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Highly Increased Cellular Accumulation of Vincristine, a Useful Hydrophobic Antitumor-drug, in Multidrug-resistant Solid Cancer Cells Induced by a Simply Reduced Taxinine

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Abstract: Regio- and/or chemo-selective reductions of taxinine (**1a**), a taxane diterpenoid readily obtainable from the needles of a Japanese yew (*Taxus cuspidata*), at the 5-*O*-cinnamoyl and 4-*exo*-methylene moieties have been accomplished by the catalytic hydrogenation over Pd/C or Rh/C to obtain 5-*O*-phenylpropionylated taxinine A (**1b**), 5-*O*-cyclohexylpropionylated taxinine A (**1c**), and 5-*O*-phenylpropionylated 4,20-dihydrotaxinine A (**2a**) in almost quantitative yields, respectively. Among them, taxoid **1b** was found to be highly effective in increasing the cellular accumulation of vincristine in the multidrug-resistant human ovarian cancer cells compared with the cases of verapamil and the previously reported taxoids.

Current attention is centered on the rapid emergence of multidrug-resistant (MDR) cancer cells in the chemotherapeutic treatment for various types of advanced solid cancers. This is mainly caused by the over-induction of P-glycoprotein,[1] a cell-membrane transporter for a variety of hydrophobic substrates, during the course of the clinical use of vincristine, paclitaxel, and etoposide which are very useful hydrophobic antitumor-drugs for cancer chemotherapy.

During the search for potent inhibitors of the P-glycoprotein efflux-function to improve the cancer-chemotherapy, a variety of heterocyclic compounds such as NK-252 (a dihydropyridine derivative),[2] MS-209 (a quinoline derivative),[3] and GF-120918 (an acridonecarboxamide derivative)[4] have been developed as functional inhibitors of the P-glycoprotein.

Along this line, we have documented that 5-*O*-benzoylated taxinine K, a photo-product of 5-*O*-benzoylated taxinine A (**1**: R= Bz), remarkably increases the cellular accumulation of vincristine in MDR human ovarian cancer cells and is a promising functional inhibitor of the P-glycoprotein.[5] More recently, taxinine 4α,20-epoxide and 5-*O*-benzyloxymethylated taxinine A (**1**: R= CH_2OCH_2Ph), which are taxane diterpenoids simply modified at the 4- or 5-position in taxinine (**1a**), were found to be more effective than the taxinine K derivative.[6] These facts encouraged us to study the reductive modifications of the taxoid **1a** which is readily obtainable from the needles of a Japanese yew (*Taxus cuspidata*).[7] We report herein that the simple reductive treatment of **1a** leading to 5-*O*-phenylpropionylated taxinine A (**1b**) is highly effective in increasing the cellular accumulation of vincristine in the MDR cancer cells.

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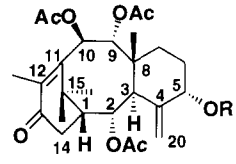
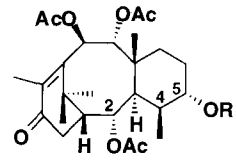


(1)

a): R= COCH=CHPh (Taxinine)
b): R= $COCH_2CH_2Ph$
c): R= $COCH_2CH_2C_6H_{11}$

Taxinine A: R= H
Taxinine H: R= Ac



(2)

a): R= $COCH_2CH_2Ph$
b): R= H
c): R= COCH=CHPh

The treatment of the taxoid **1a** with aluminum hydrides such as sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) and diisobutylaluminum hydride (DIBAL-H) has been found to cause the regioselective deacylation at the 5- or 2- position leading to taxinine A (**1**: R= H) (by Red-Al; 50% yield) or 2-deacetylated taxinine (by DIBAL-H; 53% yield).[8] On the other hand, the catalytic hydrogenation of **1a** over 10% palladium/charcoal (Pd/C) in THF under a hydrogen atmosphere at ambient pressure resulted in the chemo-selective reduction of the double bond in the cinnamoyl moiety to give the corresponding dihydrotaxinine (**1b**) (mp 240-241°C)[9] in almost quantitative yield (the completed reaction time: 4 hrs). When the reaction was carried out under a hydrogen atmosphere at higher pressure, *e.g.*, 8 atm of hydrogen pressure, the reduction of the 4-*exo*-methylene moiety also proceeded to form 5-*O*-phenylpropionylated 4,20-dihydrotaxinine A (**2a**) (mp 300-301°C).[10, 11] The structures of these taxoids **1b** and **2a** were confirmed by an ^{1}H-NMR spectral comparison with those of authentic compounds which were obtained during the course of an earlier chemical study on the structural elucidation of the nationally occurring taxoid **1a**.[9, 10] The β-orientation of the 4-methyl group in the product **2a** was supported by its NOESY spectrum, *e.g.*, the correlation of a doublet signal at δ_H 0.92 (J = 7 Hz) ppm originating from the 4-methyl group with peaks (δ_H 5.41 and δ_H 4.68 ppm) assignable to the 2β- and 5β-protons were observed.

The almost quantitative formation of the dihydrotaxinine **1b** was also observed during the catalytic hydrogenation over rhodium/charcoal (Rh/C) in THF under an ambient pressure hydrogen atmosphere. In sharp contrast to the above results, the catalytic reduction over Rh/C under 10 atm of hydrogen pressure allowed the smooth conversion of the taxoid **1a** to 5-*O*-cyclohexylpropionylated taxinine A (**1c**) (mp 169-170°C). The structure of the product **1c** is based on the microanalytical and spectral data,[12] *e.g.*, two broad singlet signals at δ_H 4.85 and δ_H 5.33 ppm and a carbon signal at δ_C 117.5 ppm unambiguously indicated retention of the *exo*-methylene moiety during the reaction. The disappearance of peaks in the low field (δ_H 6.5-7.5 ppm) and the appearance of multiplet peaks in the high field (δ_H 0.8-1.8 ppm) indicated the occurrence of the complete reduction of the 5-*O*-cinnamoyl group under the conditions employed. Under these catalytic hydrogenation conditions, the 11,12-double bond in **1a** was inert.

Table 1. Comparison of the Taxoids 1 and 2 with the case of Verapamil on the Cellular Accumulation of Vincristine in MDR Human Ovarian Cancer 2780AD Cells.

Entry	Taxoids	Relative activity	Entry	Taxoids	Relative activity
1	**1a**	77	6	**2a**	44
2	**1b**	227	7	**2b** [11]	48
3	**1c**	97	8	**2c** [11]	44
4	Taxinine A	64		**1** (R= CH_2OCH_2Ph)	158 [6]
5	Taxinine H	93		Verapamil	100

The effects of the taxoids **1** and **2** on the cellular accumulation of vincristine in MDR human ovarian cancer 2780AD cells were evaluated in comparison with the case of verapamil, a typical inhibitor of the P-glycoprotein.[13] These results are summarized in Table 1 and are expressed as values relative to that of verapamil. The table shows that (a) the reduction of the cinnamoyl double bond in **1a** is highly effective for binding to the glycoprotein to inhibit the efflux of the antitumor drug from the cancer cells (see entry 2), (b) the presence of the phenyl ring in the side-chain is effective for the protein binding (see entries 2-5), and (c) the reduction of the *exo*-methylene group results in a drastic decrease in the cellular accumulation of the antitumor drug (see entries 6-8). The present results indicate that the presence of the 4-*exo*-methylene group, which plays a significant role in the conformational fixation of the taxane ring, is a requisite for effective binding to the carrier protein. It should be noted that the taxoid **1b** has no remarkable cytotoxicity towards normal and cancer cells, indicating that this compound may be regarded as a selective functional inhibitor of the P-glycoprotein.

Acknowledgement: The inhibiting activities of the taxoids **1** and **2** were evaluated by Dr. T. Yamori of the Cancer Chemotherapy Center in Japan.

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[6] Hosoyama, H.; Shigemori, H.; Tomida, A.; Tsuruo, T.; Kobayashi, J. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 389-394. We have unpublished data contrary to their results: the 4α,20-epoxide, prepared by the oxidation of the taxoid **1a** with *m*-chloroperbenzoic acid, showed the cellular accumulation of vincristine in a low level (relative accumulation: 37%) compared with the case of verapamil. An analogous result was obtained for the case of taxinine H 4α,20-epoxide (relative accumulation: 28%).

[7] At present, the taxoid **1a** is commercially available from Wako Pure Chemicals, Ltd.

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[9] a) Baxter, J. N.; Lythgoe, B.; Scales, B.; Scrowston, R. M.; Trippett, S. *J. Chem. Soc. (C)*, **1962**, 2964-2971 (over Pd/C; mp 239°C); b) Kurono, M.; Nakadaira, Y.; Omura, S.; Sasaki, K.; Nakanishi, K. *Tetrahedron Lett.*, **1963**, 2153-2160 (over Pd/C; mp 246-7°C); c) Uyeo, S.; Ueda, K.; Yamamoto, Y.; Maki, Y. *Yakugaku Zasshi*, **1964**, *84*, 762-772 (over PtO_2; mp 237-238°C). For (**1b**): IR (KBr) 1735 and 1668 cm^{-1}; UV (EtOH) 267 nm; ^1H NMR ($CDCl_3$) δ 0.90 (3H, s, 8-Me), 1.13 (3H, s, 15β–Me) 1.63-1.70 (4H, m, 6- and 7-H), 1.75 (3H, s, 15α–Me), 2.05, 2.06, and 2.07 (each 3H, each s, 3 x OAc), 2.19 (1H, br d, *J*= 7 Hz, 1-H), 2.20 (3H, s, 12-Me), 2.27 (1H, d, *J*= 20 Hz, 14α-H), 2.46-2.62 (2H, m, 22-H), 2.78 (1H, dd, *J*= 7 and 20 Hz, 14β-H), 2.93 (2H, dd, *J*= 7 and 8 Hz, 23-H), 3.19 (1H, d, *J*= 6 Hz, 3-H), 4.85 (1H, br s, 20-H), 5.23 (1H, br s, 5-H), 5.33 (1H, br s, 20-H), 5.52 (1H, dd, *J*= 2 and 6 Hz, 2-H), 5.87 (1H, d, *J*= 10 Hz, 9-H), 6.01 (1H, d, *J*= 10 Hz, 10-H), 7.2-7.3 (5H, m, Ar-H); ^{13}C NMR ($CDCl_3$) δ 199.0, 172.3, 169.8, 169.7, 169.4, 150.2, 141.7, 140.5, 137.8, 128.5 (x 2), 128.4 (x 2), 126.1, 117.6, 78.0, 75.8, 73.3, 69.6, 48.4, 44.4, 43.0, 37.6, 37.3, 35.9, 35.5, 30.9, 28.4, 27.5, 25.1, 21.4, 20.9, 20.7, 17.4, 14.0; HR-FABMS *m/z* 609.3077 ([M + H]$^+$ (calcd for $C_{35}H_{45}O_9$ *m/z* 609.3064 ([M + H]$^+$).

[10] see, ref. 9b (over Pd/C; mp 279°C) and ref. 9c (over Pd/C; mp 276-277°C). For (**2a**): IR (KBr) 1736 and 1666 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (3H, s, 8-Me), 0.92 (3H, d, *J*= 7 Hz, 4β-Me), 1.10 (3H, s, 15β-Me), 1.40-1.90 (4H, m, 6- and 7-H), 1.70 (3H, s, 15α-Me), 2.01, 2.02, and 2.04 (each 3H, each s, 3 x OAc), 2.05 (1H, m, 4α-H), 2.15 (1H, br d, *J*= 7 Hz, 1-H), 2.16 (3H, s, 12-Me), 2.25 (1H, d, *J*= 20 Hz, 14α-H), 2.47-2.60 (3H, m, 3-H and 22-H), 2.75 (1H, dd, *J*= 7 and 20 Hz, 14β-H), 2.90 (2H, dd, *J*= 7 and 8 Hz, 23-H), 4.68 (1H, br d, *J*= 2 Hz, 5-H), 5.41 (1H, dd, *J*= 2 and 6 Hz, 2-H), 5.73 (1H, d, *J*= 10 Hz, 9-H), 5.92 (1H, d, *J*= 10 Hz, 10-H), 7.15-7.35 (5H, m, Ar-H); ^{13}C NMR ($CDCl_3$) δ 200.1, 173.5, 171.1, 170.6 (x 2), 151.8, 141.5, 138.8, 129.4 (x 2), 127.1 (x 2), 76.8, 74.6, 74.5, 70.7, 48.8, 43.0, 39.5, 38.6, 38.3, 37.5, 36.7, 36.5, 31.9, 27.9, 26.0, 22.4, 22.1, 21.8, 21.7, 19.4, 19.0, 14.7; HR-FABMS *m/z* 611.3229 ([M + H]$^+$ (calcd for $C_{35}H_{47}O_9$ *m/z* 611.3220 ([M + H]$^+$). When the reaction was carried out under 5 atm of hydrogen pressure, the formations of the reduced products **1b** and **2a** in 13% and 87% yields, respectively, were observed.

[11] Under analogous conditions, taxinine A (**1**; R= H) was smoothly converted into the corresponding 4,20-dihydrotaxinine A (**2b**; R= H) (mp 211-214°C). 4,20-Dihydrotaxinine (**2c**) (mp >300°C) was easily prepared by the condensation of **2b** with *trans*-cinnamic acid using a DCC-DMAP method.

[12] IR (KBr) 1740 and 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80-1.00 (2H, m), 0.90 (3H, s, 8-Me), 1.12 (3H, s, 15β-Me), 1.15-1.30 (4H, m), 1.48 (2H, m, 23-H), 1.59-1.82 (9H, m), 1.75 (3H, s, 15α-Me), 2.04, 2.05, and 2.07 (each 3H, each s, 3 x OAc), 2.17 (1H, dd, *J*= 2 and 7 Hz, 1-H), 2.10-2.24 (2H, m, 22-H), 2.24 (3H, s, 12-Me), 2.27 (1H, d, *J*= 20 Hz, 14α-H), 2.78(1H, dd, *J*= 7 and 20 Hz, 14β-H), 3.22 (1H, d, *J*= 6 Hz, 3-H), 4.85 (1H, br s, 20-H), 5.24 (1H, br s, 5-H), 5.33 (1H, br s, 20-H), 5.53 (1H, dd, *J*= 2 and 6 Hz, 2-H), 5.88 (1H, d, *J*= 10 Hz, 9-H), 6.03 (1H, d, *J*= 10 Hz, 10-H); ^{13}C NMR ($CDCl_3$) δ 199.0, 173.5, 170.0, 169.7, 169.4, 150.1, 141.9, 137.9, 117.5, 77.7, 75.8, 73.3, 69.8, 48.5, 44.5, 43.1, 37.6, 37.3, 36.0 (x 2), 33.0, 32.3, 31.8, 28.5, 27.6, 26.6, 26.3, 25.2, 21.4, 20.9, 20.7, 17.5, 14.0; Anal. Found C: 68.44; H: 8.33 (calcd for $C_{35}H_{50}O_9$ C: 68.38; H: 8.20).

[13] The 2780AD cells were incubated in a phosphate-buffer solution containing [^3H]-vincristine and 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid with or without the taxoids and verapamil (each 1.0 μg/mL), according to the known method (*cf.* Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.*, **1981**, *41*, 1967-1972). Radioactivity of the supernatant fluid obtained after centrifugation of the mixture was counted in a liquid scintillation system used for biological evaluations.