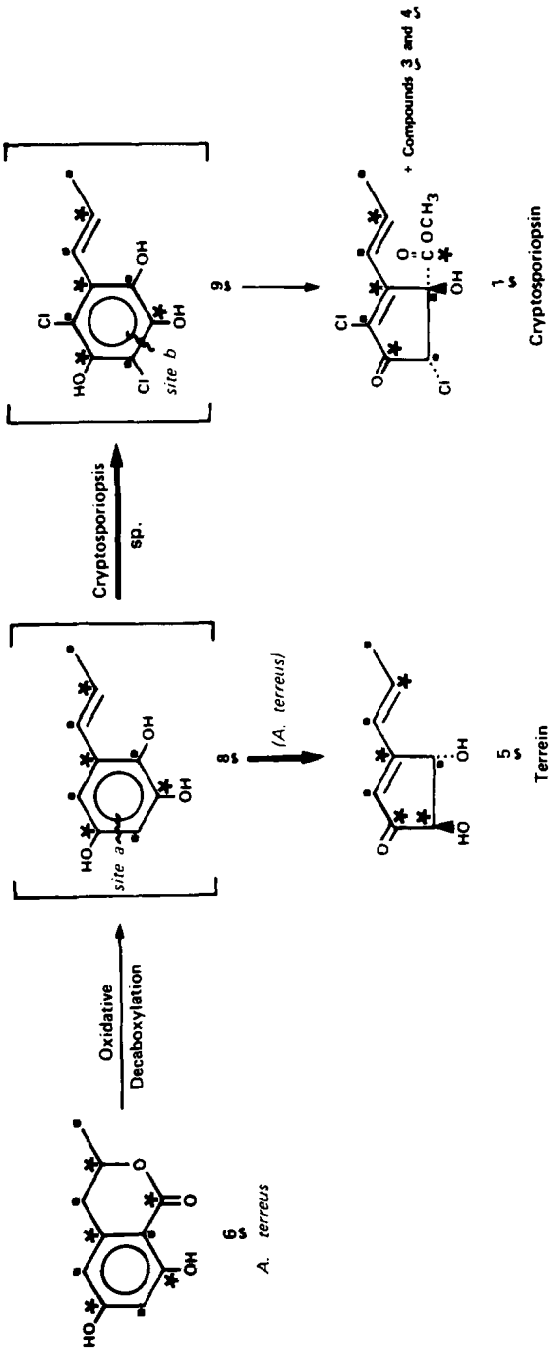


FIG. 6. Proposed pathway for cyclopentenoid metabolites formation.

reduction would then lead to terrein. On the other hand, in *Cryptosporiopsis* sp. compound **8** would first be chlorinated to give the chlorinated aromatic compound **9**. The chlorine groups on the aromatic ring then dictate the site of cleavage, which will presumably be at "site b." This surmise accounts satisfactorily for the different label distributions in terrein and the *Cryptosporiopsis* sp. metabolites. Substitution on the aromatic ring has been noted before to affect cleavage of oxygenases (see Fig. 35 of Ref. (*5*)). A method for distinguishing between the two cleavage sites of oxygenases in *A. terreus* and *Cryptosporiopsis* sp. is presently under study in our laboratories.

REFERENCES

1. R. B. WOODWARD, *Nature (London)* **162,** 155 (1948).
2. SIR ROBERT ROBINSON, *in* "The Structural Relations of Natural Products," p. 113. Oxford Univ. Clarendon Press, London/New York, 1955.
3. S. I. HEINBERGER AND A. I. SCOTT, *J. Chem. Soc. Chem. Commun.* **217** (1973).
4. R. THOMAS, Biosynthetic pathways involving ring cleavage, "Biogenesis of Antibiotic Substances" (Z. Vanek and Z. Hostalek Eds.), p. 155. Academic Press, New York, 1965.
5. L. O. ZAMIR, The biosynthesis of patulin and penicillic acid, "The Biosynthesis of Mycotoxins. A Study in Secondary Metabolism" (P. S. Steyn, Ed.), p. 223. Academic Press, New York, 1980.
6. Y. SAEKI, M. NOZAKI, AND S. SENOL, *J. Biol. Chem.* **255,** 8465 (1980).
7. J. A. GUDGEON, J. S. E. HOLKER, AND T. J. SIMPSON, *Bioorg. Chem.* **8,** 311 (1979).
8. M. A. STILLWELL, F. A. WOOD, AND G. M. STRUNZ, *Canad. J. Microbiol.* **15,** 501 (1969).
9. G. M. STRUNZ, A. S. COURT, J. KOMLOSSY, AND M. A. STILLWELL, *Canad. J. Chem.* **47,** 2087 (1969).
10. G. M. STRUNZ, A. S. COURT, J. KOMLOSSY, AND M. A. STILLWELL, *Canad. J. Chem.* **47,** 3700 (1969).
11. W. J. MCGAHREN, J. H. VAN DEN HENDE, AND L. A. MITSCHER, *J. Amer. Chem. Soc.* **91,** 157 (1969).
12. R. J. J. CH. LOUSBERG, Y. TIRILLY, AND M. MOREAU, *Experientia* **32,** 331 (1976).
13. D. GILES AND W. B. TURNER, *J. Chem. Soc. (C)* 2187 (1969).
14. L. ZAMIR AND C. C. CHIN, *J. Natural Products,* to be published.
15. H. RAISTRICK AND G. SMITH, *Biochem J.* **29,** 606 (1935).
16. N. KIRIYAMA, Y. HIGUCHI, AND Y. YAMAMOTO, *Chem. Pharmacol. Bull.* **25,** 1265 (1977).
17. R. F. CURTIS, P. C. HARRIS, C. H. HASSALL, J. D. LEVI, AND D. M. PHILLIPS, *J. Chem. Soc. (C)* 168 (1966).
18. M. J. RIX AND B. R. WEBSTER, *J. Chem. Soc. (B)* 254 (1968).
19. A. W. DUNN, I. D. ENTWISTLE, AND R. A. W. JOHNSTONE, *Phytochem.* **14,** 2081 (1975).
20. J. F. GROVE, *J. Chem. Soc.* 4693 (1954).
21. D. H. R. BARTON AND E. MILLER, *J. Chem. Soc.* 1028 (1955).
22. R. A. HILL, R. H. CARTER, AND J. STAUNTON, *J. Chem. Soc. Chem. Commun.* 380 (1975).
23. M. J. GARSON, R. A. HILL, AND J. STAUNTON, *J. Chem. Soc. Chem. Commun.* 624, (1977).
24. J. S. E. HOLKER AND K. YOUNG, *J. Chem. Soc. Chem. Commun.* 525, (1975).