Taxoids and Abietanes from Callus Cultures of *Taxus cuspidata*

Jiao Bai,[†] Nobuhide Ito,[‡] Junichi Sakai,[‡] Mutumi Kitabatake,[†] Hajime Fujisawa,[†] Liming Bai,[†] Jungui Dai,[‡] Shujun Zhang,[†] Katutoshi Hirose,[§] Akihiro Tomida,[⊥] Takashi Tsuruo,[⊥] and Masayoshi Ando*,[‡]

Graduate School of Science and Technology and Department of Chemistry and Chemical Engineering, Niigata University, Ikarashi, 2-8050, Niigata, 950-2181, Japan, Kobe Natural Products and Chemicals Co., Ltd., 527 Nishikawanabe Ichikawa-Cho Kanzaki-Gun Hyogo 679-2315, Japan, and Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan

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Seventeen known taxoids and 10 abietanes were isolated from the dark brown callus culture of *Taxus cuspidata* cultivated on a modified Gamborg's B5 medium with 0.5 or 1.0 mg/L NAA. Seven known taxoids and four abietanes were obtained from the callus culture incubated under light irradiation on the medium with 1.0 mg/L NAA. Eight taxoids and five abietanes were also separated from the callus culture on the medium with 10 mmol/L β-cyclodextrin and 1.0 mg/L NAA. The new compounds were identified by analyses of the spectroscopic data and were found to be abieta-6,8,11,13-tetraene-3β,12-diol (**1**), 3β,20-epoxy-12-methoxy-abieta-8,11,13-triene-3α,11-diol (**2**), 3α-hydroxy-9(10→20)*abeo*-abieta-1,5,8,10(20),13-pentaene-7,11,12-trione (**3**), 2-hydroxy-9(10→20)*abeo*-abieta-1,5,8,10(20),13-pentaene-3,7,11,12-tetraone (**4**), and 3,7-dioxo-9(10→20)*abeo*-12-norabieta-1,5,8,10(20),13-pentaene-11,13-lactone (**5**), respectively. The yield of paclitaxel and its analogues was markedly decreased in the calluses mentioned above compared with that of standard callus. Instead, abietanes and some taxoids related to biosynthesis of paclitaxel were produced. Taxusin (**6**) exhibited stronger MDR-reversing activity than verapamil toward 2780 AD tumor cells.

Paclitaxel (Taxol), a natural product from the yew (*Taxus*) species, is known as an important drug for the treatment of various cancers as well as HIV-associated Kaposi's sarcoma.[1] Because of the low content of paclitaxel in natural sources and the lack of a commercially viable total synthesis,[2] much attention has been directed to cell culture as a renewable resource of paclitaxel and related taxoids in order to meet the increasing demand for this drug.[3] Therefore, we established a stable and fast-growing callus culture (CR-5) of the Japanese yew, *Taxus cuspidata* Sieb. et Zucc. (Taxaceae), through optimization of the culture conditions.[4] In this callus culture, not only taxane derivatives but also abietane derivatives were produced, and the yield of taxane derivatives was markedly increased by treatment with methyl jasmonate. Several bioactive taxoids were also found in the products.[5]

As a part of our ongoing investigations of the effects of culture conditions on the secondary metabolites and production of useful natural products in the callus culture of *T. cuspidata*, we examined the dark brown callus and the callus culture incubated under light irradiation, as well as the callus culture that included β-cyclodextrin in the medium. We wish to report the structure elucidation of five new abietanes (**1**−**5**) and the MDR reversal activity of a known taxoid, taxusin (**6**), which was isolated from the above-mentioned callus cultures.

Results and Discussion

Generally, the callus cultures with good growth conditions (see Experimental Section) were selected for continuous subculture, while those with bad growth conditions (see Experimental Section) were discarded from the subculture. By analysis of the products of the callus cultures with bad growth conditions, 27 compounds, i.e., paclitaxel[6,7] and its five analogues (7-*epi*-taxol,[7,8] taxol C,[9] baccatin VI,[10−12] 1-dehydroxybaccatin VI,[12] and taxayuntin C[13]), taxuyunnanine C and its four analogues (2α,5α,10β-triacetoxy-14β-propionyloxy-4(20),11-taxadiene, 2α,5α,10β-triacetoxy-14β-isobutyryloxy-4(20),11-taxadiene, 2α,5α,10β-triacetoxy-14β-(2′-methyl)butyryloxy-4(20),11-taxadiene, and yunnanxane),[14] six other taxoids (**6**,[15] 2α-acetoxytaxusin,[16] 14β-hydroxytaxusin,[17] 2-deacetyltaxuyunnanine C,[18] 10-deacetyltaxuyunnanine C,[19] and baccatin I[10,20]), and 10 abietanes (taxamairin A,[21,22] taxamairin C,[22] abietane N-1,[23] **2**−**5**, margocilin,[24] 7-oxo-12-methoxyabieta-8,11,13-triene-3,11-diol,[25] and brevitaxin[26]) have been isolated.

The callus culture incubated in the dark retained good growth conditions, while the callus culture incubated under light irradiation showed very slow growth and turned dark brown. From the callus culture incubated under light irradiation, only seven 14-oxygenated taxoids (taxuyunnanine C and its four analogues mentioned above, 2-deacetyltaxuyunnanine C, and 10-deacetyltaxuyunnanine C) and four abietanes (taxamairin A, taxamairin C, **1**, and **5**) were obtained. The structures of the new compounds isolated from the callus cultures, abietanes **1**−**5**, were identified by analyses of spectroscopic data.

Compound **1** was isolated from the callus culture incubated under light irradiation as a white amorphous powder (yield of 0.0022%) and had the composition $C_{20}H_{28}O_2$, which was determined by a combination of HREIMS, ^{1}H NMR, and ^{13}C NMR spectra. The IR spectrum of **1** indicated the existence of hydroxyl groups (3680 and 3520 cm^{-1}) and the absence of a carbonyl group. The ^{1}H NMR spectrum had the characteristics of an abietane skeleton with an isopropyl group [δ 3.13 (1 H, sept, J = 7.0 Hz), 1.26 (3 H, d, J = 7.0 Hz), and 1.23 (3 H, d, J = 7.0 Hz)] and three C-Me groups [δ 1.08 (s), 1.02 (s), and 1.01 (s)]. No methoxyl group was present. The assignment of all protonated carbons was established by DEPT and HMQC experiments. The *cis*-

* To whom correspondence should be addressed. Tel and Fax: +81-25-262-7326. E-mail: mando@eng.niigata-u.ac.jp.
[†] Graduate School of Science and Technology, Niigata University.
[‡] Department of Chemistry and Chemical Engineering, Niigata University.
[§] Kobe Natural Products and Chemicals Co., Ltd.
[⊥] University of Tokyo.

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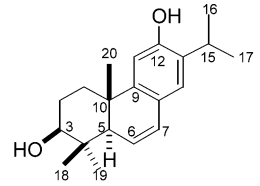

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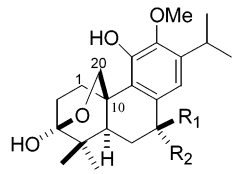

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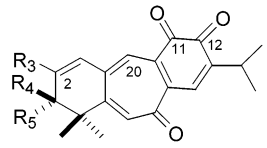
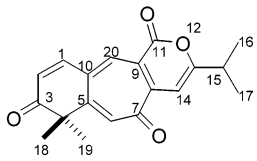
taxamairin C $R_1=R_2=O$



3 $R_3=R_4=H$, $R_5=OH$
4 $R_3=OH$, $R_4=R_5=O$



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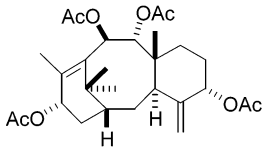

6 taxusin

olefin between C-6 and C-7 was suggested by their chemical shifts (δ_C 126.5 and 127.9, δ_H 5.86 and 6.53) and the coupling constant of their connected protons ($J = 9.8$ Hz). The positions of two hydroxyl groups at C-3 and C-12 were determined by the chemical shifts (δ 78.7 and 152.3) and the HMBC correlations of C-3 to H-2α, H-5, H-18, and H-19 and C-12 to H-11, 12-OH, H-14, and H-15, respectively. The multiple-bond $^1H-^{13}C$ correlations of six nonprotonated carbons of **1** were assigned by HMBC experiment and allowed unambiguous carbon skeletal connection. The stereochemistry of **1** was determined by NOESY experiment and consideration of the vicinal coupling constants. Accordingly, compound **1** was assigned as abieta-6,8,11,13-tetraene-3β,12-diol.

Compound **2** was isolated from the callus culture with bad growth conditions and had the composition $C_{21}H_{30}O_4$. The IR spectrum of **2** showed the existence of a hydroxyl group (3528 cm^{-1}) and the absence of a carbonyl group. The ^1H NMR spectrum was consistent with an abietane skeleton containing a methoxyl group (δ 3.74), an isopropyl group [δ 3.17 (1 H, sept, $J = 6.9$ Hz), 1.21 (3 H, d, $J = 6.9$ Hz), and 1.20 (3 H, d, $J = 6.9$ Hz)], and two methyl groups (δ 1.11 and 1.05). The $^1H-^1H$ correlations of vicinal protons and the long-range couplings of H-1α and H-20b, and H-5 and H-20a, were determined by analysis of the $^1H-^1H$ COSY spectrum. The assignment of all protonated carbons was established by DEPT and HMQC experiments. Assignments of the quaternary carbons and the attachment of functional groups were determined by HMBC experiment. The 3,20-epoxy abietane skeleton was suggested through comparison with a known compound, taxamairin C,[22] by the fact that the signals of two $-CH_2O-$ protons (δ 4.69 and 3.95) at C-20 and the downfield shift of C-3 (δ 98.4) due to a hemiacetal group were observed. Two geminal methylene protons (δ 2.66 and 2.77) at C-7 (δ 31.8) instead of the carbonyl group at C-7 of taxamairin C were also observed. The stereochemistry of **2** was confirmed by NOESY experiment and consideration of the $^1H-^1H$ coupling constants. Accordingly, compound **2** was assigned as 3β,20-epoxy-12-methoxy-abieta-8,11,13-triene-3α,11-diol.

Compound **3** ($C_{20}H_{20}O_4$) was isolated from the callus culture with bad growth conditions as a dark red solid in 0.0035% yield. The IR spectrum of **3** showed a hydroxyl group (3624 cm^{-1}) and a conjugated diketone moiety (1728 cm^{-1}). The ^1H and ^{13}C NMR spectra showed the characteristics of an *abeo*-abietane skeleton which was similar to those of taxamairin A[21,22] except for C-3, C-11, and C-12. The assignment of all protonated carbons was established by DEPT and HMQC experiments. ^{13}C NMR showed the existence of one hydroxyl and three carbonyl groups, which were assigned to C-3 (δ 73.2), C-7 (δ 180.7), C-11 (δ 199.8), and C-12 (δ 199.8) by the HMBC correlations of C-3 to H-1, H-18, and H-19; C-7 to H-6; C-11 to H-20; and C-12 to H-14, respectively. The 1,2-diketone (C-11 and C-12) but not the 1,4-diketone (C-11 and C-14) on the C ring was determined by the HMBC correlations of C-8 to H-6, H-14, and H-20. The structure of **3** was further confirmed by NOESY experiment. Accordingly, compound **3** was assigned as 3α-hydroxy-9(10→20)*abeo*-abieta-1,5,8,10(20),13-pentaene-7,11,12-trione.

Compound **4** ($C_{20}H_{18}O_5$) was isolated from the callus culture with bad growth conditions as a purple gum in 0.00024% yield. The IR spectrum of **4** showed a hydroxyl group (3630 cm^{-1}), a diketone moiety (1730 cm^{-1}), and a conjugated carbonyl group (1686 cm^{-1}). The ^1H and ^{13}C NMR spectra also showed characteristics of an *abeo*-abietane skeleton, similar to that of compound **3** except for C-2 and C-3. The ^{13}C NMR showed the existence of four carbonyl groups, which were assigned to C-3 (δ 198.8), C-7 (δ 180.0), C-11 (δ 198.9), and C-12 (δ 198.9) by the HMBC correlations of C-3 to H-1, H-18, and H-19; C-7 to H-6; C-11 to H-20; and C-12 to H-14 and H-15, respectively. The carbon (δ 141.4) was located on the C-14 according to the HMBC correlations to H-6 and H-15. The hydroxyl group was assigned to C-2 because of the chemical shift (δ 153.6) and the singlet of H-1 (δ 7.56). Thus, compound **4** was assigned as 2-hydroxy-9(10→20)*abeo*-abieta-1,5,8,10(20),13-pentaene-3,7,11,12-tetraone.

Compound **5** ($C_{19}H_{18}O_4$) was isolated from the callus culture with bad growth conditions as yellow needles in 0.0008% yield. The IR spectrum of **5** revealed an α-pyrone absorption (1730 cm^{-1}), a conjugated carbonyl group (1678 cm^{-1}), and the absence of a hydroxyl group. The ^1H NMR spectrum suggested an *abeo*-abietane skeleton very similar to taxamairin A.[21,22] The ^{13}C NMR spectrum showed 19 carbons with two conjugated carbonyl groups which were assigned to C-3 (δ 199.9) and C-7 (δ 184.4) by the HMBC experiment. Two signals (δ 163.5 and 171.6) were assigned to C-11 and C-13 of the α-pyrone moiety by the HMBC correlations of C-11 to H-20 and C-13 to H-14, H-15, H-16, and H-17. The carbon (δ 99.8) was assigned to C-14 by the HMBC correlations of C-7 to H-14 and H-6. The 9(10→20)-*abeo*-norabietane skeleton of **5** was further supported by NOESY correlations. Therefore, compound **5** was assigned as 3,7-dioxo-9(10→20)*abeo*-12-norabieta-1,5,8,10(20),13-pentaene-11,13-lactone.

We found that the yields of paclitaxel and its analogues in the callus culture with bad growth conditions were decreased markedly in comparison with those in previously reported callus culture (CR-5)[5] with good growth conditions. However, increased yields of taxuyunnanine C and its analogues, abietanes, and some biosynthetic intermediates of paclitaxel such as 14β-hydroxytaxusin and baccatin I were observed (Table S1, Supporting Information). It was interesting that the callus grown under light did not produce paclitaxel and its analogues, but the yield of abietanes increased markedly (Table S1). The biosynthetic path to abietanes appeared to be promoted under this condition, probably because of the influence of active

Table 1. Effect of Taxusin (**6**) on the Accumulation of Vincristine (VCR) in Multidrug-Resistant 2780AD Cells

	VCR accumulation[a]					evaluation
compound	concentration (μg/mL)	average[b] (dpm/well)	% of control[c]	activities[d]	verapamil (%)[e]	max. verapamil % concentration
6	0.1	361	106		102	P[f]
	1	774	228	++	157	157
	10	1562	461	++	136	1 μg/mL
verapamil	0 (control)	339	100			
	0.1	353	104	±	100	
	1	490	145	+	100	
	10	1193	352	++	100	

[a] The amount of VCR accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.1, 1, and 10 μg/mL of taxoid. [b] The values represent means of triplicate determination. [c] The values are the relative amount of VCR accumulated in the cell compared with the control experiment. [d] The indices are expressed on a scale of four by the range of the relative amount of VCR accumulation as compared with the control experiment (%): ++, 301−500%; +, 111−300%; ±, 91−110%; −, <90%. [e] The values are expressed as the relative amount of vincristine (VCR) accumulation in the cell as compared with that of verapamil. [f] P, positive: the activity is stronger than that of verapamil (verapamil% >100%).

oxygen. Addition of β-cyclodextrin into the medium also decreased the yields of paclitaxel and its analogues and increased yields of taxuyunnanine C and its analogues as well as abietane derivatives (Table S1).

The kinds of products isolated from the callus cultures and their yields largely depended on the culture conditions. The main products from calluses of Japanese yew (*T. cuspidata*) are shown in Figure S1 (Supporting Information) along with the biosynthetic path of taxanes and abietanes. The standard callus (CR-5) gives mainly taxuyunnanine C and its analogues and paclitaxel and its analogues. Addition of methyl jasmonate can promote the biosyntheses of paclitaxel and its analogues accompanied by intermediates related to the biosynthesis of paclitaxel such as baccatin I and 14β-hydroxytaxusin. On the contrary, bad growth conditions or light irradiation or β-cyclodextrin disturbs the biosynthetic route to paclitaxel and its analogues but promotes that to abietanes. These observations may be useful for the biosynthetic studies of paclitaxel.

The cellular accumulation of VCR is reduced in MDR tumor cells as compared with the parental cells. The MDR-reversing agent, verapamil, increases the accumulation in MDR cells and overcomes multidrug resistance.[27] The effect of taxusin (**6**) on the cellular accumulation of VCR in human ovarian cancer 2780 AD cells was examined, and the result is shown in Table 1. Compound **6** exhibited activity about 1.5 times that of verapamil toward VCR accumulation in MDR tumor cells.

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured using a Horiba SEPA-200 polarimeter. IR spectra were recorded in $CHCl_3$ on a Hitachi 270-30 spectrometer. UV spectra were performed with JASCO V-550 UV/vis spectrophotometer. ^{1}H NMR (499.87 MHz) and ^{13}C NMR (125.70 MHz) spectra were recorded on a Varian UNITY-PS 500 spectrometer. HREIMS spectra were obtained using a JEOL JMS HX-110 spectrometer. To describe HPLC conditions, we designated column, solvent, and retention time (t_R in min) in this order. The flow rate for HPLC was 5 mL/min, unless otherwise stated. The column codes were as follows: (A) Inertsil Prep-sil (GL Science), 25 × 1 cm i.d. stainless column; (B) Inertsil Prep-sil (GL Science), 25 × 2 cm i.d. stainless column; (C) Inertsil Prep-ODS (GL Science), 25 × 1 cm i.d. stainless column. Silica gel (70−230 mesh) was employed for column chromatography and silica gel (230−400 mesh) for flash column chromatography. The "callus culture with good growth conditions" means the callus with color degree[28] < 3 and growth rate[29] > 2 that had the ability to grow continuously through subculture, while the "callus culture with bad growth conditions" refers to the callus with color degree ≥ 3 and growth rate ≤ 2 that showed slow growth and was discarded from the subculture.

Plant Material. The plant material used for CR-5 was described in a previous paper.[5] The young stems of *T. cuspidata* Sieb. et Zucc. collected in Sendai in spring of 1996 were used as explants for CR-2 in the experiments. The stems were stripped of needles, washed with distilled H_2O, immersed for 3 min in 70% EtOH, and surface sterilized by immersion in saturated calcium hypochlorite solution for 10 min. After sterilization, the stems were rinsed three times with sterile distilled H_2O and aseptically dissected into explants 5−8 mm in length.

Induction and Culture Conditions. Gamborg's B5 medium supplemented with 2 × vitamins and 20 g/L sucrose was used with 1.0 mg/L NAA for CR-2 (all medium components were purchased from Wako Pure Chemicals, Co., Ltd., Osaka, Japan). After the pH of the media was adjusted to 5.8 with 0.1 M NaOH, 10 g/L agar was added. The media were autoclaved at 120 °C for 15 min. The explants were placed on the solidified nutrient media and incubated in darkness at 25 °C for 30−40 days. Then the initiated calluses were subcultured continuously every 60 days under the same conditions.

The callus lines of CR-5 and CR-2 were continuously subcultured 13 times and 17 times, respectively. During this period, the calluses with good growth conditions were selected for the subculture, while those with bad growth conditions were discarded from the subcultures and collected for analysis.

After CR-2 was subcultured 22 times, a part of this callus line was incubated under light irradiation for half a year and used for analysis.

After CR-5 was subcultured nine times, 10 mmol/L β-cyclodextrin was added into the medium of a part of this callus line. Then, the callus was cultured for another 60 days and harvested for analysis.

Isolation of Compounds 2, 4, and 5 and the Other Products from the Callus Culture CR-5 with Bad Growth Conditions. The freeze-dried callus (752.0 g) was macerated in 8 L of hexane for 10 h. After the supernatant was filtered and evaporated, 4.2 g of the hexane extract was obtained. The residue was then macerated in 14 L of EtOAc for 24 h, and 5.0 g of the EtOAc extract was obtained after the supernatant was filtered and evaporated. The residue was macerated in 8 L of MeOH for 4 days, the concentrated MeOH extract was diluted with 300 mL of H_2O, then extracted with $CHCl_3$ (4 × 200 mL), and 7.3 g of $CHCl_3$ extract was obtained. The crude $CHCl_3$ extract was dissolved in a mixture of MeOH−EtOAc (1:4, 100 mL) and extracted with a 0.5 M aqueous solution of H_2SO_4 (25 mL). The organic layer was successively extracted with 29% aqueous solution of NH_4OH until the pH of the aqueous solution became 9.0, washed with a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, and concentrated to give a crude neutral fraction of the $CHCl_3$ extract (4.2 g).

The EtOAc extract was first separated into six fractions (EF1−EF6) by flash column chromatography using a gradient elution from hexane−EtOAc (1:1) to MeOH. EF3 (1.4 g) was further separated into five fractions (EF3-1−EF3-5) by flash column chromatography using a gradient elution from hexane−EtOAc (8:2) to MeOH. Purification of EF3-3 (27.4 mg) by normal-phase HPLC [column B, hexane−EtOAc (7:3), flow rate 9 mL/min] afforded compound **4** (t_R 25.3 min, 1.8 mg).

The neutral $CHCl_3$ extract was separated into seven fractions (CF1−CF7) by flash column chromatography using a gradient elution from hexane−EtOAc (8:2) to MeOH. CF2 (873 mg) was applied to normal-phase HPLC [column A, hexane−EtOAc (8:2)] to afford compound **5** (t_R 15.1 min, 6 mg). CF4 (386 mg) was separated by normal-phase HPLC [column B, hexane−EtOAc (6:4), flow rate 9 mL/min] to afford a mixture (t_R 17.5−19.0 min, 16 mg), which was purified using normal-phase HPLC [column A, hexane−EtOAc (72:28)] to afford compound **2** (t_R 21.0 min, 3.0 mg). Similarly, the isolation of the other products from the hexane, EtOAc, and $CHCl_3$ extracts was performed by the same operation mentioned above, and their yields are shown in Table S1.

Isolation of Compound 3 from the Callus Culture CR-2 with Bad Growth Conditions. The freeze-dried callus (99.7 g) was defatted in 8 L of hexane for 1 h. After the supernatant was removed, the residue was macerated in 15 L of EtOAc and stirred for 3 × 1 h. Then 1.45 g of the EtOAc extract was obtained after the supernatant was filtered and evaporated.

The EtOAc extract was first separated into 11 fractions (F1−F11) by flash column chromatography using a gradient elution from hexane−EtOAc (9:1) to MeOH. F7 (129 mg) was further applied to normal-phase HPLC [column A, hexane−EtOAc (6:4)] to afford F7-3 (t_R 12.6 min, 6 mg). F8 (126 mg) was further separated by reversed-phase HPLC [column C, MeOH−H_2O−MeCN (1:2:2)] to give F8-4 (t_R 16.7−19.3 min, 16 mg). Purification of F7-3 and F8-4 by normal-phase HPLC [column A, hexane−EtOAc (5:5)] afforded compound **3** (t_R 6.7 min, 3.5 mg).

Extraction and Isolation of Compound 1 and the Other Products from the Callus Culture in Light Irradiation. The freeze-dried callus (55.0 g) was extracted with hexane (3 × 1.1 L) for 3 h to give 233 mg of the hexane extract. The remaining cell mass was successively extracted with EtOAc (3 × 1.1 L) for 18 h, and 511 mg of the EtOAc extract was obtained after the supernatant was filtered and evaporated. The remaining cell mass was further extracted with MeOH (3 × 1.1 L) for 4 h, the concentrated MeOH extract was diluted with 100 mL of H_2O, then extracted with $CHCl_3$ (3 × 200 mL), and a crude $CHCl_3$ extract was obtained. Subsequently, the crude $CHCl_3$ extract was dissolved in a mixture of MeOH−EtOAc (1:4, 25 mL) and extracted with a 0.5 M aqueous solution of H_2SO_4 (5 mL). The organic layer was successively extracted with a 29% aqueous solution of NH_4OH until the pH of the aqueous solution became 9.0, washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, and concentrated to give a neutral fraction of the $CHCl_3$ extract (608 mg).

The neutral $CHCl_3$ extract was separated into five fractions (LCF1−LCF5) by flash column chromatography using a gradient elution from hexane−EtOAc (1:1) to MeOH. LCF3 (38.9 mg) was separated by normal-phase HPLC [column A, hexane−EtOAc (55:45)] to give a subfraction (t_R 7.0−13.0 min, 15.8 mg), which was purified by normal-phase HPLC [column A, hexane−EtOAc (3:7)] to afford compound **1** (t_R 20.3 min, 1.2 mg). Similarly, the isolation of the other products was performed by the same operation mentioned above, and their yields are shown in Table S1.

Extraction and Isolation of the Products from Callus Culture with β-Cyclodextrin Added to the Medium. The fresh callus (491.4 g) was freeze-dried for 2 days to give a dry callus (47.2 g). It was defatted with hexane (3 × 1 L) for 3 h to give a crude hexane extract (298 mg). The remaining cell mass was successively extracted with EtOAc (3 × 1 L) for 3 h to give a crude EtOAc extract (506 mg). The remaining cell mass was further extracted with MeOH (3 × 1 L) for 3 h. After removal of the solvent the MeOH extract was diluted with a saturated aqueous solution of NaCl (500 mL) and extracted with $CHCl_3$ (3 × 100 mL) to give a crude $CHCl_3$ extract (1.8 g). The products isolated from these extracts and their yields are shown in Table S1.

Compound 1: white amorphous powder; mp 129−131 °C; $[\alpha]^{20}_D$ −5.57° (*c* 0.269, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 275.5 (2.30), 240.5 (2.56) nm; IR ($CHCl_3$) ν_{max}, 3680, 3520, 1500 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.90 (1H, s, H-14), 6.56 (1H, s, H-11), 6.53 (1H, dd, J = 9.8, 3.2 Hz, H-7), 5.86 (1H, dd, J = 9.8, 2.7 Hz, H-6), 4.73 (1H, s, 12-OH), 3.36 (1H, br dd, J = 11.2, 4.4 Hz, H-3), 3.13 (1H, sept, J = 7.0 Hz, H-15), 2.12 (1H, m, H-1β), 2.09 (1H, dd, J = 3.2, 2.7, H-5), 1.87 (1H, m, H-2α), 1.79 (1H, m, H-2β), 1.74 (1H, m, H-1α), 1.26 (3H, d, J = 7.0 Hz, H-16), 1.23 (3H, d, J = 7.0 Hz, H-17), 1.08 (3H, s, H-19), 1.02 (3H, s, H-18), 1.01 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 152.3 (C, C-12), 146.5 (C, C-9), 131.2 (C, C-13), 127.9 (CH, C-7), 126.5 (CH, C-6), 126.1 (C, C-8), 124.6 (CH, C-14), 109.5 (CH, C-11), 78.7 (CH, C-3), 50.3 (CH, C-5), 38.3 (C, C-4), 37.5 (C, C-10), 34.2 (CH_2, C-1), 27.8 (CH_3, C-18), 27.7 (CH_2, C-2), 26.7 (CH, C-15), 22.8 (CH_3, C-16), 22.4 (CH_3, C-17), 20.2 (CH_3, C-20), 16.5 (CH_3, C-19); HREIMS *m/z* 300.2093 (calcd for $C_{20}H_{28}O_2$, 300.2089).

Compound 2: white amorphous powder; mp 198−200 °C; $[\alpha]^{20}_D$ +109.74° (*c* 0.154, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 276.5 (2.87), 209.0 (4.07) nm; IR ($CHCl_3$) ν_{max}, 3528, 1472 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.49 (1H, s, H-14), 6.02 (1H, d, J = 1.7 Hz, 11-OH), 4.69 (1H, br d, J = 8.8 Hz, H-20a), 3.95 (1H, dd, J = 8.8, 2.7 Hz, H-20b), 3.74 (3H, s, 12-OC*H*$_3$), 3.34 (1H, td, J = 12.5, 5.7 Hz, H-1β), 3.17 (1H, sept, J = 6.9 Hz, H-15), 2.77 (1H, br dt, J = 14.7, 3.0 Hz, H-7β), 2.66 (1H, m, H-7α), 2.23 (1H, m, H-2α), 1.84 (1H, td, J = 12.5, 3.3 Hz, H-2β), 1.79 (1H, m, H-6α), 1.61 (1H, m, H-6β), 1.61 (1H, m, H-5), 1.45 (1H, tt, J = 12.5, 3.3 Hz, H-1α), 1.21 (3H, d, J = 6.9 Hz, H-16), 1.20 (3H, d, J = 6.9 Hz, H-17), 1.11 (3H, s, H-18), 1.05 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 147.9 (C, C-11), 142.6 (C, C-12), 139.0 (C, C-13), 135.3 (C, C-8), 121.9 (C, C-9), 117.7 (CH, C-14), 98.4 (C, C-3), 67.3 (CH_2, C-20), 61.8 (CH_3, O*C*H$_3$), 50.4 (CH, C-5), 40.8 (C, C-4), 36.7 (C, C-10), 31.8 (CH_2, C-7), 30.0 (CH_2, C-1), 29.1 (CH_2, C-2), 26.9 (CH_3, C-18), 26.4 (CH, C-15), 23.6 (CH_3, C-16), 23.6 (CH_3, C-17), 20.5 (CH_2, C-6), 18.1 (CH_3, C-19); HREIMS *m/z* 346.2145 (calcd for $C_{21}H_{30}O_4$, 346.2144).

Compound 3: dark red solid; mp 282−284 °C; $[\alpha]^{20}_D$ −118.84° (*c* 0.138, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 520.0 (3.04), 306.0 (3.04), 241.0 (3.82) nm; IR ($CHCl_3$) ν_{max} 3624, 1728, 1616, 1598, 1554 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.63 (1H, d, J = 1.7 Hz, H-14), 7.43 (1H, s, H-6), 6.98 (1H, s, H-20), 6.54 (1H, br d, J = 9.5 Hz, H-1), 6.29 (1H, dd, J = 9.5, 5.1 Hz, H-2), 4.03 (1H, br d, J = 5.1 Hz, H-3), 2.72 (1H, sept d, J = 7.0, 1.7 Hz, H-15), 2.03 (1H, br s, 3-OH), 1.43 (3H, s, H-18), 1.24 (3H, s, H-19), 1.19 (3H, d, J = 7.0 Hz, H-17), 1.17 (3H, d, J = 7.0 Hz, H-16); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 199.8 (C, C-11), 199.8 (C, C-12), 180.7 (C, C-7), 153.6 (C, C-5), 150.3 (C, C-8), 144.6 (C, C-13), 141.8 (CH, C-14), 141.8 (CH, C-6), 141.4 (C, C-10), 133.6 (CH, C-2), 132.6 (CH, C-1), 130.0 (C, C-9), 125.2 (CH, C-20), 73.2 (CH, C-3), 43.9 (C, C-4), 27.8 (CH_3, C-19), 25.3 (CH, C-15), 23.9 (CH_3, C-18), 21.4 (CH_3, C-16), 21.3 (CH_3, C-17); HREIMS *m/z* 296.1415 $[M - CO]^+$ (calcd for $[C_{19}H_{20}O_3]^+$, 296.1413).

Compound 4: purple gum; UV (MeOH) λ_{max} (log ϵ) 552.5 (3.37), 320.5 (3.53), 239.5 (3.84) nm; IR ($CHCl_3$) ν_{max} 3630, 1730, 1686, 1596, 1556 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.70 (1H, d, J = 1.7 Hz, H-14), 7.56 (1H, s, H-1), 7.54 (1H, s, H-6), 7.33 (1H, s, H-20), 2.79 (1H, sept d, J = 7.0, 1.7 Hz, H-15), 1.61 (3H, s, H-18), 1.61 (3H, s, H-19), 1.22 (3H, d, J = 7.0 Hz, H-17), 1.22 (3H, d, J = 7.0 Hz, H-16); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 198.9 (C, C-11), 198.9 (C, C-12), 198.8 (C, C-3), 180.0 (C, C-7), 154.3 (C, C-5), 153.6 (C, C-2), 146.6 (C, C-13), 144.6 (CH, C-1), 141.5 (CH, C-6), 141.4 (CH, C-14), 138.3 (C, C-8), 132.2 (C, C-10), 129.8 (CH, C-20), 129.1 (C, C-9), 52.1 (C, C-4), 27.5 (CH_3, C-19), 27.5 (CH_3, C-18), 25.6 (CH, C-15), 21.3 (CH_3, C-16), 21.3 (CH_3, C-17); HREIMS *m/z* 338.1596 (calcd for $C_{20}H_{18}O_5$, 338.1155).

Compound 5: bright yellow needles; mp 159−161 °C; UV (MeOH) λ_{max} (log ϵ) 396.0 (3.78), 262.5 (3.91), 208.0 (3.99) nm; IR ($CHCl_3$) ν_{max}, 1730, 1678, 1632, 1554 cm^{-1}; ^1H NMR ($CDCl_3$,

500 MHz) δ 7.91 (1H, s, H-20), 7.31 (1H, d, J = 9.9 Hz, H-1), 7.15 (1H, s, H-6), 6.82 (1H, s, H-14), 6.20 (1H, d, J = 9.9 Hz, H-2), 2.86 (1H, sept, J = 7.0 Hz, H-15), 1.49 (3H, s, H-18), 1.49 (3H, s, H-19), 1.32 (3H, d, J = 7.0 Hz, H-17), 1.32 (3H, d, J = 7.0 Hz, H-16); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 199.9 (C, C-3), 184.4 (C, C-7), 171.6 (C, C-13), 163.5 (C, C-11), 154.8 (C, C-5), 148.3 (C, C-10), 146.4 (CH, C-1), 134.9 (C, C-9), 134.7 (CH, C-20), 134.3 (CH, C-6), 125.5 (CH, C-2), 121.3 (C, C-8), 99.8 (CH, C-14), 50.8 (C, C-4), 33.2 (CH, C-15), 27.8 (CH_3, C-19), 27.3 (CH_3, C-18), 20.0 (CH_3, C-16), 20.0 (CH_3, C-17); HREIMS *m/z* 310.1201 (calcd for $C_{19}H_{18}O_4$, 310.1205).

Cellular Accumulation of [^{3}H]-VCR. The MDR 2780AD cells were maintained in PRMI-1640 medium (Nissui, Tokyo, Japan) supplemented with 5% heat-inactivated fetal bovine serum and 100 μg/mL kanamycin. 2780AD cells (1×10^6 cells/well) were seeded in a 24-well plate and cultured for 18 h before the assay. The cells were treated with 1×10^5 dpm of [^{3}H]-VCR (222 Gbq/mmol; Amersham Pharmacia Biotech, Tokyo, Japan) in the presence or absence of verapamil or taxoids. Immediately after incubation for 2 h at 37 °C, the cells were washed five times with ice-cold phosphate-buffered saline containing 0.1 mg/mL of nonradioactive VCR and lysed with 500 μL of 0.2 M NaOH. After incubation for 45 min at 56 °C, the lysates were neutralized with 2 M acetic acid, and the radioactivity was counted in ACS II (Amersham Pharmacia Biotech).

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Supporting Information Available: Compounds list, Table S1, Figure S1, ^{1}H and ^{13}C NMR spectra, and full NMR data (Tables S2–S6) of compounds **1**–**5** in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) The color of callus cultures was evaluated in five degrees: 1 (colorless) to 5 (dark brown).

(29) The growth rate of callus cultures was calculated by the formula (growth rate = final weight of fresh callus/initial weight of fresh callus) in a period of subculture.

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