Journal of Natural Products
Vol. 50, No. 5, pp. 903-909, Sep-Oct 1987

MICROBIAL TRANSFORMATION STUDIES ON ARTEANNUIN B

SAYED A. ELMARAKBY, FAROUK S. EL-FERALY,[1] HALA N. ELSOHLY, EDWARD M. CROOM, and CHARLES D. HUFFORD*

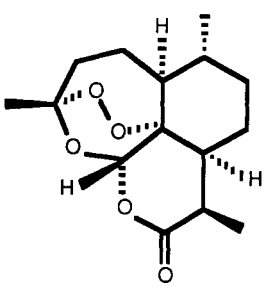
Department of Pharmacognosy and Research Institute of Pharmaceutical Sciences, University of Mississippi, University, Mississippi 38677

ABSTRACT.—The microbial transformation of the sesquiterpene lactone arteannuin B [**3**] using *Aspergillus flavipes* produced dihydroarteannuin B [**4**] as the main transformation product. Preparative-scale fermentation of **3** with *Beauveria bassiana*, on the other hand, has resulted in the production of two metabolites, 3β-hydroxyarteannuin B [**5**] and 13-hydroxy-11-*epi*-dihydroarteannuin B [**6**]. The structure of these metabolites, all of which are new compounds, was established using chemical and spectroscopic techniques. The isomeric dihydrocompound, 11-*epi*-dihydroarteannuin B [**7**] and an isomer of arteannuin B [**8**] were also prepared chemically. All compounds were subjected to 2D-nmr experiments and full ^{1}H- and ^{13}C-nmr assignments were made.

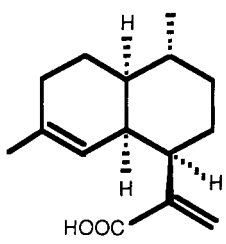
Recently, artemisinin [**1**], the active constituent of *Artemisia annua* L., (Compositae), has received considerable attention because of its promising antimalarial activity (1,2). During the course of ongoing studies conducted to obtain large quantities of **1**, two other sesquiterpenes, namely, artemisinic acid [**2**] and arteannuin B [**3**], have also been isolated in large amounts. The structure of **3**, an epoxy-α-methylene-butyrolactone of the cadinane type was first established by Stefanovic and co-workers (3) and later confirmed by X-ray studies (4,5). The molecule has also been the target of several synthetic efforts (6,7).

Several reports describing the microbial transformation of sesquiterpenes have been published (8-10). The usefulness of such a technique in carrying out a variety of chemical conversions is well documented in the literature (11-13). The microbial transformations of **3** were undertaken to examine its possible microbiological conversion into some useful metabolites. These results should not only be of value for future biosynthetic studies of artemisinin (14), but also may provide some key intermediates towards its total synthesis.

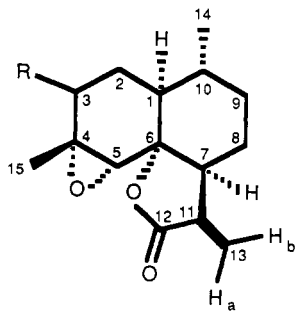
In the present study, three biotransformation products of arteannuin B [**3**] were isolated. On the basis of chemical and spectroscopic data, in particular 2D-nmr techniques, they were identified as dihydroarteannuin B [**4**], 3β-hydroxyarteannuin B [**5**], and 13-hydroxy-11-*epi*-dihydroarteannuin B [**6**]. The isolation and structure elucidation of these new metabolites are described herein.



1



2



3 R=H
5 R=β-OH

[1]Present Address: King Saud University, College of Pharmacy, PO Box 2457, Riyadh 11451, Saudi Arabia.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on either a Fisher-Digital melting point analyzer model 355 or on a Thomas-Hoover uni-melt capillary melting point apparatus. Optical rotations were measured in $CHCl_3$ using a Perkin-Elmer 141 polarimeter. The ir spectra were obtained on a Perkin-Elmer 281B recording spectrophotometer in $CHCl_3$ solution (0.1 mm NaCl cells). ^1H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ on a Varian VXR-300 FT spectrometer operating at 300 MHz and 75 MHz, respectively. The chemical shift values are reported in (ppm) units, and the coupling constants are in Hz. Abbreviations for nmr signals are as follows: s=singlet, d=doublet, t=triplet, q=quartet, dd=double doublet, m=multiplet, br=broad. Standard pulse sequences were used for COSY (15), HETCOR (16), DEPTGL (17), and APT (18) experiments. Low resolution electron impact mass spectra were obtained using an E.I. Finnigan model 3200 (70 eV ionization potential) with INCOS data system. High resolution mass spectra were obtained through The University of Utah, Salt Lake City, Utah. Microanalyses were performed by Scandinavian Microanalytical Laboratories, Herlev, Denmark.

CHROMATOGRAPHY.—Tlc chromatographic analyses were carried out on pre-coated Si gel G-25 UV_{254} plates (0.25 mm). The adsorbent used for column chromatography was Si gel 60/70-270 mesh (Macherey Nagel & Co). Visualization of the tlc plates was done using anisaldehyde-H_2SO_4 spray reagent (19).

ORGANISMS.—The cultures (Table 1) were obtained from the University of Mississippi, Department of Pharmacognosy Culture Collection and were originally from the American Type Culture Collection (ATCC), Rockville, Maryland, or from Northern Regional Research Laboratories (NRRL), Peoria, Illinois. UI cultures were obtained from Dr. John Rosazza, University of Iowa.

MEDIA.—All the preliminary screening and transformation experiments were carried out in a

TABLE 1. Cultures Used for Preliminary Screening[a] of Arteannuin B[3]

Organism	Metabolite production
Aspergillus niger (ATCC 16888)	+
Aspergillus flavus (ATCC 9170)	+
Chaetomium cochloides (ATCC 10195)	−
Cunninghamella blakesleeana (ATCC 8688a)	+
Cunninghamella echinulata (NRRL 3655)	+
Mucor mucedo (UI 4605)	−
Streptomyces roseochromogenus (ATCC 13400)	+
Beauveria bassiana (ATCC 7159)	+
Calonetria decora (ATCC 14767)	+
Cladosporium resinae (ATCC 22712)	+
Nocardia corallina (ATCC 19070)	−
Rhodotorula rubra (ATCC 20129)	+
Sepedonium chrysospermum (ATCC 13378)	−
Sporobolomyces pararoseus (ATCC 11386)	−
Aspergillus flavipes (ATCC 1030)	+
Aspergillus flavipes (ATCC 11013)	+
Aspergillus flavus (NRRL 501)	+
Aspergillus flavus (ATCC 24741)	+
Aspergillus niger (ATCC 10549)	−
Aspergillus niger (ATCC 10581)	+
Aspergillus parasiticus (ATCC 15517)	−
Cunninghamella elegans (ATCC 9245)	−
Nocardia minima (ATCC 19150)	−
Nocardia petroleophilia (ATCC 15777)	−
Pencillium chrysogenum (ATCC 9480)	−
Saccharomyces cerevisiae (ATCC 9763)	−
Streptomyces punipalus (NRRL 3529)	−
Streptomyces rimosus (ATCC 23955)	+

[a]Metabolite production denoted by (+) indicates one or more metabolites were produced, as shown by tlc.

medium consisting of (per liter of H_2O) dextrose, 20 g; yeast extract, 5 g; peptone, 5 g; NaCl, 5g; and K_2HPO_4, 5 g. Stock cultures of fungi and bacteria were stored on slants of Mycophil and Eugon agar, respectively, at 4°.

FERMENTATION PROCEDURES.—Microbial transformation studies were carried out by placing the cultures on rotary shakers, model G-10 gyrotory shaker (New Brunswick, New Jersey), operating at 250 rpm at 24-26°. Preliminary screening was carried out in 125-ml stainless steel-capped Delong culture flasks containing 25 ml of medium. The media were sterilized at 121° and 18 psi for 15 min. A standard two-stage fermentation protocol was employed in all experiments as described previously (20). In general, the substrate was added to the incubation media 24 h after the inoculation of stage II cultures as a 10% solution in EtOH at a concentration of 0.2 mg/ml of stage II medium.

Substrate controls were composed of sterile medium to which the substrate was added and incubated without microorganisms. Culture controls consisted of fermentation blanks in which the organisms were grown under identical conditions but without adding the substrate.

Arteannuin B [**3**] used in this study was isolated from locally grown *A. annua* and possessed physical and spectral data consistent with those reported in the literature (3). ^{1}H-nmr assignments, inferred from COSY experiments, were in accordance with those previously reported for **3** (7). In addition, the ^{13}C-nmr assignments for **3**, which were based on ^{1}H-^{13}C shift correlated 2D-nmr spectroscopy (HETCOR), are reported for the first time (Table 2). Multiplicities were confirmed using the DEPTGL pulse sequence (17).

MICROBIAL TRANSFORMATION OF ARTEANNUIN B [**3**] BY *ASPERGILLUS FLAVIPES*.—Arteannuin B [**3**] (440 mg) was dissolved in 4.4 ml EtOH and distributed evenly among eleven 1-liter, 24-h *Aspergillus flavipes* (ATCC 1030) stage II culture flasks, each containing 200 ml of medium. After 5 days, the incubation mixtures were pooled, filtered (2.2 liters), and the culture filtrates were extracted 3 times with EtOAc (2.2 liters, 2×1.1 liters). The extracts were backwashed with H_2O, dried over anhydrous Na_2SO_4, and concentrated (in vacuo) to afford 0.611 g of dark brown residue. The residue was adsorbed onto Si gel and was loaded on a 60 g (2.5×72 cm) column of Si gel. Elution was carried out with Et_2O-hexane (4:1), and 15-ml fractions were collected. Tlc of fractions 6-14 showed a single spot with Rf=0.73 (Et_2O-hexane, 4:1, system A) (Rf of **3**=0.84). These fractions were combined and concentrated to give 254 mg of pure dihydroarteannuin B [**4**], (42%). Recrystallization from Et_2O/hexane afforded colorless crystals of **4**, mp 131-132°; $[\alpha]^{23}D$ −7.3° (c=0.4, $CHCl_3$), ir ($CHCl_3$) 3020, 2935, 2405, 1771, 1452, 1420, 1380, 1225, 1150, 1127, 1002 cm^{-1}; ^{1}H nmr 0.92 (3H, d, J=6.0 Hz, Me-14), 1.20 (1H, m, H-9$_{ax.}$), 1.30 (1H, m H-1), 1.32 (3H, s, Me-15), 1.34 (3H, d, J=8.0 Hz, Me-13), 1.40-1.56 (3H, m, H-10, H-8$_{ax}$, H-2), 1.57 (1H, OH), 1.58-1.75 (2H, m, H-3, H-2), 1.76-1.92 (3H, m, H-8$_{eq.}$, H-9$_{eq.}$, H-3), 2.20

TABLE 2. ^{13}C-nmr Chemical Shift Assignments for Compounds **3-8**[a,b]

Carbon No.	Compounds					
	3	**4**	**5**[c]	**6**	**7**	**8**
1	43.9(1)	45.9(1)	46.5(1)	44.5(1)	44.8(1)	47.6(1)
2	16.4(2)	16.4(2)	26.8(2)	16.4(2)	16.4(2)	16.9(2)
3	24.5(2)	24.4(2)	68.0(1)	24.5(2)	24.7(2)	24.3(2)
4	58.2(0)	57.9(0)	61.0(0)	58.1(0)	57.8(0)	57.0(0)
5	58.7(1)	59.6(1)	62.6(1)	58.1(1)	58.1(1)	59.0(1)
6	81.0(0)	82.9(0)	80.4(0)	81.7(0)	80.8(0)	83.1(0)
7	52.8(1)	50.3(1)	52.3(1)	48.9(1)	55.0(1)	163.0(0)
8	21.8(2)	21.7(2)	21.8(2)	22.9(2)	22.9(2)	25.8(2)
9	34.0(2)	35.0(2)	34.2(2)	34.6(2)	34.8(2)	35.3(2)
10	30.7(1)	30.6(1)	32.3(1)	30.6(1)	30.8(1)	30.3(1)
11	138.7(0)	38.7(1)	138.6(0)	45.6(1)	37.8(1)	120.2(0)
12	169.5(0)	179.0(0)	169.3(0)	176.9(0)	177.9(0)	173.4(0)
13	117.2(2)	12.6(3)	117.6(2)	59.9(2)	12.9(3)	8.3(3)
14	18.6(3)	18.6(3)	18.6(3)	18.5(3)	18.6(3)	17.9(3)
15	22.7(3)	23.1(3)	19.0(3)	22.9(3)	22.9(3)	22.6(3)

[a]The number in parenthesis indicates the number of hydrogens attached to the corresponding carbon, and was determined from DEPT experiments.

[b]Assignments are based on ^{1}H-^{1}H and ^{1}H-^{13}C Shift Correlated 2D-nmr Spectroscopy.

[c]APT pulse sequence was used to confirm the multiplicities.

(1H, ddd, J=3.0, 8.0, 12.8 Hz, H-7), 2.73 (1H, dq, J=8.0, 8.0 Hz, H-11), 2.98 (1H, s, H-5); ^{13}C nmr (Table 2); ms *m/z* (rel. int.) 250 (M^+) (2.6); (anal. calcd for $C_{15}H_{22}O_3$: C, 71.97, H, 8.86; found: C, 71.92, H, 8.90).

MICROBIAL TRANSFORMATION OF ARTEANNUIN B [**3**] BY *BEAUVERIA BASSIANA*.—*Beauveria bassiana* (ATCC 7159) was grown in thirteen 1-liter Erlenmeyer flasks each containing 200 ml of medium. A total of 520 mg of arteannuin B [**3**] (in 5.2 ml EtOH) was evenly distributed among the 24-h-old stage-II cultures. After 5 days, the incubation mixtures were pooled, filtered to remove the cells (2.6 liters), then extracted three times with EtOAc (2.6 liters, 2×1.3 liters). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness (in vacuo) to afford a dark brown residue (0.596 g).

ISOLATION AND CHARACTERIZATION OF 3β-HYDROXYARTEANNUIN B [**5**].—The residue (0.596 g) was purified by column chromatography over a Si gel column (2.5×72 cm, 60 g) using Et_2O-hexane (4:1) as eluting system and 10-ml fractions were collected. Monitoring of fractions was done on silica plates developed in system A. Fractions 24-47, yielding a single spot with Rf=0.37, were pooled and evaporated to dryness to give 25 mg of pure **5** (5%). Crystallization from Et_2O/hexane gave colorless needles, mp 207-209°; $[\alpha]^{23}D$ −40° (c=0.4, $CHCl_3$), ir ($CHCl_3$) 3575, 3020, 1775, 1670, 1450, 1382, 1220, 1040, 1000 cm^{-1}; ^{1}H nmr 1.05 (3H, d, J=6.6 Hz, Me-14), 1.49 (3H, s, Me-15), 1.56 (1H, m, H-2), 2.02 (1H, m, H-2), 2.72 (1H, dddd, J=3.0, 3.1, 3.3, 12.0 Hz, H-7), 2.90 (1H, s, H-5), 3.87[2] (1H, m, H-3), 5.46 (1H, d, J=3.3 Hz, H-13b), 6.18 (1H, d, J=3.0 Hz, H-13a); ^{13}C nmr (Table 2); ms (high resolution): M^+ at *m/z* 264.1362, consistent with $C_{15}H_{20}O_4$ (calcd 264.1372).

ISOLATION AND CHARACTERIZATION OF 13-HYDROXY-11-*EPI*-DIHYDROARTEANNUIN B [**6**].—Fractions 64-150 from the above column yielded metabolite **6** as a single spot with Rf=0.24 (system A), (153 mg, 27%). Recrystallization from Et_2O/hexane afforded colorless crystals of **6**, mp 108-109°; $[\alpha]^{23}D$ −67.8° (c=0.5, $CHCl_3$), ir ($CHCl_3$) 3540, 2925, 1763, 1452, 1382, 1305, 1230, 1190, 1165, 1130, 995 cm^{-1}; ^{1}H nmr[3] 0.95 (3H, d, J=6.5 Hz, Me-14), 1.20 (1H, dddd, J=3.0, 4.5, 12.3, 12.9 Hz, H-9_{ax}), 1.34 (1H, br. m, H-1), 1.36 (3H, s, Me-15), 1.39-1.53 (2H, m, H-8, H-10), 1.54-1.71 (2H, m, H-2), 1.72-1.98 (4H, m, H-3, H-9_{eq}, H-3, H-8), 2.17 (1H, ddd, J=2.7, 13.3, 13.4 Hz, H-7), 2.58 (1H, br. t, exch., OH), 2.83 (1H, ddd, J=4.1, 5.2, 13.4 Hz, H-11), 2.85 (1H, s, H-5), 3.78 (1H, m, H-13b), 3.99 (1H, m, H-13a) ^{13}C nmr (Table 2); ms *m/z* (rel. int.) 266 (M^+) (1.59); (anal. calcd for $C_{15}H_{22}O_4$: C, 67.65, H, 8.33; found: C, 67.83, H, 8.36).

11-*EPI*-DIHYDROARTEANNUIN B [**7**].—This compound was prepared by $NaBH_4$ reduction of [**3**] as follows: Compound **3** (46 mg) was dissolved in 2 ml of absolute EtOH and stirred for 20 min with $NaBH_4$ (12 mg) at room temperature. The reaction mixture was neutralized with 10% HOAc, and the solvent was evaporated. The $CHCl_3$ solution of the residue was washed with H_2O then evaporated to dryness to afford 45 mg of **7**. An analytical sample of **7** was obtained by purification through a short Si gel column. Crystals of **7** (Et_2O/hexane) had mp, 180-181°; ir ($CHCl_3$) 3016, 2933, 2408, 1773, 1453, 1423, 1370, 1224, 1130, 1000 cm^{-1}; ^{1}H nmr 0.96 (3H, d, J=6.6 Hz, Me-14), 1.24 (3H, d, J=6.8 Hz, Me-13), 1.36 (3H, s, Me-15), 2.67 (1H, dq, J=6.8, 13.6 Hz, H-11), 2.82 (1H, s, H-5); ^{13}C nmr (Table 2); (anal. calcd for $C_{15}H_{22}O_3$: C, 71.97, H, 8.86; found: C, 72.14, H, 8.89).

CATALYTIC REDUCTION OF ARTEANNUIN B [**3**].—To 30 mg of 10% Pd-C in EtOH (5 ml), previously saturated with hydrogen, was added 200 mg of **3** in EtOH (10 ml), and the mixture was allowed to hydrogenate with stirring for 2 h at room temperature. The solution was filtered and evaporated to give a solid residue (184 mg, 92% yield). Tlc analysis of the residue, using hexane-EtOAc (7:3) as developing system, indicated a mixture of 3 spots (Rf 0.38, 0.32, 0.28; Rf of **3**=0.40 using this system). This mixture was further purified on a Si gel column using hexane-EtOAc (7:3) as eluting system to give 3 fractions. Fractions 1 (67 mg) and 2 (41 mg) were found to be identical to **7** and **4**, respectively (mp, tlc, and 300 MHz ^{1}H nmr). Crystallization (Et_2O/hexane) of fraction 3 (24 mg) afforded the endocyclic isomer of arteannuin B [**8**] as pure, colorless needles, mp 97-98°. Ir ($CHCl_3$) 3010, 2960, 2330, 1735, 1680, 1450, 1380, 1350, 1210, 1150, 1010 cm^{-1}; ^{1}H-nmr 0.92 (3H, d, J=6.6 Hz, Me-14), 1.02-1.07 (2H, m, H-1, H-9), 1.36 (3H, s, Me-15), 1.48-1.60 (2H, m, H-10, H-2), 1.67-1.80 (2H, m, H-3_{ax}, H-2), 1.81 (3H, s, Me-13), 1.86-2.00 (2H, m, H-3, H-9), 2.10 (1H, m, H-8), 2.70 (1H, s, H-5), 2.80 (1H, ddd, J=2.7, 4.0, 13.9 Hz, H-8_{eq}); ^{13}C nmr (Table 2); ms *m/z* (rel. int.) 248 (M^+) (0.3); (anal. calcd for $C_{15}H_{20}O_3$: C, 72.55, H, 8.12; found: C, 72.38, H, 8.23).

[2]By adding D_2O, this signal simplified into a simple dd (6.5, 9.0 Hz).

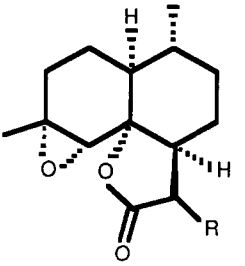
[3]By adding D_2O, the signal at 2.58 disappeared; the signals at 3.78 and 3.99, each simplified into a simple dd, with J=5.2, 11.6 and 4.1, 11.6, respectively.

RESULTS AND DISCUSSION

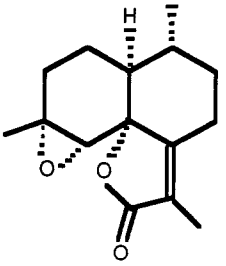
The microbial transformation products of 30 cultures (Table 1), were initially examined by tlc for their ability to biotransform **3**. Among several other cultures, *A. flavipes* (ATCC 1030) and *B. bassiana* (ATCC 7159) were found efficiently to convert **3** to more polar metabolites. Preparative-scale incubation of **3** with *A. flavipes* gave a fermentation product which was found to consist of one major metabolite identified as dihydroarteannuin B [**4**]. The elemental analysis and a display of a molecular ion peak at *m/z* 250 indicated a molecular formula of $C_{15}H_{22}O_3$ for metabolite **4**. In the ^{1}H-nmr spectrum a three proton doublet at δ 1.34 was observed while the characteristic pair of low field doublets (H-13) were missing. The absence of the olefinic signals (C-11 and C-13) and the presence of an additional Me-group at δ 12.6 in the ^{13}C nmr of **4** (Table 2) strongly suggested that the metabolite was dihydroarteannuin B. The stereochemistry at C-11 was deduced from the observed splitting pattern of H-11 and the value of the coupling constant between H-11 and H-7. H-11 appears as a dq (J=8.0, 8.0 Hz) indicating equal coupling to both H-7 and the C-11 methyl, which requires a pseudo-equatorial configuration for H-11 (α) (21). These connectivities of H-11 were confirmed in the COSY spectrum of **4**. The remaining ^{1}H- and ^{13}C-nmr assignments were established by comparison with **3** whose ^{13}C-nmr assignments have been fully established.

Reduction of **3** with $NaBH_4$ provided, as expected (22), the C-11-methyl epimer [**7**]. Full ^{1}H-nmr analysis of **7** established unambiguously the epimeric relationship (at C-11) between compounds **4** and **7**. In the case of **7**, H-11 was found as a dq (J=7.0, 13.5 Hz), which is consistent with a pseudo-axial configuration of H-11 (β). The J values and the distinctive coupling patterns of the H-11 have been used previously to determine the configuration of the C-11-methyl in similar systems (21,23).

Catalytic hydrogenation of **3** resulted in the production of a mixture of dihydroderivatives, one of which was identical to **7** and the other identical to the metabolite **4**. In addition, a minor product **8** was also produced. Compound **8** had a molecular ion peak at *m/z* 248, which indicates it to be isomeric with **3**. Comparison of ^{13}C nmr of **8** with that of **3** (Table 2) indicated that the exocyclic methylene in **3** was converted into a vinyl methyl group (The DEPT experiment established that the two olefinic signals, 120.2 and 163.3, were quaternary and that the new signal at 8.3 was a methyl group). The ^{1}H-nmr data were also consistent with the structure shown for **8**. Numerous examples, in which the migration of the double bond has occurred during catalytic reductions, have been reported (24-26).



4 R=β-Me
6 R=α-CH_2OH
7 R=α-Me



8

The second organism chosen for preparative-scale study, *B. bassiana*, was capable of converting **3** into two metabolites [**5**, **6**]. The ms spectrum of metabolite **5** was 16 mass units higher than that of **3**. Hrms data were consistent with a molecular formula of

$C_{15}H_{20}O_4$, suggesting that metabolite **5** was a monohydroxylated derivative of **3**. An exchangeable signal at 1.57 in the ^{1}H nmr as well as a band at 3575 cm^{-1} in the ir spectra of **5** confirmed the presence of a hydroxyl substituent. Since all the ^{13}C-nmr assignments of **3** were made, the position of the hydroxyl group in **5** was established from the ^{13}C-nmr data. As shown in Table 2, the resonance at 24.5 (t) for C-3 of **3** disappeared and was replaced by a new doublet at 68.0 ppm which suggested that the hydroxyl group was at C-3. This was substantiated by the observed downfield shifts (β-effect) for both carbons 2 and 4. Cross peaks establishing the connectivity between H-3 and both protons at position 2 in the COSY spectrum of **5** confirmed unambiguously the assignment of the hydroxyl group to position 3. The stereochemistry of the hydroxyl group at C-3 was assigned to the β-configuration based on the observed J values for H-3 (dd, J=6.5, 9.0 Hz) which is consistent with an equatorial (α) H-3.

Metabolite **6** had a molecular formula of $C_{15}H_{22}O_4$ (elemental analysis and ms). Comparison of the 300 MHz ^{1}H nmr of **6** with that of **3** (see Experimental section) again revealed the absence of the characteristic exocyclic protons at C-13 from the metabolite spectrum. Other salient features included the presence of an exchangeable signal (D_2O) at 2.58 and two geminally-coupled methylene protons at 3.78 and 3.99, each of which appeared as a double doublet. The ir spectrum confirmed the presence of a hydroxyl group (3540 cm^{-1}) and a saturated γ-lactone (1763 cm^{-1}). These data suggested a hydroxylated dihydroderivative of **3**. The ^{13}C-nmr data of **6** were similar to that of **3**, with the exception of the olefinic signals (C-11 and 13) in **3**, which were replaced by two new signals, a doublet at 45.6 and a triplet at 59.9. The conclusion that hydroxylation occurred at C-13 was further supported by the fact that, except for C-7, which underwent an upfield shift (γ-interaction), all the other carbon resonances remained essentially unchanged when compared with **3**. The stereochemistry of the hydroxymethyl side chain at C-11 was determined to be α based on a similar analysis as discussed with **7**. H-11 represents an X-part of an ABX system, the AB-part being the methylene protons at position-13 (3.78, 3.99). It is coupled to both H-13 protons (4.1 and 5.2 Hz) and further split by H-7 with a coupling constant of 13.4 Hz. The large H-7, H-11 coupling value would require H-11 to be pseudo-axial (β). These observations were all confirmed by examination of the COSY spectrum of **6**.

LITERATURE CITED

1. D.L. Xuan and C.S. Chia, *Med. Res. Rev.*, **7**, 29 (1987).
2. D.L. Klayman, *Science*, **228**, 1049 (1985).
3. D. Jeremic, A. Jokic, A. Behbud, and M. Stefanovic, *Tetrahedron Lett.*, 3039 (1973).
4. M.R. Uskokovic, T.H. Williams, and J.F. Blount, *Helv. Chim. Acta*, **57**, 600 (1974).
5. D.G. Leppard, M. Rey, and A.S. Dreiding, *Helv. Chim. Acta*, **57**, 602 (1974).
6. X. Xu, Z. Jie, and C. Weishan, *Kexue tongbao*, **28**, 859 (1983).
7. O. Goldberg, I. Deja, M. Rey, and A.S. Dreiding, *Helv. Chim. Acta*, **63**, 2455 (1980).
8. A.M. Clark and C.D. Hufford, *J. Chem. Soc., Perkin I*, 3022 (1979).
9. H. Hikino, Y. Tokuoka, Y. Hikino, and T. Takemoto, *Tetrahedron*, **24**, 3147 (1968).
10. H. Hikino, Y. Tokuoka, and T. Takemoto, *Chem. Pharm. Bull.*, (Tokyo), **16**, 1088 (1968).
11. K. Kieslisch, "Microbial Transformations of Non-steroid Cyclic Compounds," Wiley-Georg Thieme, Stuttgart, 1976.
12. J.P. Rosazza, in: "Anticancer Agents based on Natural Product Models," Ed. by J. Cassady, and J. Douros, vol. 16, Academic Press, New York, 1980, p. 437.
13. A. Garcia-Granados, A. Martinez, M.E. Onorato, and J.M. Arias, *J. Nat. Prod.*, **49**, 126 (1986).
14. F.S. El-Feraly, I.A. Al-Meshal, M.A. Al-Yahya, and M.S. Hifnawy, *Phytochemistry*, **25**, 2778 (1986).
15. A. Bax, R. Freeman, and G.A. Morris, *J. Magn. Reson.*, **42**, 169 (1981).
16. A. Bax, *J. Magn. Reson.*, **53**, 512 (1983).
17. O.W. Sorensen, S. Donstrup, H. Bildsoe, and J.H. Jakobsen, *J. Magn. Reson.*, **55**, 347 (1983).
18. S.L. Patt and J. Shoolery, *J. Magn. Reson.*, **46**, 535 (1982).
19. F.S. El-Feraly and C.D. Hufford, *J. Org. Chem.*, **47**, 1527 (1982).

20. S.A. Elmarakby, A.M. Clark, J.K. Baker, and C.D. Hufford, *J. Pharm. Sci.*, **75**, 614 (1986).
21. R.W. Doskotch, C.D. Hufford, and F.S. El-Feraly, *J. Org. Chem.*, **37**, 2740 (1972).
22. N.H. Fischer, E.J. Olivier, and H.D. Fischer, *Fortschr. Chem. Org. Naturst.*, **38**, 48 (1979).
23. C.R. Narayanan and N.K. Venkatasubramanian, *J. Org. Chem.*, **33**, 3156 (1968).
24. R.W. Doskotch and C.D. Hufford, *J. Org. Chem.*, **35**, 486 (1970).
25. R.W. Doskotch and F.S. El-Feraly, *J. Org. Chem.*, **35**, 1928 (1970).
26. W. Herz, H. Watanabe, M. Miyazaki, and Y. Kishida, *J. Am. Chem. Soc.*, **84**, 2601 (1962).

Received 16 March 1987