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*J. Nat. Prod.*, **1994**, 57 (7), 882-889• DOI: 10.1021/np50109a002 • Publication Date (Web): 01 July 2004

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Journal of Natural Products
Vol. 57, No. 7, pp. 882-889, July 1994

# HYDROXYLATION OF SYNTHETIC ABIETANE DITERPENES BY *ASPERGILLUS* AND *CUNNINGHAMELLA* SPECIES: NOVEL ROUTE TO THE FAMILY OF DITERPENES ISOLATED FROM *TRIPTERYGIUM WILFORDII*

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ABSTRACT.—Several strains of filamentous fungi and yeasts were screened for their ability to oxidize the butenolide **1** (19-hydroxy-18(4→3)-*abeo*-abieta-3,8,11,13-tetraen-18-oic acid lactone), which is a synthetic diterpene analogue of compounds isolated from the perennial herb, *Tripterygium wilfordii*. Novel products **7–13** were obtained from the filamentous fungus, *Cunninghamella elegans*, grown in SSBF medium and their structures were determined by $^{1}$H- and $^{13}$C-nmr, ms, ir, and uv spectroscopy. *C. elegans* hydroxylated butenolide **1** at C-5 or C-7 on ring B and/or on the isopropyl side-chain at C-15 or C-16. Changing the growth medium to PDB resulted in hydroxylation of **1** at C-7 only.

Hot $H_2O$ or EtOH extracts of the perennial herb, *Tripterygium wilfordii* Hook. f. (Celastraceae) have been used in traditional Chinese medicine as antitumor and antiinflammatory preparations (1). The extracts have been shown to be antiinflammatory both in vitro and in vivo. For example, they inhibit antigen and mitogen-stimulated proliferation of T and B cells, block the release of interleukin-2 by T cells (2,3), and prevent Type II collagen-induced arthritis in mice (4). Recent investigations have revealed that such extracts also possess anti-spermatogenic effects in rats and humans (5–7).

The potential therapeutic usefulness of *T. wilfordii* extracts has stimulated research on the isolation of the pharmacologically active components from these extracts. In 1972, Kupchan *et al.* isolated lactone triepoxide diterpenes of the abietane type, triptolide and tripdiolide, which they showed possessed significant antileukemic activity (8). Subsequently, Kutney *et al.* established *T. wilfordii* in plant cell culture from which they have isolated several other novel diterpenes and triterpenes (9–12). More recently, a kaurene-type diterpene, tripterifordin, has been isolated from *T. wilfordii* which was found to have significant anti-HIV activity in vitro (13).

The clinical usefulness of the diterpene triepoxides, triptolide and tripdiolide, is therefore promising. However, iv administration of triptolide to dogs (20–160 μg/kg/day) resulted in pathological or functional changes in the heart, liver, and gastrointestinal tract (14). Therefore, related compounds are sought which retain the pharmacological activity without side effects. Structure-activity relationships have indicated that the pharmacological activity of the diterpene epoxides requires some functionality in ring A (possibly the butenolide ring) and hydroxylation and/or epoxidation of the B-C ring system (15). The production of potentially active congeners of triptolide and tripdiolide in yields adequate for pharmacological screening can perhaps best be achieved by a combination of chemical synthesis and microbial transformation. For example, previous studies in our laboratory (16) showed that cultures of the filamentous fungus,

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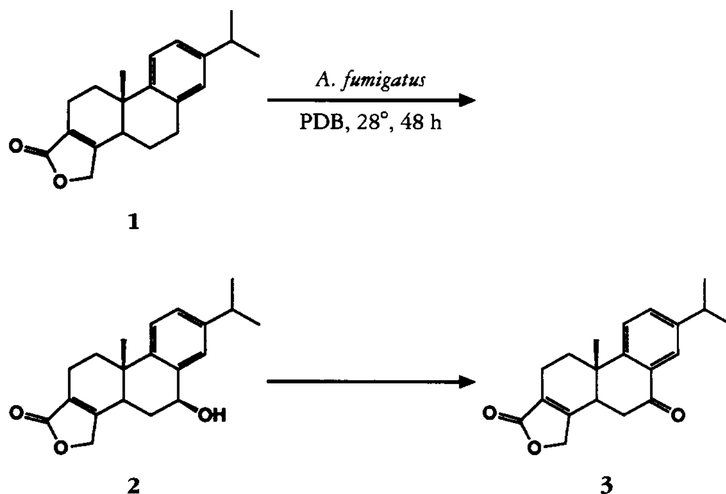
*Syncephalastrum racemosum*, were able to hydroxylate the B ring or the isopropyl side-chain of a synthetic lactone diterpene precursor, 19-hydroxy-18-(4→3)-*abeo*-abieta-3,8,11,13-tetraen-18-oic acid lactone [**1**]. In the study described here, other fungal species capable of oxidizing the butenolide substrate **1** were identified and the structures of the products formed by these strains have been determined.

## RESULTS AND DISCUSSION

From a screen of 15 strains of yeast and filamentous fungi, the following were found to metabolize butenolide **1**: *Cunninghamella elegans* var. *chibaensis* Kuwabara et Hoshino, *Cunninghamella echinulata* (Thaxter) Thaxter var. *echinulata, Cunninghamella* sp. (isolate UBC#95 from the University of British Columbia collection, Dr. R. Bandoni), and *Aspergillus fumigatus* Fresenius. None of the yeasts tested was capable of oxidizing compound **1**. The metabolism of diterpenes by filamentous fungi may be related to their exposure in nature to plant-derived diterpenes (phytoalexins) (17). Interestingly, another strain of *C. echinulata* (UBC#92) was unable to metabolize butenolide **1**. Moreover, *A. fumigatus* but not *A. niger* showed biotransformation activity. Thus, the ability to oxidize butanolide **1** was not uniform within a genus nor was it present in different strains of the same species.

Scheme 1 depicts the oxidation of **1** by *A. fumigatus*. Butenolide **1** (0.1 mg/ml) was added to 48-h-old cultures of *A. fumigatus* (see Experimental) and incubation for a further 48 h resulted in the production of the 7β-hydroxy-derivative **2** and the 7-ketobutenolide **3**. This metabolic profile was different from that determined previously for *S. racemosum*, which formed all of the 7β- [**2**], 7-keto- [**3**], and 7α-hydroxy- [**6**] derivatives as well as oxidizing the isopropyl side-chain to yield the 15-OH derivative (structure not shown) (16). Whether the formation of the 7-keto- group was due to autooxidation of the 7-OH or was enzymatically oxidized has not been tested directly; however, the pure 7β-hydroxybutenolide **2** did not autooxidize when added to liquid cultures of *C. elegans* and it was maintained at 28° and 240 rpm for 96 h, which suggests that the 7-ketobutenolide **3** was produced enzymatically.

When **1** was added to *A. fumigatus* cultures during logarithmic phase (30 h), only 18% of the parent compound was oxidized after 48 h (Figure 1). In contrast, over 90%




SCHEME 1. Biotransformation of the synthetic butenolide **1** by *A. fumigatus*. Butenolide **1** (100 mg–0.1 mg/ml) was dissolved in EtOH, added to the culture, and incubated for 24–48 h.

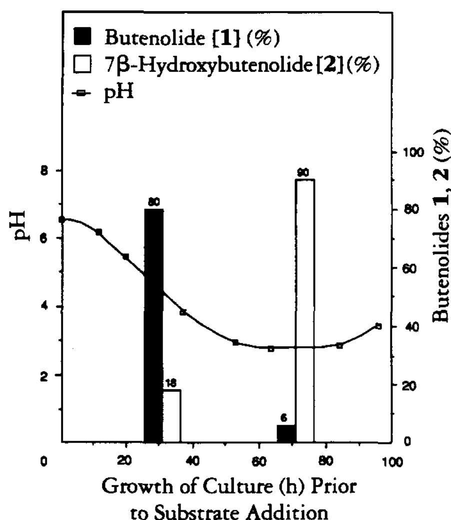



FIGURE 1. The influence of culture age on the biotransformation of the butenolide **1** by *A. fumigatus*. Butenolide **1** (0.1 mg/ml) was added to cultures grown for 30 h (pH 5) or 70 h (pH 2.8–3.0). Total biotransformation time was 48 h.

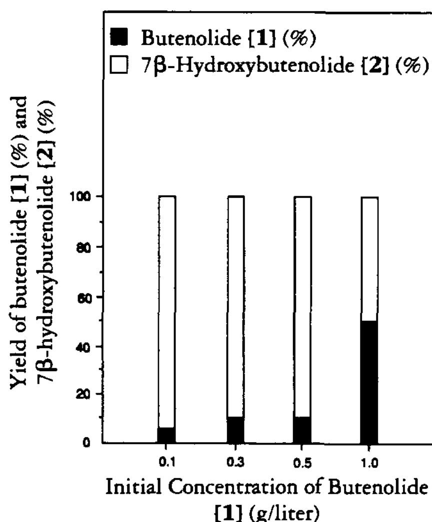


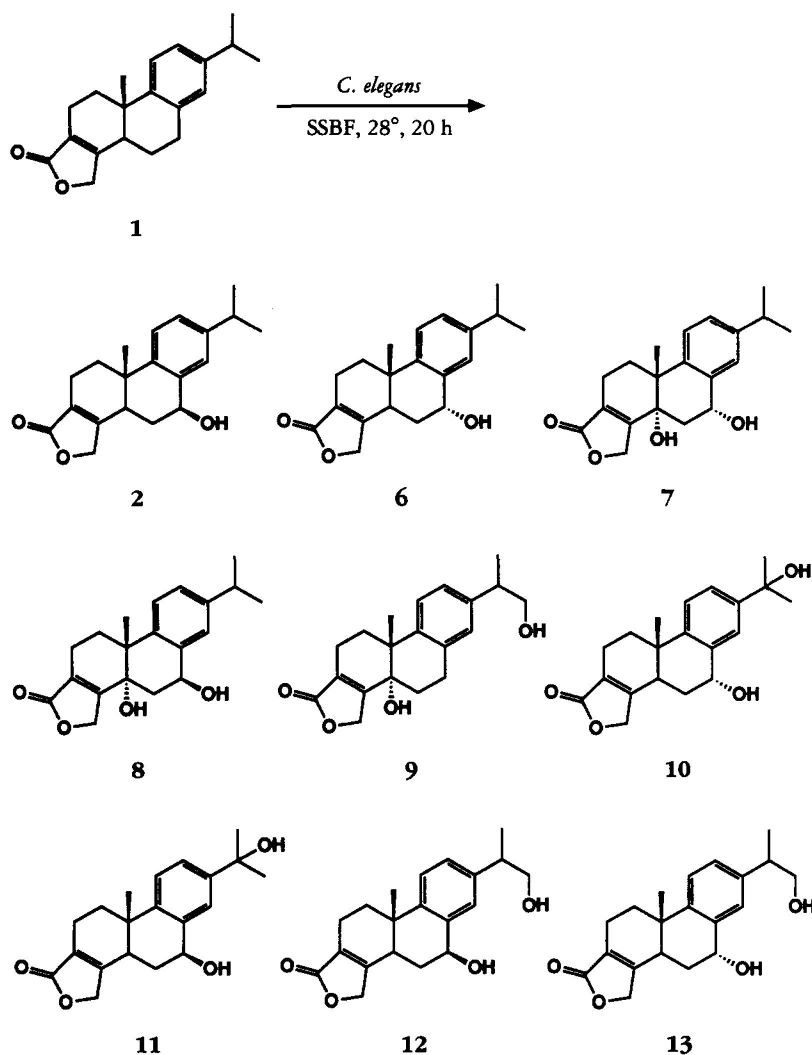

FIGURE 2. The effect of varying the butenolide concentration on the yield of 7β-hydroxybutenolide. Butenolide **1** was added to 72-h old cultures of *A. fumigatus* and the yield of 7β-hydroxybutenolide [**2**] (after 48 h) was determined.

of the butenolide was converted to the 7β-hydroxybutenolide **2** in 48 h if the substrate was added during the stationary phase (70 h). It is probable that the greater conversion was due to the presence of a more active or a higher concentration of hydroxylase during stationary phase.

Substrate concentrations of 0.1–0.5 g/liter of **1** resulted in a 90% conversion to **2** after 48 h. However, when 1.0 g/liter was used, only 50% of the butenolide parent compound was oxidized (Figure 2). Substrate saturation of the hydroxylase or substrate-induced cytotoxicity could account for the observed percentage decrease in bioconversion. However, the latter explanation is less likely as there was no apparent inhibition of *A. fumigatus* growth by the higher concentration of the butenolide (data not shown). More likely, the solubility of **1** may have been the limiting step because this in $H_2O$ is only 0.015 g/100 g $H_2O$.

Incubation of stationary phase cultures of *C. elegans* with **1** for 20 h yielded nine products (of which seven were novel compounds) that were extracted from the broth and the mycelia and separated using Si gel chromatography followed by flash chromatography. Scheme 2 presents the structures of all metabolites from *C. elegans*. Pertinent $^1$H-nmr and ms data for the butenolide [**1**] and for the novel metabolites [**7–13**] isolated from *C. elegans* are noted below. *C. echinulata* (ATCC #9244) also oxidized the butenolide **1** but afforded only one product, **2** (data not shown). *C. elegans* has been shown to generate regio- and stereoselective modifications to a variety of organic substrates. For example, *Cunninghamella* spp. are able to oxidize polycyclic aromatic hydrocarbons (18), sesquiterpenes (19), and the diterpene, sclareol (20), as well as an acronycine alkaloid (21).

Compounds **2** and **6** showed a molecular ion peak at *m/z* 312 in their ms, which was 16 mass units larger than that of butenolide **1**. The $^1$H-nmr spectrum of **6** showed a signal due to H-7 at δ 4.92 (dd, $J$=5 and 1 Hz) and **2** at δ 5.02 (1H, dd, $J$=8 and 8 Hz). Therefore, **2** and **6** were deduced to be a 7β-hydroxybutenolide and a 7α-hydroxybutenolide, respectively. In addition, the $^1$H-nmr spectrum of **2** was identical




SCHEME 2. The biotransformation of the butenolide **1** by *C. elegans*. After extraction and chromatographic separation, the polar products were isolated. Compounds **7–13** are novel products.

to the spectrum of the 7β-hydroxybutenolide described previously (16,22). Characterization of **6** as a 7α-hydroxybutenolide was also based on the *J* value of the H-7 signals of the $^1$H-nmr spectrum.

Metabolites **7** and **8** showed a molecular ion peak at *m/z* 328 in the ms which was 32 mass units greater than that of butenolide **1**. The $^{13}$C-nmr spectrum of both products showed a tertiary hydroxyl group on C-5 (**7**, δ 69.2; **8**, δ 69.2) and a secondary hydroxyl group at C-7 (**7**, δ 4.97, d, $J$=5 Hz and **8**, δ 5.15, dd, $J$=8 and 8 Hz). Finally, from the coupling constants of each signal on the $^1$H-nmr spectrum, **7** was characterized as a 5α,7α-dihydroxybutenolide and **8** as a 5α,7β-dihydroxybutenolide. Compound **9** showed a molecular ion peak at *m/z* 328 in the ms which was 32 mass units greater than that of butenolide **1**. The $^1$H-nmr spectrum of **9** indicated the existence of a tertiary hydroxyl group on C-5 (no proton signal due to C-5) and a primary hydroxyl group on C-16 (δ 3.72, d, $J$=8 Hz). Finally, from the coupling constants of each signal on the $^1$H-nmr spectrum, **9** was determined to be a 5α,16-dihydroxybutenolide.

Compounds **10** and **11** showed an ms molecular ion peak at $m/z$ 328, which was 32 mass units greater than that of butenolide **1**. The $^1$H-nmr spectrum of both products showed the existence of a secondary hydroxyl group at C-7 (**10**, δ 4.95, dd, $J$=5 and 1 Hz; **11**, δ 5.03, dd, $J$=8 and 8 Hz) and a tertiary hydroxyl group at C-15 [neither compound showed a proton signal due to C-15 and both had signals due to C-16 and C-17 as singlets (**10**, δ 1.60; **11**, δ 1.61)]. From a determination of the coupling constants of each signal on the $^1$H-nmr spectrum, **10** was established as a 7α,15-dihydroxybutenolide and **11** as a 7β-15-dihydroxybutenolide.

Compounds **12** and **13** both showed a molecular ion peak at $m/z$ 328 in the ms which was 32 mass units greater than that of butenolide **1**. The $^1$H-nmr spectrum of both products showed the existence of a secondary hydroxyl group on C-7 (**12**, δ 5.00, dd, $J$=8 and 8 Hz; **13**, δ 4.92, br s) and a primary hydroxyl group on C-16 (**12**, δ 3.72; **13**, δ 3.74, d, $J$=8 Hz). From the $^1$H-nmr coupling constants, **12** was determined to be a 7β,16-dihydroxybutenolide and **13** a 7α,16-dihydroxybutenolide.

Oxidation of the aromatic ring C was not observed in these experiments. However, Biellmann *et al.* have found that certain bacteria can hydroxylate the aromatic ring of a related diterpene structure, dehydroabietic acid, which has a carboxylic acid group at C-4 on ring A. In their studies, *Flavobacterium resinovorum, Pseudomonas* sp., and *Alcaligenes eutrophus* degraded dehydroabietic acid by C-7 hydroxylation followed by aromatic hydroxylation and subsequent cleavage of ring B (23,24). We therefore screened *F. resinovorum* (ATCC #12524) for its ability to oxidize the butenolide **1** but found no biotransformation by this organism (data not shown).

The composition of the culture medium influenced the ability of fungi to metabolize **1**. For example, *A. fumigatus* metabolized **1** when cultured in PDB but no metabolites were formed by this fungus in SSBF (see Experimental for the composition of the media). Conversely, nine metabolites were formed by *C. elegans* in SSBF (Scheme 2) whereas only two metabolites, **2** and trace amounts of **3**, were formed when *C. elegans* was cultured in PDB (data not shown).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps (with recrystallization solvents given in parentheses) were determined using a Kofler block melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 141 automatic polarimeter in $CHCl_3$ solution using a quartz cell of 1 dm path length with the concentration (in g/100 ml) given in parentheses. The ir spectra were recorded on Perkin-Elmer 710, 710B, and 1710 spectrometers in $CHCl_3$ solution (using NaCl cells of 0.1 mm path length) or as a thin film (using NaCl plates). The uv spectra were recorded on Cary 15 or Unicam SP800 spectrometers using quartz cells of 1 cm path length. The $^1$H-nmr spectra were recorded on Bruker WH-400, AE-200, or Varian XL-300 spectrometers (with solvents given in parentheses) and the chemical shifts reported in the delta (δ) scale in ppm relative to TMS. Assignments, where given, are based on a combination of chemical shift, coupling constant, decoupling, and nOe difference nmr data. The $^{13}$C-nmr spectra were recorded on Bruker AE-20 or Varian XL-300 spectrometers and the chemical shifts are reported on the delta (δ) scale in ppm relative to TMS. The mass spectra were recorded on AEI-MS-9 or Kratos-MS-50 (using eims for low- and high-resolution analysis) or Delsi Nermag R10-1OC (cims) spectrometers.

Elemental analyses were determined using a combustion technique.

FUNGAL MATERIAL.—The following strains were able to oxidize **1** to polar products: *Aspergillus fumigatus* Fresenius (ATCC# 13073) and *Cunninghamella elegans* var. *chibaensis* Kuwabara et Hoshino (ATCC# 20230).

GROWTH CONDITIONS.—Fungi were stored on potato dextrose agar slants under sterile mineral oil. Erlenmeyer flasks were inoculated with a spore suspension prepared from these slants to a final spore concentration of $2.5\times10^6$ spores/ml. The ratio of flask volume to medium volume was 5:1. *A. fumigatus* spores were inoculated into 1 liter of PDB medium containing 2% glucose and grown for 48 h at 28°, 240 rpm on a rotary shaker. *C. elegans* spores were inoculated into 3 liters of SSBF medium containing 2% glucose and the cells were grown at 28°, 240 rpm for 72 h. Butenolide **1** was dissolved in EtOH (final volumes of

EtOH were not more than 2% of the total medium volume) and added to the culture and further incubated for various periods which are noted on the figures. The composition of the media used for filamentous fungi were: PDB; glucose 2%, freshly prepared potato extract 23%, yeast extract 0.5% (pH 6.5), and SSBF; glucose 2%, soya bean flour containing 1% fat 0.5%, NaCl 0.5%, $KH_2PO_4$ 0.5%, yeast extract 0.5% (pH 7.0) (20). The EtOAc extracts of cell suspensions or separate broth and mycelia fractions were analyzed for metabolites of compound **1** by tlc as previously described (16).

PURIFICATION OF METABOLITES FROM EXTRACTS OF *A. FUMIGATUS*.—After 48 h of biotransformation of **1** (100 mg) in 1 liter of PDB medium, the broth and mycelia were separated by filtration through Miracloth and extracted with three volumes of EtOAc. The solvent was then dried over $MgSO_4$ and evaporated under vacuum. The crude residue was dissolved in hexane-EtOAc (4:1) and chromatographed on Si gel 60 (230–400 mesh). The products from this separation were examined by tlc (Si gel 60 $F_{254}$, mobile phase EtOAc-hexane, 7:3); **2** had an $R_f$ value 0.26, **3** had a value of 0.47. The $^1H$ nmr and ms of the metabolites were identical to those of authentic samples (16,22). The yields of **2** and **3** from 100 mg of starting material [**1**] were 72 mg (72%) and 3 mg (3%), respectively; 5 mg (5%) of unreacted compound [**1**] was also recovered. Overall recovery was 80%.

PURIFICATION OF METABOLITES FROM EXTRACTS OF *C. ELEGANS*.—Butenolide **1** (500 mg) was dissolved in 45.0 ml of EtOH and added directly to 3.0 liters of SSBF medium containing a 72-h culture of *C. elegans* (0.17 mg/ml), which had been inoculated from a spore suspension. The culture was further incubated for 20 h at 240 rpm and 28°. After this period, the cell suspension was filtered through Miracloth in a Buchner funnel and the filtrate extracted with EtOAc (3×1.5 liters). The combined extracts were washed with $H_2O$ (750 ml), NaCl (750 ml), and then dried over anhydrous $MgSO_4$. This material was concentrated under vacuum to yield a crude broth extract (664 mg). The crude extract was chromatographed over Si gel with hexane-$Me_2CO$ (95:5, 9:1, 8:2, 1:1) followed by MeOH, which afforded 6 fractions (Fractions 1 and 2 were obtained with hexane-$Me_2CO$, 95:5, Fraction 3 was obtained with 9:1, Fraction 4 was obtained with 8:2, Fraction 5 was obtained with 1:1, and Fraction 6 was obtained with MeOH). Fractions 1–6 were further chromatographed as follows: Fraction 1 ($CHCl_3$-$Me_2CO$, 95:5) yielded compounds **1**, **2**, **6**, and **7**; Fraction 2 ($CHCl_3$-$Me_2CO$, 9:1) yielded compound **8**; Fraction 3 (hexane-$Me_2CO$, 2:1) yielded compound **9**; Fraction 4 (EtOAc) yielded compounds **10** and **11**, and Fraction 5 ($CH_2Cl_2$-$Me_2CO$, 1:1) yielded compounds **12** and **13**. After the evaporation of solvent, the percent yields were determined by weighing the vacuum-dried, pure compounds, the mole percent of starting material was then calculated: **1**, 44 mg (9%); **2**, 54 mg (10%); **6**, 37 mg (7%); **7**, 80 mg (14%); **8**, 53 mg (10%); **9**, 21 mg (4%); **10**, 17 mg (3%); **11**, 14 mg (3%); **12**, 12 mg (2%), and **13**, 15 mg (3%). The total recovery from the extract of the broth was therefore 65%.

To separate the metabolites contained in the fungal biomass from this incubation, 2 liters of EtOAc were added to the combined cell material and the resulting suspension was allowed to extract overnight at 4°. The cell suspension was filtered through Miracloth and the filtrate washed with $H_2O$ (500 ml) and then NaCl (500 ml) and dried over anhydrous $MgSO_4$. Concentration of the extract *in vacuo* resulted in 1.08 g of crude mixture which was chromatographed over Si gel using hexane-$Me_2CO$ (19:1). Yields of compounds were **1**, 10 mg (2%); **2**, 10 mg (2%); **6**, 8 mg (2%); and **7**, 20 mg (4%), respectively. The recovery from the extract of the cell material was 10%. The overall recovery from the broth and cell material was 75%.

The physical properties of the metabolites obtained from the biotransformaton of **1** by *C. elegans* are as follows:

*Compound* **2**.—White powder (hexane/$Me_2CO$), mp 63–67°; $[\alpha]^{19.3}D$ +76.0° ($c$=1.00, $CHCl_3$); ir $\nu$ max ($CHCl_3$) 3470, 2960, 1740 $cm^{-1}$; uv $\lambda$ max (MeOH) (log $\epsilon$) 216 (4.31) nm; $^1H$ nmr ($CDCl_3$) $\delta$ 1.12 (3H, s, $CH_3$-20), 1.17 (6H, d, $J$=8 Hz, $CH_3$-16 and -17), 1.70 (1H, m, $H_A$-1), 1.94 (1H, ddd, $J$=12, 12, and 8 Hz, $H_B$-6), 2.34 (1H, ddd, $J$=12, 8, and 2 Hz, $H_B$-6), 2.38 (1H, m, $H_A$-2), 2.50 (1H, m, $H_B$-1), 2.53 (1H, m, $H_B$-2), 2.81 (1H, br d, $J$=12 Hz, H-5), 2.92 (1H, m, H-15), 4.77 (1H, br d, $J$=16 Hz, $H_A$-19), 4.84 (1H, br d, $J$=16 Hz, $H_B$-19), 5.02 (1H, dd, $J$=8 and 8 Hz, H-7$\alpha$), 7.18 (1H, dd, $J$=8 and 2 Hz, H-12), 7.28 (1H, d, $J$=8 Hz, H-11), 7.44 (1H, d, $J$=2 Hz, H-14); $^{13}C$ nmr ($CDCl_3$) $\delta$ 32.7 (C-1), 18.1 (C-2), 125.8 (C-3), 162.1 (C-4), 36.8 (C-5), 30.7 (C-6), 69.3 (C-7), 137.4 (C-8), 147.5 (C-9), 40.8 (C-10), 126.3 (C-11), 124.3 (C-12), 142.4 (C-13), 126.1 (C-14), 33.7 (C-15), 24.0 (C-16), 23.8 (C-17), 174.0 (C-18), 70.4 (C-19), 23.0 (C-20); ms $m/z$ 312 $[M]^+$, 294, 279, 237 (base peak); hrms calcd for $C_{20}H_{24}O_3$ 312.1726; found 312.1717.

*Compound* **6**.—White powder (hexane/$Me_2CO$), mp 132–136°; $[\alpha]^{19.3}D$ +24.8° ($c$=1.00, $CHCl_3$); ir $\nu$ max ($CHCl_3$) 3485, 2965, 1740 $cm^{-1}$; uv $\lambda$ max (MeOH) (log $\epsilon$) 216.7 (4.37) nm; $^1H$ nmr ($CDCl_3$) $\delta$ 1.00 (3H, s, $CH_3$-20), 1.28 (6H, d, $J$=8 Hz, $CH_3$-16 and 17), 1.77 (1H, m, $H_A$-1), 2.01 (1H, ddd, $J$=12, 1, and 1 Hz, $H_A$-6), 2.17 (1H, ddd, $J$=12, 12, and 5 Hz, $H_B$-6), 2.40 (1H, m, $H_A$-2), 2.51 (1H, m, $H_B$-1), 2.54 (1H, m, $H_B$-2), 2.91 (1H, m, H-15), 3.20 (1H, br d, $J$=12 Hz, $H_A$-5), 4.76 (1H, br d, $J$=16 Hz, $H_A$-19), 4.89 (1H, br d, $J$=16 Hz, $H_B$-19), 4.92 (1H, dd, $J$=5 and 1 Hz, H-7$\beta$), 7.21 (1H, dd, $J$=8 and 2 Hz, H-

12), 7.24 (1H, d, $J$=2 Hz, H-14), 7.32 (1H, d, $J$=8 Hz, H-11); $^{13}$C nmr ($CDCl_3$) δ 32.5 (C-1), 18.2 (C-2), 125.4 (C-3), 163.2 (C-4), 36.7 (C-5), 29.4 (C-6), 67.2 (C-7), 136.2 (C-8), 147.6 (C-9), 36.6 (C-10), 126.9 (C-11), 124.6 (C-12), 142.4 (C-13), 128.3 (C-14), 33.6 (C-15), 24.0 (C-16), 13.8 (C-17), 174.1 (C-18), 70.5 (C-19), 22.1 (C-20); ms *m/z* 312 [M]$^+$, 294, 279, 237 (base peak); hrms calcd for $C_{20}H_{24}O_3$, 312.1726; found 312.1566. *Anal.* calcd for $C_{20}H_{24}O_3$: C 76.89, H 7.74; found: C 76.66, H 7.69.

*Compound* **7**.—Colorless prisms (hexane-$Me_2CO$, 9:1), mp 156–158°; $[\alpha]^{19.3}D$ −89.3° ($c$=0.44, $CHCl_3$); ir ν max ($CHCl_3$) 3434, 2934, 1724 cm$^{-1}$; uv λ max (MeOH) (log ε) 214 (4.36) nm; $^1$H nmr ($CDCl_3$) δ 1.07 (3H, s, $CH_3$-20), 1.28 (6H, d, $J$=8 Hz, $CH_3$-16 and -17), 2.20 (1H, m, $H_A$-1), 2.25 (1H, d, $J$=13 Hz, $H_A$-6), 2.38 (1H, m, $H_B$-1), 2.43 (1H, m, $H_A$-2), 2.52 (1H, dd, $J$=13 and 8 Hz, $H_B$-6), 2.58 (1H, m, $H_B$-2), 2.93 (1H, m, H-15), 4.82 (1H, br d, $J$=16 Hz, $H_A$-19), 4.97 (1H, d, $J$=5 Hz, H-7β), 5.07 (1H, dt, $J$=16 and 2 Hz, $H_B$-19), 7.22 (1H, dd, $J$=8 and 1 Hz, H-12), 7.31 (1H, d, $J$=8 Hz, H-11), 7.36 (1H, d, $J$=1 Hz, H-14); $^{13}$C nmr ($CDCl_3$) δ 34.0 (C-1), 18.0 (C-2), 127.0 (C-3), 161.1 (C-4), 69.2 (C-5), 26.1 (C-6), 67.0 (C-7), 135.9 (C-8), 147.4 (C-9), 41.7 (C-10), 128.4 (C-11 or C-14), 124.6 (C-12), 139.3 (C-13), 127.0 (C-14 or C-11), 33.6 (C-15), 24.0 (C-16), 23.8 (C-17), 174.1 (C-18), 70.5 (C-19), 27.1 (C-20); ms *m/z* 328 [M]$^+$, 310, 189 (base peak); hrms calcd for $C_{20}H_{24}O_4$ 328.1675; found 328.1682. *Anal.* calcd for $C_{20}H_{24}O_4$: C 73.14, H 7.37; found C 73.06, H 7.40.

*Compound* **8**.—Colorless plates (hexane-$Me_2CO$, 5:1), mp 198–203°; $[\alpha]^{19.3}D$ −13.9° ($c$=1.00, $CHCl_3$); ir ν max ($CHCl_3$) 3430, 2930, 1720 cm$^{-1}$; uv λ max (MeOH) (log ε) 214 (4.31) nm; $^1$H nmr ($CDCl_3$) δ 1.22 (3H, s, $CH_3$-20), 1.26 (6H, d, $J$=8 Hz, $CH_3$-16 and -17), 2.04 (1H, m, $H_A$-1), 2.19 (1H, dd, $J$=13 and 8 Hz, $H_A$-6), 2.35 (1H, m, $H_B$-1), 2.41 (1H, m, $H_A$-2), 2.52 (1H, dd, $J$=13 and 10 Hz, $H_B$-6), 2.55 (1H, m, $H_B$-2), 2.90 (1H, m, H-15), 4.80 (1H, ddd, $J$=16, 4, and 2 Hz, $H_A$-19), 5.00 (1H, dd, $J$=8 and 8 Hz, H-7α), 5.15 (1H, dt, $J$=16 and 3 Hz, $H_B$-19), 7.18 (1H, dd, $J$=8 and 1 Hz, H-12), 7.24 (1H, d, $J$=8 Hz, H-11), 7.38 (1H, d, $J$=1 Hz, H-14); $^{13}$C nmr ($CDCl_3$) δ 36.4 (C-1), 17.9 (C-2), 126.6 (C-3), 161.3 (C-4), 69.2 (C-5), 26.1 (C-6), 66.7 (C-7), 136.9 (C-8), 147.6 (C-9), 41.4 (C-10), 127.0 (C-11 or C-14), 124.5 (C-12), 139.6 (C-13), 126.8 (C-14 or C-11), 33.7 (C-15), 24.0 (C-16), 23.8 (C-17), 173.9 (C-18), 72.0 (C-19), 27.4 (C-20); ms *m/z* 328 [M]$^+$, 310, 43 (base peak); hrms calcd for $C_{20}H_{24}O_4$, 328.1675; found 328.1670. *Anal.* calcd for $C_{20}H_{24}O_4$: C 73.14, H 7.37; found C 72.98, H 7.42.

*Compound* **9**.—White powder (hexane-$Me_2CO$, 5:1), mp 72–75°; $[\alpha]^{19.3}D$ −37.9° ($c$=1.00, $CHCl_3$); ir ν max ($CHCl_3$) 3430, 2950, 1750 cm$^{-1}$; uv λ max (MeOH) (log ε) 215 (4.31) nm; $^1$H nmr ($CDCl_3$) δ 1.13 (3H, s, $CH_3$-20), 1.28 (3H, d, $J$=8 Hz, $CH_3$-17), 2.03 (1H, ddd, $J$=14, 8, and 3 Hz, $H_A$-6), 2.13 (1H, m, $H_A$-1), 2.25 (1H, ddd, $J$=14, 8, and 8 Hz, $H_B$-6), 2.35 (1H, m, $H_B$-1), 2.43 (1H, m, $H_A$-2), 2.60 (1H, m, $H_B$-2), 2.92 (1H, m, H-15), 3.12 (2H, m, H-7β), 3.72 (2H, d, $J$=8 Hz, $CH_2$-16), 4.83 (1H, ddd, $J$=16, 4, and 2 Hz, $H_A$-19), 5.02 (1H, ddd, $J$=16, 2, and 2 Hz, $H_B$-19), 7.06 (1H, d, $J$=2 Hz, H-14), 7.11 (1H, dd, $J$=8 and 2 Hz, H-12), 7.30 (1H, d, $J$=8 Hz, H-11); ms *m/z* 328 [M]$^+$, 310, 297, 280 (base peak), 251; hrms calcd for $C_{20}H_{24}O_4$, 328.1675; found 328.1675. *Anal.* calcd for $C_{20}H_{24}O_4$: C 73.14, H 7.37; found C 73.00, H 7.50.

*Compound* **10**.—White powder (hexane-$Me_2CO$, 5:1), mp 193–195°; $[\alpha]^{19.3}D$ +24.9° ($c$=1.00, $CHCl_3$); ir ν max ($CHCl_3$) 3460, 2990, 1750 cm$^{-1}$; uv λ max (MeOH) (log ε) 216 (4.35) nm; $^1$H nmr ($CDCl_3$) δ 1.00 (3H, s, $CH_3$-20), 1.60 (6H, s, $CH_3$-16 and -17), 1.77 (1H, m, $H_A$-1), 2.19 (2H, m, $H_A$-6), 2.42 (1H, ddd, $J$=12, 12, and 5 Hz, $H_B$-6), 2.51 (1H, m, $H_B$-1), 2.54 (1H, m, $H_B$-2), 3.20 (1H, br d, $J$=12 Hz, H-6), 4.78 (1H, br d, $J$=16 Hz, $H_A$-19), 4.88 (1H, br d, $J$=16 Hz, $H_B$-19), 4.92 (1H, dd, $J$=5 and 1 Hz, H-7β), 7.24 (1H, d, $J$=2 Hz, H-14), 7.32 (1H, d, $J$=8 Hz, H-11), 7.39 (1H, dd, $J$=8 and 2 Hz, H-12); ms *m/z* 328 [M]$^+$, 313, 310, 295, 292, 277 (base peak); hrms calcd for $C_{20}H_{24}O_4$ 328.1675; found 328.1667.

*Compound* **11**.—White powder (hexane-$Me_2CO$, 5:1), mp 92–95°; $[\alpha]^{19.3}D$ +41.1° ($c$=1.00, $CHCl_3$); ir ν max ($CHCl_3$) 3450, 2950, 1740 cm$^{-1}$; uv λ max (MeOH) (log ε) 216 (4.39) nm; $^1$H nmr ($CDCl_3$) δ 1.12 (3H, s, $CH_3$-20), 1.61 (6H, s, $CH_3$-16 and -17), 1.70 (1H, m, $H_A$-1), 1.95 (1H, ddd, $J$=12, 12, and 8 Hz, $H_A$-6), 2.35 (1H, ddd, $J$=12, 8, and 2 Hz, $H_B$-6), 2.39 (1H, m, $H_A$-2), 2.51 (1H, m, $H_B$-1), 2.53 (1H, m, $H_B$-2), 2.82 (1H, br d, $J$=12 Hz, $H_B$-19), 5.03 (1H, dd, $J$=8 and 8 Hz, H-7α), 7.32 (1H, d, $J$=8 Hz, H-11), 7.42 (1H, dd, $J$=8 and 2 Hz, H-12), 7.71 (1H, $J$=2 Hz, H-14); ms *m/z* 328 [M]$^+$, 313, 310, 295, 292, 277 (base peak); hrms calcd for $C_{20}H_{24}O_4$, 328.1675; found 328.1669.

*Compound* **12**.—White powder (hexane-$Me_2CO$, 5:1), mp 93–95°; $[\alpha]^{19.3}D$ +63.8° ($c$=1.00, $CHCl_3$); ir ν max ($CHCl_3$) 3445, 2945, 1745 cm$^{-1}$; uv λ max (MeOH) (log ε) 216 (4.34) nm; $^1$H nmr ($CDCl_3$) δ 1.12 (3H, s, $CH_3$-20), 1.29 (3H, d, $J$=8 Hz, $CH_3$-17), 1.70 (1H, m, $H_A$-1), 1.94 (1H, ddd, $J$=13, 13, and 4 Hz, $H_A$-6), 2.32 (1H, ddd, $J$=13, 8, and 2 Hz, $H_B$-6), 2.40 (1H, m, $H_A$-2), 2.50 (1H, m, $H_B$-1), 2.52 (1H, m, $H_B$-2), 2.80 (1H, m, H-15), 2.98 (1H, br d, $J$=13 Hz, H-5), 3.72 (2H, d, $J$=8 Hz, $CH_2$-16), 4.75 (1H, br d, $J$=16 Hz, $H_A$-19), 4.84 (1H, br d, $J$=16 Hz, $H_B$-19), 5.00 (1H, dd, $J$=8 and 8 Hz, H-7α), 7.18 (1H,

dd,$J$=8 and 2 Hz, H-12), 7.31 (1H, d,$J$=8 Hz, H-11), 7.45 (1H, d,$J$=2 Hz, H-11); ms $m/z$ 328 $[M]^+$, 310, 297 (base peak); hrms calcd for $C_{20}H_{24}O_4$, 328.1675; found 328.1675.

*Compound* **13**.—White powder (hexane-$Me_2CO$, 5:1) mp 83–85°; $[\alpha]^{19.3}D$ +42.1° ($c$=1.00, $CHCl_3$); ir $\nu$ max ($CHCl_3$) 3440, 2950, 1740 $cm^{-1}$; uv $\lambda$ max (MeOH) (log $\epsilon$) 217 (4.52) nm; $^1H$ nmr ($CDCl_3$) $\delta$ 1.00 (3H, s, $CH_3$-20), 1.30 (3H, d,$J$=8 Hz, $CH_3$-17), 1.74 (1H, m, $H_A$-1), 2.01 (1H, d,$J$=13 Hz, $H_A$-6), 2.17 (1H, ddd,$J$=13, 13, and 4 Hz, $H_B$-6), 2.41 (1H, m, $H_A$-2), 2.52 (1H, m, $H_B$-1), 2.55 (1H, m, $H_B$-2), 2.96 (1H, m, H-15), 3.19 (1H, br d,$J$=13 Hz, H-5), 3.74 (2H, d,$J$=8 Hz, $H_2$-16), 4.75 (1H, br d,$J$=16 Hz, $H_A$-19), 4.89 (1H, br d,$J$=16 Hz, $H_B$-19), 4.92 (1H, br s, H-7$\beta$), 7.21 (1H, dd,$J$=8 and 2 Hz, H-12), 7.27 (1H, d,$J$=2 Hz, H-14), 7.37 (1H, d,$J$=8 Hz, H-11); ms $m/z$ 328 $[M]^+$, 310, 297 (base peak); hrms calcd for $C_{20}H_{24}O_4$, 328.1675; found 328.1676.

ACKNOWLEDGMENTS

The authors are grateful for cooperation and discussions with Professor J.P. Kutney and his co-workers in the Department of Chemistry, University of British Columbia. The synthetic precursors utilized in these studies were kindly provided by Mr. Kang Han, Department of Chemistry, University of British Columbia. This work was supported by grants to M.M. and J.P.K. from the British Columbia Health Research Foundation and the Natural Sciences and Engineering Research Council of Canada.

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*Received 28 October 1993*