Biotransformation of 2α,5α,10β,14β-Tetraacetoxy-4(20),11-taxadiene by the Fungi *Cunninghamella elegans* and *Cunninghamella echinulata*

Shanghui Hu,* Xufang Tian, Weihua Zhu, and Qicheng Fang

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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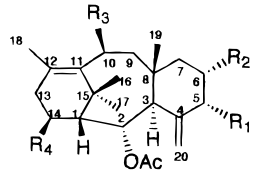
The biotransformations of the taxane diterpene 2α,5α,10β,14β-tetraacetoxy-4(20),11-taxadiene (**1**) by the fungi *Cunninghamella elegans* AS 3·2033 and *Cunninghamella echinulata* AS 3·1990 were investigated. Incubation of compound **1** with *C. elegans* afforded three new hydroxylated derivatives: 5α,10β-dihydroxy-2α,6α,14β-triacetoxy-4(20),11-taxadiene (**2**), 6α-hydroxy-2α,5α,10β,14β-tetraacetoxy-4(20), 11-taxadiene (**3**), and 5α,6α,10β-diacetoxy-4(20),-11-taxadiene (**4**). The two new taxane metabolites, 5α,10β,14β-trihydroxy-2α-acetoxy-4(20),11-taxadiene (**5**) and 5α,6α,10β,14β-tetrahydroxy-2α-acetoxy-4(20),11-taxadiene (**6**), were isolated from the incubation supernatant of *C. echinulata* with compound **1**.

The diterpenoid Taxol[1] is an important new drug in cancer chemotherapy.[2,3] The biotransformation of taxol is an important area of study because a knowledge of its mammalian metabolism is an essential feature of its clinical pharmacology. The metabolism of taxol in rats and humans has been reported.[4−7] In humans, the metabolites of taxol are different from those in rats, and 6α-hydroxytaxol is the major human metabolite of Taxol. Furthermore, the biotransformation of Taxol and other taxoids offers potential pathways to the preparation of chemically inaccessible metabolites. Few studies of the metabolism of Taxol and other taxoids by microbial and other biological systems however, have been reported so far; the first example is site-specific enzymatic hydrolysis of taxanes at C-10 and C-13.[8]

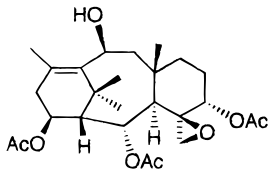
Recently, a series of new taxoids with an oxygen substituent at C-14 was isolated from the stem bark and roots of *Taxus yunnanenis*, as well as from cell cultures of *Taxus chinensis* (Pilger) Rehd var. *mairei*.[9−11] In previous work, we isolated the taxane diterpene 2α,5α,-10β,14β-tetraacetoxy-4(20),11-taxadiene (**1**) from cell cultures of *T. yunnanensis* in high amount (ca. 1−2% dry wt).[10] We reported that the fungus *Cunninghamella echinulata* could transform **1** to give 6α,10β-dihydroxy-2α,5α,14β-triacetoxy-4(20),11-taxadiene (**7**) as a main product (in 33% yield), together with three minor products when the culture pH was maintained at 6.0 during the incubation.[12] In the course of our continuing search for new potent anticancer agents with a taxane skeleton, we have examined the biotransformation of **1** by *Cunninghamella elegans* and the effect of the pH of the culture on the transformation of **1** by *C. echinulata*.

Results and Discussion

Compound **1** was actively metabolized by *C. elegans* under the incubation conditions. After 9 days' incubation, three new products were isolated and identified as 5α,10β-dihydroxy-2α,6α,14β-triacetoxy-4(20),11-taxadiene (**2**), 6α-hydroxy-2α,5α,10β,14β-tetraacetoxy-4(20),-11-taxadiene (**3**), and 5α,6α,10β-trihydroxy-2α,14β-diacetoxy-4(20),11-taxadiene (**4**), respectively. Three other products-the triacetate **7**, 10β-hydroxy-2α,5α,14β-triacetoxy-4(20),11-taxadiene (**8**), and 10β-hydroxy−4β,-20-epoxy-2α,5α,14β-triacetoxy-11-taxene (**9**), previously obtained from incubation of **1** with *C. echinulata*[11]-were also measured.



1 $R_1 = R_3 = R_4 = OAc$, $R_2 = H$;
2 $R_1 = R_3 = OH$, $R_2 = R_4 = OAc$;
3 $R_1 = R_3 = R_4 = OAc$, $R_2 = OH$;
4 $R_1 = R_2 = R_3 = OH$, $R_4 = OAc$;
5 $R_1 = R_3 = R_4 = OH$, $R_2 = H$;
6 $R_1 = R_2 = R_3 = R_4 = OH$;
7 $R_1 = R_4 = OAc$, $R_2 = R_3 = OH$;
8 $R_1 = R_4 = OAc$, $R_2 = H$, $R_3 = OH$



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FABMS of **2** showed a protonated molecular ion at *m/z* 479 corresponding to the molecular formula $C_{26}H_{38}O_8$, the same as **7**. The ^{1}H-NMR spectrum of **2** was similar to that of **7**. The C-10 proton signal was at δ 5.08 (dd, $J = 5.7$, 11.7 Hz), suggesting that a hydroxyl group rather than an OAc group was present at C-10. The H-5β resonance was at δ 3.98 (d, $J = 2.0$ Hz), strongly suggesting the presence of a free hydroxyl group at C-5 in **2**. The H-6α or -6β multiplet ($J = 2.9$ Hz) at δ 1.80 in **1** was replaced by a 1H multiplet at δ 4.85 (br t, $J = 2.1$ Hz), suggesting an acetoxy group at C-6. These proposed locations were supported by ^{1}H-^{1}H COSY NMR spectra. The highfield H-5β doublet at δ 3.98 was coupled with the H-6 multiplet at δ 4.85, which in turn was coupled with the 1H doublet of doublets at 2.15 ppm assigned to H-7; the H-7 is geminally coupled. The structure of **2** was also supported by ^{13}C-NMR signals at δ 70.6 (C-2), 77.7 (C-5), 73.8 (C-6), 67.5 (C-10), and 70.8 (C-14) and by direct comparison with the ^{13}C-NMR spectrum of **7**, whose

* To whom correspondence should be addressed. Phone: 086 010 63013366-468. Fax: 086 010 63017757.
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Table 1. ^{1}H-NMR Data of Compounds **2**–**6** ($CDCl_3$, 500 MHz)

carbon	2	3	4	5	6
1	1.86 (d, 2.2)	1.93 (d, 2.3)	1.87 (d, 2.3)	1.72 (d, 1.7)	1.74 (d, 1.7)
2	5.37 (dd, 6.3, 2.4)	5.36 (dd, 6.6, 2.3)	5.36 (dd, 6.4, 2.5)	5.40 (dd, 6.1, 1.9)	5.44 (dd, 2.1, 6.0)
3	3.25 (d, 6.4)	2.95 (d, 6.4)	3.29 (d, 6.3)	3.16 (d, 5.9)	3.32 (d, 5.9)
5	3.98 (d, 2.0)	5.05 (br s)	3.95 (d, 3.1)	4.17 (br t)	3.89 (d, 1.9)
6	4.85 (br t, 2.1)	3.91 (br t, 2.0)	3.88 (br s)	1.72 (m), 1.25 (m)	3.84 (m)
7	1.40 (d, 15.1), 2.15 (dd, 4.2, 15.1)	1.50 (d, 14.9), 2.03 (m)	1.35 (d, 14.8), 2.12 (dd, 4.2, 14.8)	1.08 (m), 2.03 (m)	1.33 (m), 2.16 (m)
9	1.62 (dd, 15.0, 5.6), 2.21 (dd, 11.7, 14.9)	1.69 (m), 2.28 (dd, 12.2, 14.9)	1.60 (dd, 14.9, 5.5), 2.19 (dd, 11.6, 14.4)	1.62 (dd, 14.8, 5.6), 2.29 (dd, 11.9, 14.7)	1.59 (dd, 14.9, 5.6), 2.18 (dd, 11.7, 14.9)
10	5.08 (dd, 5.7, 11.7)	6.01 (dd, 5.7, 12.0)	5.08 (dd, 5.7, 11.6)	5.13 (dd, 5.6, 11.7)	5.09 (dd, 5.6, 11.7)
13	2.35 (dd, 3.6, 17.6), 2.75 (dd, 18.9, 9.3)	2.43 (dd, 4.5, 19.0), 2.82 (dd, 19.0, 9.2)	2.35 (dd, 3.8, 18.6), 2.75 (dd, 18.8, 9.3)	2.43 (dd, 4.7, 18.2), 2.64 (dd, 18.4, 9.1)	2.19 (dd, 4.9, 18.4), 2.68 (dd, 17.5, 8.4)
14	5.04 (dd, 9.3, 4.7)	5.01 (dd, 9.2, 5.0)	5.05 (dd, 9.2, 4.8)	4.08 (dd, 9.0, 5.2)	4.18 (dd, 9.1, 5.1)
16	1.70 (s)	1.66 (s)	1.71 (s)	1.70 (s)	1.69 (s)
17	1.17 (s)	1.13 (s)	1.18 (s)	1.22 (s)	1.25 (s)
18	2.05 (s)	2.20 (s)	2.05 (s)	2.07 (s)	2.11 (s)
19	0.95 (s)	1.05 (s)	1.02 (s)	0.80 (s)	1.02 (s)
20	5.17 (br s), 4.92 (br s)	5.42 (s), 4.97 (br s)	5.26 (s), 4.93 (d, 5.2)	5.09 (s), 4.77 (s)	5.21 (br s), 4.92 (br s)
2-OAc	2.02 (s)	2.05 (s)	2.03 (s)	1.98 (s)	1.98 (s)
5-OAc		2.05 (s)			
6-OAc	1.99 (s)				
10-OAc		2.05 (s)			
14-OAc	1.93 (s)	2.05 (s)	1.94 (s)		

assignments were determined by HETCOR NMR.[12] The HMBC spectrum of **2** exhibited a three-bond correlation between the C-19 methyl signal at δ 0.95 and the carbon signal at δ 39.15 (C-3). This same proton signal also exhibited a two-bond coupling with the carbon signal at δ 39.69, which was therefore assigned to the quaternary carbon at C-8. The carbon signal at δ 37.52 was assigned to the quaternary carbon C-15 due to its two three-bond correlations with the proton resonances at 5.08 (H-10α) and 5.04 (H-14α). The stereochemistry of the 6-acetoxy group in **2** was deduced to be α, on the basis of a ^{1}H-NMR coupling pattern analogus to that of **5**.[12]

FABMS of **3** showed $[MH]^+$ at m/z 521, consistent with the molecular formula $C_{28}H_{40}O_9$, which was 16 atomic mass units greater than that of **1**. These data indicated that **3** is a monohydroxylated derivative of **1**. The ^{1}H-NMR spectrum was also close to that of **1** and **2** and showed the presence of four acetyl groups. The disappearance of the signals for H-6α and H-6β at δ 1.80 and the appearance their place of a broad 1H triplet at δ 3.91 (br t, J = 2.0 Hz) revealed that a hydroxyl group had been introduced at C-6, and this conclusion was supported by the appearance of a carbon resonance at δ 70.0 in the ^{13}C-NMR spectra of **3**. Assignments of the ^{1}H- and ^{13}C-NMR spectra of compound **3** were established by direct comparison with those of **1**, **2**, and **7**.

FABMS of **4** showed $[MH]^+$ at m/z 437, consistent with the molecular formula $C_{24}H_{36}O_7$. The ^{1}H- and ^{13}C-NMR spectra of **4** showed only two acetyl resonances. A free hydroxyl group was present at C-10 (δ 5.08) instead of the acetoxyl group observed in **1**. As in **3** and **5**, a broad 1H triplet at δ 3.88 appeared and was assigned to CHOH at C-6. Furthermore, the ^{1}H-NMR spectrum showed that H-5β at δ 3.95 was shifted upfield by 1.34 ppm from the corresponding signal of **1**, indicating the presence of a free hydroxyl group at C-5. All these proposed locations of the protons in the ^{1}H-NMR spectra of **4** were supported by carbon resonances at δ 80.67 (C-5), 72.29 (C-6), and 67.56 (C-10) in direct comparison with those of **2** and **7**. The configuration of the 6-hydroxyl group in **4** was determined as α by comparing the coupling pattern of H-6 with that of **2** and **7**.

Because compounds **2** and **7** are isomeric acetates of a 1,2-diol, the question arises as to whether one of them is a true metabolite and the other the product of a chemical isomerization. To test this hypothesis, the isomerization of **7** into **2** was studied under the conditions of cell culture but without the cells, and it was found that isomerization did indeed occur to give a mixture of 41% **2** and 58% **7**. This ratio differs significantly from that found with biotransformation of **1** (14% **2** to 86% **7**), and this fact suggests that there may be an enzymatic component to the production of **2**. Alternatively, **7** is the only true enzymatic product and is incompletely isomerized to **2** under the experimental conditions.

These results indicate that the biotransformation of **1** by *C. elegans* produces a similar pattern of metabolites to that of *C. Echinulata*. The production of 6α-hydroxylated metabolites was the usual result. Unfortunately, the 6α-hydroxylated derivatives **2**, **3**, and **4** exhibited poor cytotoxicities, presumably due to the absence of the oxetane ring and C-13 side chain of taxol.[3]

In the second part of our study, we investigated the effect of the pH of the culture medium on the biotransformation of **1** by *C. echinulata*. When pH was uncontrolled with HCl, it usually rose from an initial value of 5.5 to 8.0 after 11 days of incubation with **1**. Simultaneously, the yield of **7** was reduced drastically (from 33.0% to 8.5%), while another product was afforded in large amount (21.4% yield), together with two minor products that are different from those observed at pH 6.0.

Seven metabolites of **1** were isolated from incubation of **1** for 11 days with *C. echinulata* under conditions without pH control. Among them, the four compounds **7, 8, 9**, and the 6β-hydroxy epimer of **7**(**10**) were identical to those previously isolated at pH 6.0. The remaining two products were two new metabolites, identified as 5α,10β,14β-trihydroxy-2α-acetoxy-4(20),-11-taxadiene (**5**) and 5α,6α,10β,14β-tetrahydroxy-2α-acetoxy-4(20),11-taxadiene (**6**), respectively.

The ^{1}H-NMR spectrum of **5** was similar to those of **1** and **8** but indicated only one acetyl group. The ^{1}H-NMR spectrum of **5** was similar to those of **1** and **8** but indicated only one acetyl group. The 1H proton at δ 5.40 (dd, J = 6.1, 1.9 Hz) suggested that this acetyl group was at C-2, which was confirmed by the ^{13}C-NMR resonance at δ 71.8 attributed to C-2. The two doublets of doublets at δ 5.13 and 4.08 were assigned to H-10α and H-14α, respectively, which indicated two hydroxyl groups attached to C-10 and C-14. In the ^{1}H−^{1}H COSY spectra, the broad triplet at δ 4.17 assigned to H-5β was coupled with the H-6 multiplet at δ 1.70. The latter signal was also correlated with the 1H multiplet at δ 2.03 assigned to H-7. All complete assignments of ^{1}H-NMR spectra data of **5** were unambiguously determined by analysis of ^{1}H−^{1}H COSY spectra of **5**. The assignments of the ^{13}C-NMR spectrum of **5** were established based upon a DEPT experiment and by direct comparison with those of **3**, which supported the structure elucidation of **5**. In addition, chemical methanolysis of **3** with K_2CO_3 also afforded **5**.

The structure of **6** was closely related to **1** and **5** based on ^{1}H-NMR spectral data. Furthermore, the ^{1}H-NMR spectrum of **6** indicated the presence of an extra secondary hydroxyl group in **6** in comparison with **1** and **5**, due to the appearance of the multiplet at δ 3.84 assigned to the proton at C-6. The two 1H doublets of doublets at δ 5.09 and 4.18, assigned to H-10β and H-14β, respectively, suggested the presence of two free hydroxyl groups at C-10 and C-14. The 1H doublet of doublets at δ 5.44 indicated the presence of an acetyl group at C-2. A hydroxyl group at C-5 was shown by the broad doublet at δ 3.89. The assignments of these resonances were supported by ^{1}H−^{1}H COSY spectra. All complete assignments of ^{13}C-NMR spectra of **6** were achieved by a DEPT experiment and by comparing ^{13}C-NMR spectral data with those of **1**, **2**, **5**, and **6**. These data further confirmed the proposed structure assignment of **6**. The configuration of the hydroxyl group at C-6 was concluded to be α, on the basis of an observed ^{1}H-NMR coupling pattern of H-6 similar to that found in **7** and **10**.

The fact that the new metabolites **5** and **6** observed at pH 8.0 are simple hydrolysis products of **1** and **7**, respectively, raised the question of whether these compounds are true metabolites. To answer this question we incubated **1** and **7** with culture medium at pH 8.0 but without cells for the same time as the original incubation. Neither hydrolysis of **1** to **5** nor that of **7** to **6** was observed. It is thus concluded that the conversion of **1** to **5** and **6** is a true enzymatic process and that the higher pH conditions favor the action of the hydrolyses in *C. echinulata*. The maintenance of the pH around 6.0 is clearly necessary for the efficient production of 6α-hydroxy metabolites of **1** by *C. echinulata*.

Experimental Section

General Experimental Conditions. Mps were determined with Yanaco apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were obtained on a Perkin-Elmer 683 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with Brucker AM-500 spectrometer (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz) in CD_3OD with TMS as the internal standard. FABMS were performed with JMS-DX 300 MS mass spectrometer. CIMS, were performed with VG ZAB-2f MS mass spectrometer. Macroporous styrene resin DA-201 was purchased from the Agriculture Chemicals Factory of Tianjing, China. Si gel F_{254} and Si gel (200−300 mesh) were obtained from Qingdao Marine Chemical Factory, Qingdao, China.

Substrate. 2α,5α,10β,14β-Tetraacetoxy-4(20),11-taxadiene (**1**) was isolated from cell cultures of *T. yunnanensis* in ca. 1−2% dry wt. Its structure was fully characterized by spectral methods.[10,11]

Microorganisms and Biotransformation Procedure. *C. elegans* AS 3·2033 and *C. echinulata* AS 3·1990 were purchased from the Institute of Microbiology, the Chinese Academy of Sciences (AS), Beijing, and maintained on potato-agar slant and stored in a refrigerator at 4 °C.

Biotransformation experiments were carried out as follows: *C. elegans* AS 3·2033 and *C. echinulata* AS 3·1990 were cultured in five 5-L Erlenmeyer flasks each containing 1 L of the liquid medium (g/L: glucose, 30; cornsteep liquor, 10; K_2HPO_4, 2; KH_2PO_4, 1; $NaNO_3$, 2; KCl, 0.5; $MgSO_4$·7 H_2O, 0.5; $FeSO_4$·7 H_2O, 0.02.) at 27 °C on a reciprocal shaker (80 rpm). After 48 h of cultivation, 160 mg of **1** in 10 mL of Me_2CO (containing 50 μl/mL Tween 80) was added to each flask, and the incubation was continued for 9 and 11 additional days, respectively. During the incubation, the pH values were measured at regular intervals.

At this time the culture broth from *C. elegans* was filtered, and the filtrate was passed over a macroporous resin column that was washed with aqueous EtOH. The combined EtOH fractions containing the metabolites of **1** were evaporated to afford a crude residue (2.0 g). After extraction with EtOAc and evaporation of the solvent, the crude extract (1.5 g) was separated by Si gel chromatography. After elution with petroleum ether−Me_2CO (a gradient from 9:1 to 1:1), the products were obtained as follows: residual substrate **1** (12 mg, 1.5%), **8** (77 mg, 10.5%), **9** (20 mg, 2.6%), **3** (75 mg, 9.1%), **2** (9 mg, 1.2%), and **7** (55 mg, 7.2%). The fraction consisting of a mixture of **4** and several other products was separated by repeated preparative TLC, eluting with CH_2Cl_2−Me_2CO (100:1 to 10:1) to afford 9 mg of pure **4** (1.3%). In addition, 250 mg of unchanged substrate (31.3%) was also isolated from the mycelium of *C. elegans*.

After the usual treatment for the culture broth from *C. echinulata*, the crude extract (1.0 g) was separated by Si gel chromatography. After elution with petroleum ether−Me_2CO−EtOAc (a gradient from 90:8:2 to 35:63:2), the products were obtained as follows: fractions 1−4 contained the known compounds **1** (49 mg, 6.1%), **8** (80 mg, 10.9%), **9** (35 mg, 4.6%), and **7** (42 mg, 5.5%), respectively. Fraction 5 afforded a mixture of **7** and **5** (164 mg), which was further purified by flash chromatography using a mixture of CH_2Cl_2−Me_2CO−MeOH (95:2.5:2.5, 85:7.5:7.5) to yield pure **7** (23 mg, 3.0%) and pure **5** (123 mg, 18.4%). Fraction 6 was composed of a mixture of **5** and **10** that was further purified by flash chromatography using CH_2Cl_2−Me_2CO (100:1 to 10:1) to afford pure **5** (20 mg, 3.0%) and **10** (20 mg, 2.6%). Fraction 9 contained **4** as the main product, which was also purified as for fraction 6. Finally, fraction 10

Table 2. ^{13}C NMR Data of Compounds **2**−**6** ($CDCl_3$, 125 MHz)

carbon	2	3	4	5	6
1	59.07	58.84	59.07	63.51	63.98
2	70.63	70.24	70.60	71.75	73.07[c]
3	39.15	41.65	40.91	39.92	40.62
4	143.56	138.50	144.06	148.20	146.08
5	77.66	81.69	80.67	76.50	81.40
6	73.81	70.01	72.29	31.17	73.47[c]
7	39.23	41.37	40.03	33.11	41.56
8	39.67	37.36	39.58	40.04	40.91
9	47.10	44.35	47.44	47.06	48.65
10	67.52	70.07	67.56	67.58	68.08
11	137.88	135.57	137.98	137.86	139.10
12	133.66	134.63	133.64	134.20	134.96
13	37.96	39.45	39.29	42.27	42.66
14	70.75	70.53	70.81	68.00	68.32
15	37.52	39.51	37.54	37.96	39.04
16	25.37	25.43	25.38	25.70	26.32
17	32.17	31.73	32.22	31.94	32.45
18	20.85	21.56	20.90	21.10	21.23
19	24.56	25.30	25.38	22.23	25.92
20	117.09	120.47	117.20	113.30	116.62
2-OAc	170.01, 21.40	170.16, 21.40	169.86, 21.42[b]	169.74, 21.58	171.91, 21.55
5-OAc		169.97, 20.92			
6-OAc	170.23, 21.05[a]				
10-OAc		169.45, 21.40			
14-OAC	169.88, 21.35[a]	169.97, 21.36	169.86, 21.47[b]		

[a−c] Interchangeable assignments.

afforded pure **6** (20 mg, 3.1%), and fraction 11 afforded a mixture of **6** and one unidentified product.

6α,10β-Dihydroxy-2α,5α,14β-triacetoxy-taxadiene (2): white amorphous solid; IR (dry film) ν max 3447, 2922, 1738, 1638, 1383, 1096 cm^{-1}; FABMS *m/z* $[MH + glycerol]^+$ 571 (19), $[MH]^+$ 479 (3), 461 (2), 419 (8), 401 (100), 341 (11); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2.

6α-Hydroxy-2α,5α,10β,14β-tetraacetoxy-taxadiene (3): white amorphous solid, mp 208−210 °C; $[\alpha]^{25}_D$ +22.3 (*c* 0.166, MeOH); IR (dry film) ν max 3439, 2916, 1736, 1715, 1371, 1020 cm^{-1}; FABMS *m/z* $[MH]^+$ 521 (21), 503 (30), 460 (42), 401 (100); 341 (43), 281 (30); CIMS *m/z* $[MH - HOAc]^+$ 461 (8), 401 (45), 383 (4), 341 (100), 281 (100); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2.

5α,6α,10β-Trihydroxy-2α,14β-diacetoxy-taxadiene (4): white amorphous solid, mp 96−98 °C; $[\alpha]^{25}_D$ +31.7° (*c* 0.069, MeOH); IR (dry film) ν max 3474, 2926, 1713, 1653, 1267, 1026 cm^{-1}; FABMS *m/z* $[MH + glycerol]^+$ 529 (10), $[MH]^+$ 437 (1), $[MH - H_2O]^+$ 419 (12), $[MH - H_2O - HOAc]^+$ 359 (100), 299 (30); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2.

5α,10β,14β-Trihydroxy-2α-4(20),11-taxadiene (5): white amorphous solid, mp 116−118 °C; $[\alpha]^{22}_D$ +53.5° (*c* 0.331, MeOH); IR (dry film) ν max 3427, 2922, 1701, 1641, 1381, 1277, 1024 cm^{-1}; CIMS *m/z* $[MH - H_2O - HOAc]^+$ 301 (12), 283 (40), 265 (12), 41 (100); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2.

5α,6α,10β-Tetrahydroxy-2α-acetoxy-4(20),11-taxadiene (6): IR (dry film) ν max 3447, 2922, 1701, 1630, 1466 cm^{-1}; CIMS *m/z* $[MH]^+$ 395 (4), $[MH - 2 \times H_2O]^+$ 359 (2), $[MH - 3 \times H_2O]^+$ 341 (2), 299 (16), 281 (10), 257 (50), 41 (100); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2.

Chemical Conversion of 7 to 2. Compound **7** (16 mg) in 1 mL of Me_2CO was added to 100 mL of culture medium at pH 8.0 but without the cells, and the incubation was conducted as unusual for 9 days. After the usual treatment, the crude extract was separated over a Si gel column and eluted with petroleum ether−Me_2CO (8:2 and 7:3) to afford **2** (6.5 mg, 41%) and residual **7** (9.2 mg, 58%).

Chemical Hydrolysis of 1. Compound **1** (8 mg) in 0.5 mL of Me_2CO was added to 50 mL of culture medium at pH 8.0 but without the cells, and the incubation was conducted as usual for 11 days. No hydrolysis to **5** was observed.

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References and Notes

(1) The name Taxol was a common usage until recently, when it was trademarked by Bristol-Myers Squibb. Accordingly, each time the word Taxol is used in this manuscript, the trademark rights of Bristol-Myers Squibb are acknowledged.
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