BIOACTIVE TAXOIDS FROM JAPANESE YEW *TAXUS CUSPIDATA* AND TAXOL BIOSYNTHESIS

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Abstract- A series of new taxoids, named taxuspines A ~ H and J ~ Z (**1** ~ **25**), have been isolated together with 37 known taxoids (**26** ~ **62**) including taxol (**42**) from the Japanese yew *Taxus cuspidata* Sieb. *et* Zucc. These new taxoids possessed various skeletons containing 5/7/6, 6/10/6, 6/5/5/6, 6/8/6, or 6/12-membered ring systems. Among the new taxoids, some non-taxol-type compounds remarkably reduced $CaCl_2$-induced depolymerization of microtubules, or increased cellular accumulation of vincristine in multidrug-resistant tumor cells as potent as verapamil. Here we describe our recent results on the isolation, structure elucidation, and bioactivity of these new and known taxoids as well as recent development of taxol biosynthesis.

1. INTRODUCTION

On account of promising anticancer activity of taxol (**42**) and some related compounds, considerable attention has been recently given to the taxoids. Chemical studies on constituents of different yew trees have resulted in isolation of a large number of new taxoids.[1,2] Taxol (**42**) exhibits potent antitumor activity against different cancers which have not been effectively treated by existing antitumor drugs. This is due to unique activity of taxol (**42**) that it inhibits remarkably microtubule depolymerization process. Many researchers have reported that an *N*-acylphenylisoserine group at C-13 and an oxetane ring at C-4 and C-5 should play important roles for this unique activity of taxol

(**42**). Recent reports[3] on clinical trials of taxol (**42**), however, have disclosed that this hightly effective drug is inactive against colon cancer and renal cell carcinoma, which are originated from tissues that express constitutively the MDR1 gene. Thus, the observed lack of activity against these tumors could be partly due to the fact that this drug is subjected to multidrug-resistance (MDR).[3,4] In our search for new bioactive taxoids, we examined extracts of the Japanese yew *Taxus cuspidata* Sieb. *et* Zucc. and obtained new taxoids, taxuspines A ~ H and J ~ Z (**1** ~ **25**),[5~12] together with known taxoids (**26** ~ **62**)[13] containing taxol (**42**) and taxol-type compounds (**43** ~ **50**) with an *N*-acylphenylisoserine group at C-13 and an oxetane ring at C-4 and C-5. Among the taxoids, non-taxol-type compounds (**4**) and (**38**) were found to be remarkably reduced $CaCl_2$-induced depolymerization of microtubules. On the other hand, some non-taxol-type taxoids increased cellular accumulation of vincristine (VCR) in MDR tumor cells, while taxol (**42**) and taxol-type compounds did not show such an activity. The present review provides our recent results on the isolation, structure elucidation, and bioactivity of these new taxoids, as well as recent development of biosynthetic study of taxol (**42**) and findings of new microtubule-stabilizing natural compounds with a completely different structure from taxol (**42**).

2. ISOLATION AND STRUCTURES OF NEW TAXOIDS

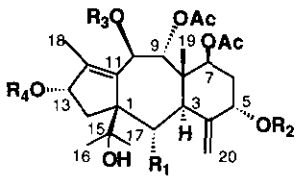
The methanolic extract of stems and leaves of the yew collected at Sapporo was partitioned between toluene and water, and then aqueous layer was partitioned with $CHCl_3$. The toluene and $CHCl_3$ soluble portions were subjected to a silica gel column followed by reversed-phase and silica gel column chromatographies and centrifugal partition chromatography to afford taxuspines A ~ H and J ~ Z (**1** ~ **25**) together with known taxoids (**26** ~ **62**) (Figure 1).

1) 11(15→1)-Abeotaxanes (5/7/6-membered ring system)

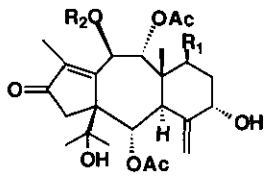
Taxuspine A (**1**), $C_{42}H_{48}O_{11}$, was isolated in 0.0017% yield from stems of the yew. Detailed analyses of the 2D NMR data (^1H-^1H COSY, HMQC, and HMBC) led to the

structure of **1** which was constructed from a 5/7/6-membered ring system with a hydroxyisopropyl, a cinnamoyl, a benzoyl, and three acetyl groups. The relative stereochemistry of a hydroxyisopropyl, a cinnamoyl, three acetyl, and a benzoyl groups at C-1, C-5, C-7, C-9, C-10, and C-13 and the ring conformation in **1** was elucidated on the basis of the NOESY data and ^{1}H-^{1}H coupling constants (Figure 2). Taxuspines J (**9**), M (**12**), O (**14**), and Y (**24**) were also assigned as 11(15→1)-abeotaxanes on the basis of 2D NMR data. Taxuspines J (**9**) and M (**12**) were 10-debenzoyl-10-acetyltaxuspine A and 10-debenzoyltaxuspine A, respectively, while taxuspines O (**14**) and Y (**24**) contained a carbonyl group at C-13. Taxuspine Q (**16**) was also an 11(15→1)-abeotaxane with an oxetane ring at C-4 and C-5 and a tiglic acid group at C-10.

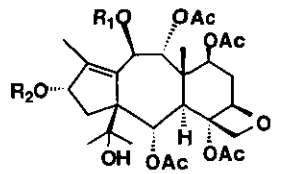
5/7/6-Membered ring system



1 R_1=H, R_2=*trans*-COCH=CHPh, R_3=Bz, R_4=Ac
9 R_1=H, R_2=*trans*-COCH=CHPh, R_3=R_4=Ac
12 R_1=H, R_2=*trans*-COCH=CHPh, R_3=H, R_4=Ac
52 R_1=OAc, R_2=R_4=H, R_3=Bz
53 R_1=OAc, R_2=H, R_3=Bz, R_4=Ac

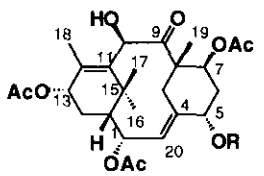


14 R_1=OH, R_2=Ac
24 R_1=H, R_2=Bz



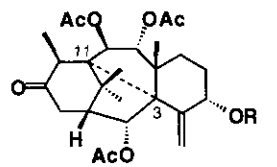
16 R_1=tigloyl, R_2=H
51 R_1=Bz, R_2=*trans*-COCH=CHPh
56 R_1=Ac, R_2=H
57 R_1=Bz, R_2=H

6/10/6-Membered ring system



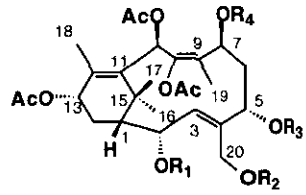
2 R=*trans*-COCH=CHPh
22 R=H
58 R=COCH(OH)CH(NMe_2)Ph

6/5/5/6-Membered ring system



3 R=*trans*-COCH=CHPh
8 R=$COCH_2CH(NMe_2)Ph$
63 R=*cis*-COCH=CHPh

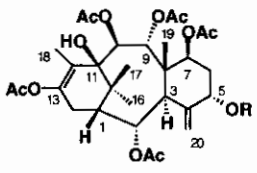
6/12-Membered ring system



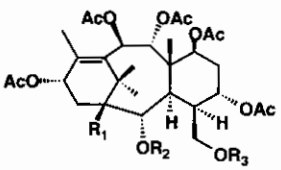
20 R_1=R_2=R_4=H, R_3=Ac
23 R_1=R_2=R_4=Ac, R_3=*trans*-COCH=CHPh

Figure 1

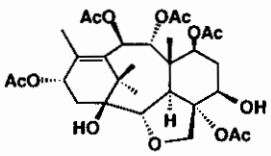
6/8/6-Membered ring system



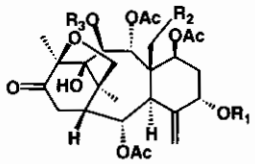
4 R=*trans*-COCH=CHPh
15 R=COCH$_2$CH(NMe$_2$)Ph



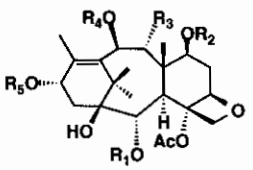
11 R$_1$=R$_3$=H, R$_2$=Ac
17 R$_1$=OH, R$_2$=H, R$_3$=Ac



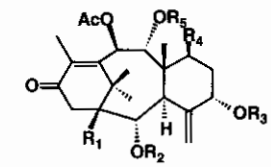
10



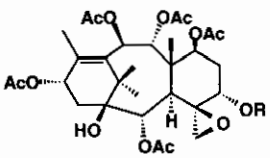
18 R$_1$=*trans*-COCH=CHPh, R$_2$=OH, R$_3$=Ac
19 R$_1$=*trans*-COCH=CHPh, R$_2$=OAc, R$_3$=H
38 R$_1$=*trans*-COCH=CHPh, R$_2$=H, R$_3$=Ac
39 R$_1$=*trans*-COCH=CHPh, R$_2$=OBz, R$_3$=Ac
40 R$_1$=H, R$_2$=H, R$_3$=Ac
41 R$_1$=H, R$_2$=OBz, R$_3$=Ac
55 R$_1$=H, R$_2$=OAc, R$_3$=Ac



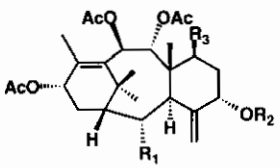
5 R$_1$=Bz, R$_2$=Ac, R$_4$=R$_5$=H, R$_3$=OH
13 R$_1$=R$_2$=Ac, R$_3$=OAc, R$_4$=Bz, R$_5$=COCH$_2$CH(NMe$_2$)Ph
59 R$_1$=Bz, R$_2$=R$_4$=R$_5$=H, R$_3$=O



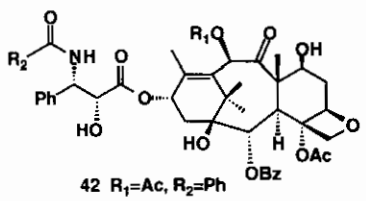
6 R$_1$=R$_3$=H, R$_2$=R$_5$=Ac, R$_4$=OAc
7 R$_1$=R$_2$=R$_3$=R$_4$=H, R$_5$=Ac
26 R$_1$=R$_4$=H, R$_2$=R$_5$=Ac, R$_3$=*trans*-COCH=CHPh
27 R$_1$=R$_3$=R$_4$=H, R$_2$=R$_5$=Ac
28 R$_1$=H, R$_2$=R$_5$=Ac, R$_3$=*trans*-COCH=CHPh, R$_4$=OAc
29 R$_1$=OH, R$_2$=R$_5$=Ac, R$_3$=*trans*-COCH=CHPh, R$_4$=H
30 R$_1$=H, R$_2$=R$_5$=Ac, R$_3$=COCH$_2$CH(NMe$_2$)Ph, R$_4$=H
54 R$_1$=OH, R$_2$=R$_5$=Ac, R$_3$=COCH$_2$CH(NMe$_2$)Ph, R$_4$=H
61 R$_1$=OH, R$_2$=R$_5$=Ac, R$_3$=R$_4$=H
62 R$_1$=R$_4$=R$_5$=H, R$_2$=Ac, R$_3$=*trans*-COCH=CHPh



21 R=H
37 R=Ac



25 R$_1$=OH, R$_2$=COCH$_2$CH(NMe$_2$)Ph, R$_3$=H
31 R$_1$=H, R$_2$=COCH$_2$CH(NMe$_2$)Ph, R$_3$=OAc
32 R$_1$=H, R$_2$=*trans*-COCH=CHPh, R$_3$=H
33 R$_1$=H, R$_2$=*trans*-COCH=CHPh, R$_3$=OAc
34 R$_1$=R$_3$=H, R$_2$=COCH$_2$CH(NMe$_2$)Ph
35 R$_1$=OAc, R$_2$=Ac, R$_3$=H
36 R$_1$=R$_3$=OAc, R$_2$=H
60 R$_1$=OH, R$_2$=H, R$_3$=OAc



42 R$_1$=Ac, R$_2$=Ph
43 R$_1$=H, R$_2$=Ph
44 R$_1$=Ac, R$_2$=C$_4$H$_7$
45 R$_1$=H, R$_2$=C$_4$H$_7$
46 R$_1$=Ac, R$_2$=*n*-C$_5$H$_{11}$
47 R$_1$=H, R$_2$=*n*-C$_5$H$_{11}$
48 R$_1$=Ac, R$_2$=*n*-C$_3$H$_7$
49 R$_1$=H, R$_2$=Ph, C-7 epimer
50 R$_1$=Ac, R$_2$=Ph, C-7 epimer

Figure 1 (continued)

2) 2(3→20)-Abeotaxanes (6/10/6-membered ring systems)

Taxuspine B (**2**), $C_{35}H_{42}O_{10}$, was obtained in 0.00097% yield, and its structure consisting of a 6/10/6-membered ring system with a cinnamoyl, a carbonyl, and three

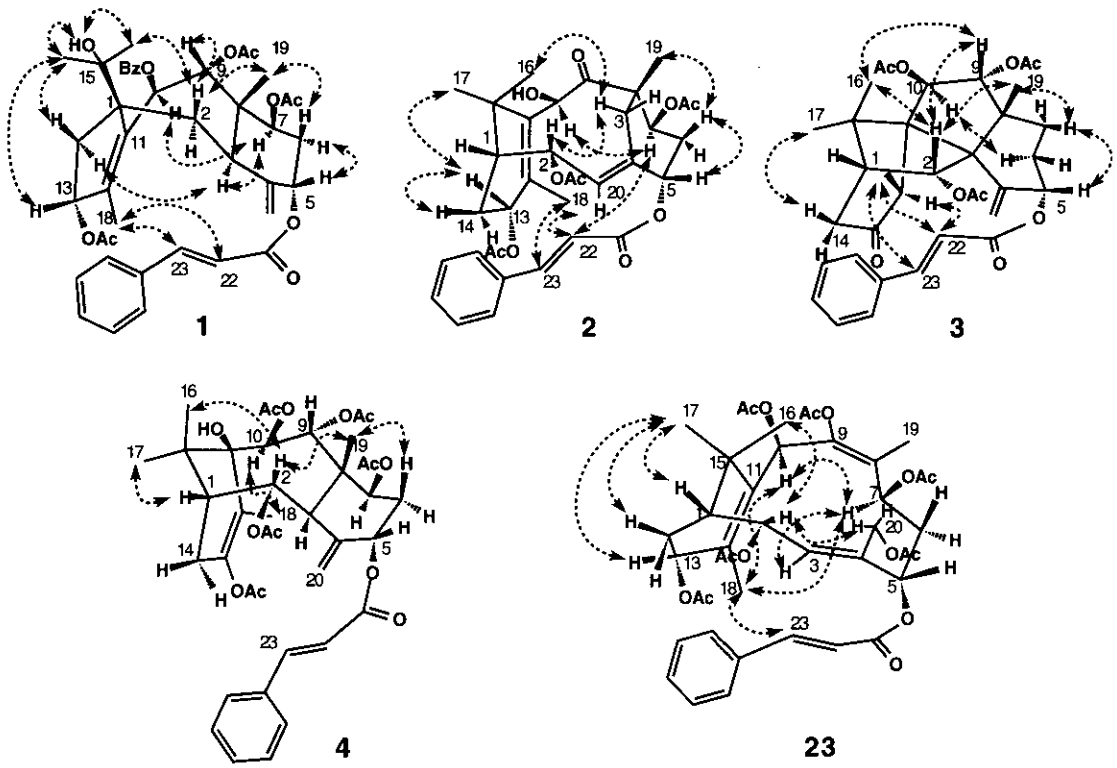


Figure 2. Stereostructures of taxuspines A ~ D (**1** ~ **5**) and X (**23**)
Dotted arrow denote NOESY correlation

acetyl groups was elucidated by applying several types of 2D NMR techniques. The relative stereochemistry of the functional groups and the ring conformation in **2** were elucidated on the basis of the NOESY data and ^{1}H-^{1}H coupling constants (Figure 2). Taxuspine W (**22**) was assigned to be a 5-decinnamoyltaxuspine B by NMR data, possessing a marked caged conformation with an hydrogen bonding between the hydroxy group at C-5 and the acetyl group at C-13.

3) 3,11-Cyclotaxanes (6/5/5/6-membered ring system)

Taxuspine C (**3**), $C_{35}H_{42}O_9$, was obtained in 0.0017% yield. The structure of **3** was interpreted by the extensive analysis of its spectroscopic data to be composed of a 6/5/5/6-membered ring system containing a cinnamoyl, a carbonyl, and three acetyl

groups. Relative stereochemistry of **3** was elucidated by the NOESY spectrum and ^{1}H-^{1}H coupling constants (Figure 2). The absolute stereochemistry of **3** was established by the following photochemical reaction[14]; irradiation of taxinine (**26**), of which absolute stereochemitry has been determined by X-Ray analysis,[15] in dioxane with an Hg-lamp gave rise to compounds (**3**) and (**63**), the latter is 22-*Z*-form of **3** (Figure 3). All spectral data ($[\alpha]_D$, ^{1}H and ^{13}C NMR, IR, UV, and EIMS) of **3** derived from taxinine (**26**) were identical with those of taxuspine C (**3**). Thus the absolute stereochemistry of taxuspine C (**3**) was concluded to be the same as that of taxinine (**26**). The rearrangement, which formally involves a hydrogen transfer from C-3 to C-12 and bond formation between C-3 and C-11, might be a triplet reaction. A concerted $\sigma_s^2 + \pi_s^2$ route was proposed to the photochemical reaction.[14] Taxuspine H (**8**) was elucidated to be a 5-decinnamoyl-5-(3-*N*,*N*-dimethylamino-3-phenylpropanoyl)taxuspine C by NMR data and photochemical reaction from taxine II (**30**) into **8**.

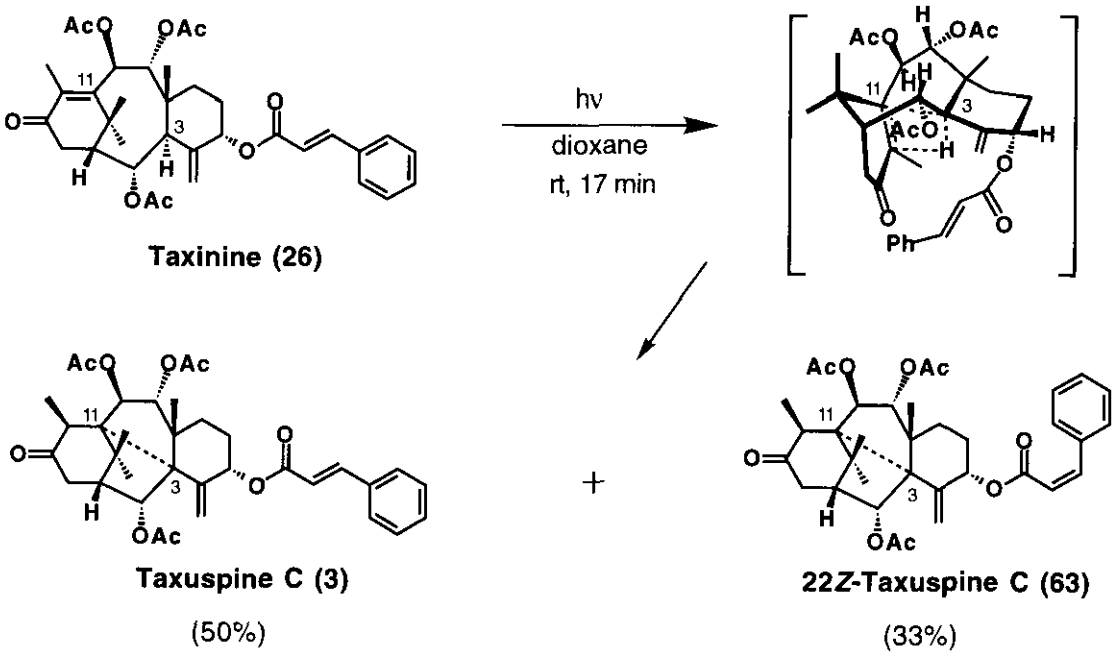
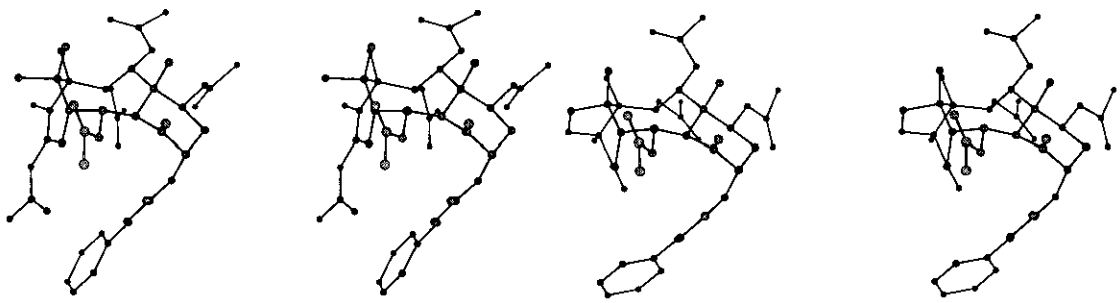


Figure 3. Photochemical reaction of taxinine (**26**)

4) Taxanes (6/8/6-membered ring system)

Most taxoids have a 6/8/6-membered ring system. A known major taxoid, taxinine (**26**)[16], has been isolated from the Japanese yew *Taxus cuspidata* Sieb. *et* Zucc. The structures of taxuspines F (**6**) and G (**7**) were elucidated by comparison of the NMR data with taxinine (**26**). These compounds have a cyclohexenone (ring A) and C-4(20)-exomethylene. Taxuspines D (**4**) and P (**15**) are the first examples of taxoids involving an enol acetate moiety in ring A. The conformation of ring B in these compounds was different from those of other taxoids having a 6/8/6-membered ring system, judging from comparison of the ^{1}H-^{1}H coupling constants ($J_{9,10}$ = 4.8 Hz) of **4** and **15** with those ($J_{9,10}$ = *ca.* 10 Hz) of other taxanes such as taxinine (**26**) and **27** ~ **36** (Figures 2 and 4). Taxuspines L (**11**) and R (**17**) were rare example of taxoids involving a hydroxymethyl group at C-4. Taxuspine K (**10**) was the first example of taxoids containing a 6/8/6-membered ring system with a tetrahydrofuran ring at C-2, C-3, C-4,



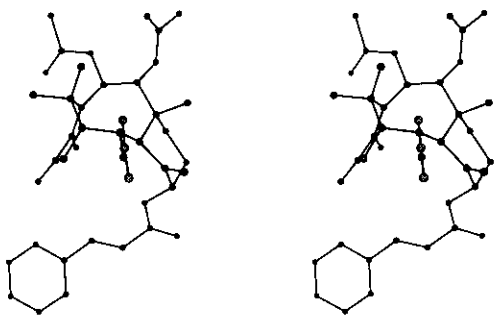


Figure 4. Three-dimensional structures of **4**, **26**, and **38** by MM2 calculation

and C-20, while it has been reported that taxoids containing an oxetane ring at C-4 and C-5 could be converted into taxoids with a tetrahydrofuran ring at C-2, C-3, C-4, and C-20.[17] A chair-like conformation of ring B in **10** was deduced from the coupling constant (4.5 Hz) between H-9 and H-10 and NOESY correlations. Taxuspines S (**18**) and T (**19**) have a tetrahydrofuran ring in ring A, which were similar to taxagifine (**38**). The conformation of ring B in **18** and **19** was similar to those of taxuspines D (**4**) and P (**15**), judging from ^{1}H-^{1}H coupling constant ($J_{9,10}$ = 2.8 Hz for **18** and $J_{9,10}$ = 3.8 Hz for **19**) and NOESY correlations (Figure 4). Taxuspines E (**5**) and N (**13**) possessed an oxetane ring between C-4 and C-5 whose structures were similar to that of 10-deacetylbaccatin III (**59**), while **5** and **13** differed in the functional group at C-9 (a hydroxy and an acetoxy group for **5** and **13**, respectively; a carbonyl group for 10-deacetylbaccatin III (**59**)). Taxuspine N (**13**) was the first example of taxoids containing a 6/8/6-membered ring system with a 3-*N*,*N*-dimethylamino-3-phenylpropanoyl group at C-13 from yew trees. Taxuspines H (**8**), N (**13**), P (**15**), and Z (**25**) contained a 3-*N*,*N*-dimethylamino-3-phenylpropanoyl group (Winterstein's acid). The absolute stereochemistry of this group was determined to be all *R* by chiral HPLC analysis (SUMICHIRAL OA-5000) of the acid hydrolysates of taxuspines H (**8**), N (**13**), P (**15**), and Z (**25**) (Fig. 5). The structure of taxuspine V was assigned to be **21** by NMR data and acetylation of **21** into 1β-hydroxybaccatin I (**37**).[18]

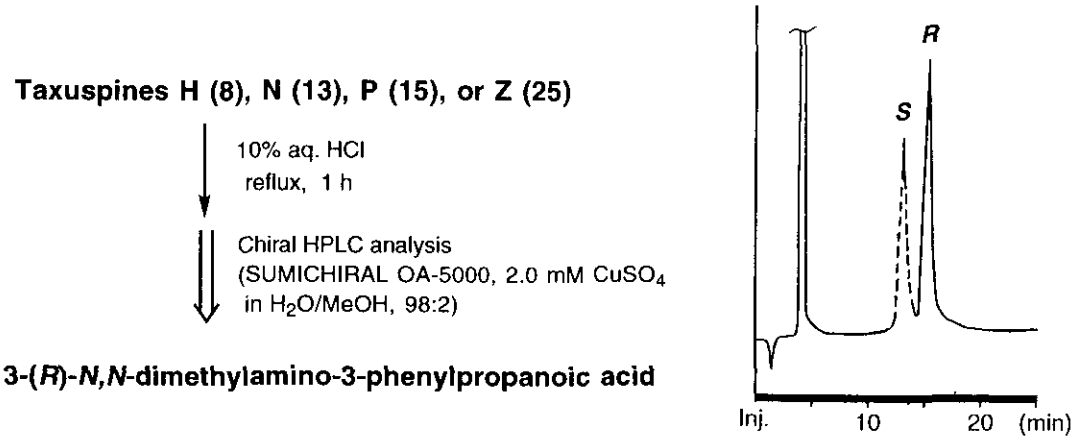


Figure 5. Chiral HPLC analysis of 3-*N*,*N*-dimethylamino-3-phenylpropanoic acid in taxoids

5) Bicyclic taxane related diterpenes (6/12-membered ring system)

Taxuspine U (**20**), $C_{28}H_{40}O_{11}$, was isolated in 0.000017% yield from stems of the yew. Analyses of the ^1H and ^{13}C NMR data and HMQC spectrum of **20** provided four acetyls, five oxymethines, one oxymethylene, one trisubstituted olefin, two tetrasubstituted olefins, and four methyl groups. Since seven out of nine unsaturations were thus accounted for, **20** was inferred to contain two rings. Detailed analyses of the 2D NMR data (^1H-^1H COSY, HMQC, and HMBC) led to the structure of taxuspine U (**20**) as constructed from a 6/12-membered ring system. NOESY correlations (Figure 6) of H-1/H-14a, H-1/H_3-17, H-13/H-14a, and H-13/H_3-17 indicated a boat conformation of ring A, while those of H-1/H-2 and H-2/H3-16 implied that a hydroxy group at C-2 was α-oriented. The α-orientation of H-10 was deduced from a NOESY correlation of H-10/H_3-18. The relative stereochemistries at C-5 and C-7 were investigated by

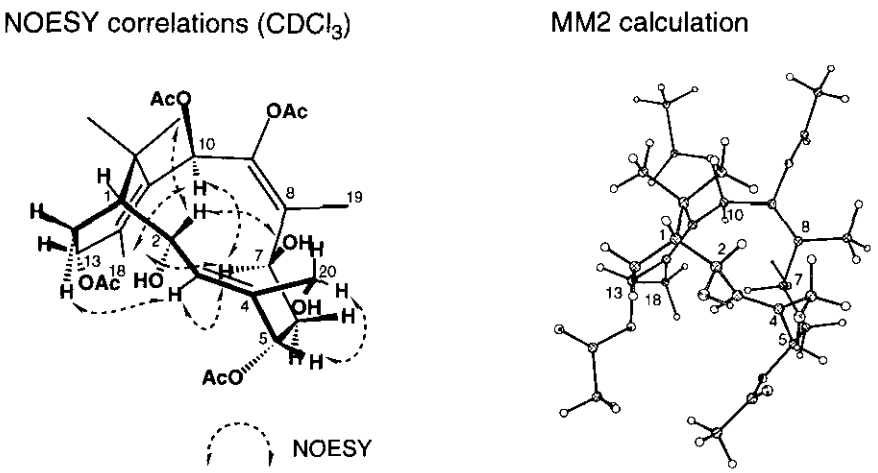


Figure 6. Relative stereochemistry of taxuspine U (**20**)

Table 1. Molecular Mechanics Calculations for Diastereomers (**20a-d**)

diastereomer	H-7/H-10 (Å)	H-7/H-18 (Å)	distance[a] H-5/H-20b (Å)
20a (5*R** 7*S**)	2.42	2.98	2.23
20b (5*R** 7*R**)	3.61	3.70	2.43
20c (5*S** 7*S**)	2.45	2.66	3.61
20d (5*S** 7*R**)	3.70	3.97	3.42

a) Distances for the lowest energy conformers.

combination of the NOESY data and molecular mechanics calculations, in which four diastereomers (**20a**, 5S*7S*; **20b**, 5S*7R*; **20c**, 5R*7S*; **20d**, 5R*7R*) were considered, and systematic conformational searching[19] for each diastereomer was carried out by using Macromodel program.[20] The calculation of the distances between H-7/H-10, H-7/H_3-18, and H-5/H-20b of conformational isomers within 3 kcal/mol molecular energy for each diastereomer (**20a** ~ **20d**) was summarized in Table 1. The distances of H-7/H-10, H-7/H_3-18, and H-5/H-20b for **20a** were within 3.0 Å, while those of **20b** ~ **20d** were over 3.0 Å. These results suggested that relative stereochemistry of ring B in taxuspine U (**20**) was 2R*, 5S*, 7S*, and 10R* (Figure 6). The structure of taxuspine X (**23**) was similar to that of taxuspine U (**20**) except for acetyl groups at C-2, C-7, and C-20 and a cinnamoyl group at C-5 in **23**. It was easy to elucidate the relative stereochemistry of **23** by NOESY correlations (Figure 2). The skeletons of these compounds and related taxoids are similar to that of verticillene (Figure 9).

3. BIOACTIVITY

1) Inhibitory Activity of Ca^{2+}-Induced Microtubule Depolymerization by Taxoids.

Taxol (**42**) is unique among antimitotic drugs in that it promotes the polymerization of tubulin α,β-heterodimers by binding to and stabilizing the resulting microtubule polymer. It differs from antimitotic drugs such as colchicine, podophyllotoxin, and the vinca alkaloids, which inhibit microtubule assembly. Microtubules polymerized in the presence of taxol are resistant to depolymerization by Ca^{2+} ions.[21] The effect of taxoids (**1** ~ **62**) was examined against the $CaCl_2$-induced depolymerization of microtubules. Microtubule proteins were polymerized under normal polymerization condition[22] in the absence and the presence of taxol (**42**) or taxoids (**1** ~ **41**) and (**43** ~ **62**), and, after 30 minutes incubation, $CaCl_2$ was added. Microtubule polymerization and depolymerization were monitored by the increase and the decrease in turbidity. The results were summarized in Fig. 7 as the changes in the relative absorbance at 400 nm. The $CaCl_2$-induced depolymerization of microtubules (shown as control) was completely inhibited by 10 mM of taxol (**42**). Among the tested taxoids, taxuspine D (**4**) and

taxagifine (**38**) remarkably reduced the depolymerization process, suggesting that these compounds have taxol-like activity to microtubule systems. The potency of **4** and **38** in inhibition of the depolymerization process corresponded to half to one third of that of taxol (**42**). Compounds (**2**), (**28**), and (**51**) exhibited moderate activity, while the other compounds showed little or no such effect. On the other hand, taxol-type compounds (**43** ~ **50**) inhibited the depolymerization process as potent as taxol (**42**). It is noted that compounds **4** and **38** lacking both an oxetane ring and an *N*-acylphenylisoserine moiety exhibited potent activity. Since these active compounds (**2**), (**4**), (**28**), and (**38**) possessed a cinnamoyl group at C-5, the cinnamoyl group may play an important role for binding to microtubules like the acyl group at C-13 in taxol (**42**). In addition, the acetoxy group at C-10 and the hydroxy group at C-11 in **4** and **38** may be important in

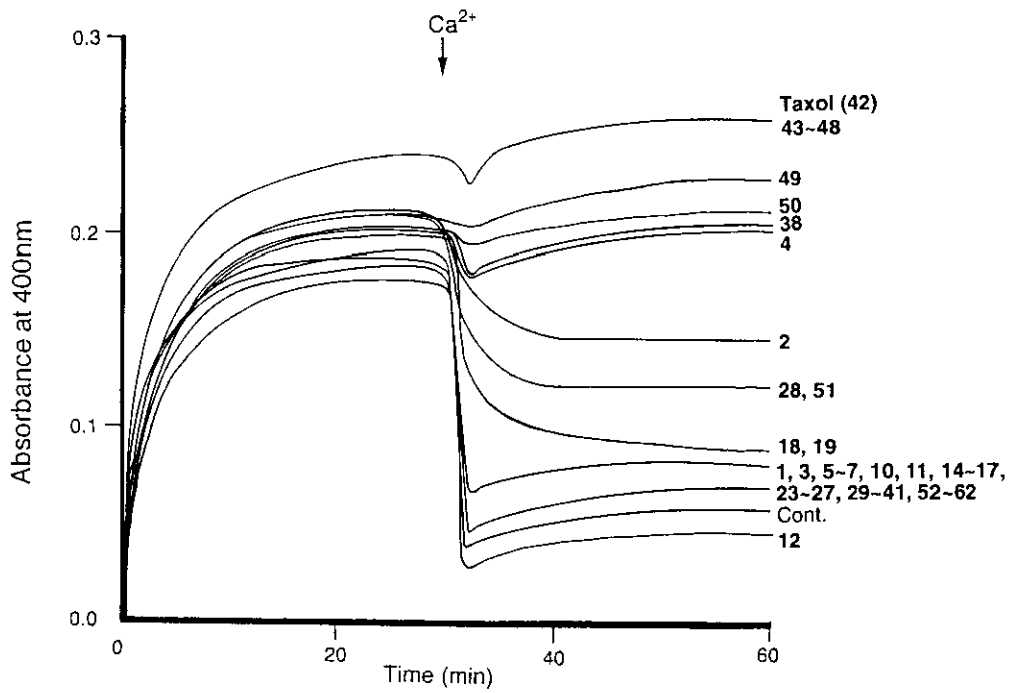


Figure 7. Effects of taxoids on Ca^{2+}-induced microtubule depolymerization. The temperature was held at 37 °C and changes in turbidity were monitored at 400 nm. For the drug-protein studies, 10 mM of drug dissolved in DMSO was added to 1 mL buffer solution containing 2 mg microtubule protein. The final DMSO concentration was less than 1%. After 30 min incubation of the test mixture, 4 mM $CaCl_2$ was added, and the mixture was further incubated for another 30 min. The turbidity changes were monitored throughout the incubation time.

the inhibition of microtubule depolymerization like the oxetane moiety at C-4 and C-5 and the acetoxy group at C-4 in taxol (**42**). It is noted that the conformation of **4** is very similar to that of **38** by macromodel caluculations (Figure 4), while the conformation of **26** is different from those of **4** and **38**. These results suggested that it may be important for inhibition of microtubule depolymerization by both functional groups and the conformation of **4** and **38**.

2) Increased Cellular Accumulation of Vincristine in Multidrug-Resistant Cells by Taxoids.

The cellular accumulation of vincristine (VCR) is reduced in multidrug-resistant (MDR) tumor cells as compared with the parental cells.[23] MDR-reversing agents such as verapamil increase the reduced accumulation of antitumor agents in MDR cells and overcome multidrug resistance.[24,25] The effect of taxoids (**1** ~ **62**) on the cellular accumulation of VCR in multidrug-resistant human ovarian cancer 2780AD cells was examined and the results were shown in Table 2. Verapamil at 1 and 10 μg/mL increased the VCR accumulation in a dose dependent manner. Compounds (**2**, **3**, **9**, **23**, **24**, **30**, **31**, **33**, and **34**) increased the VCR accumulation as potent as verapamil. Compounds (**12**, **25**~**29**, **32**, **39**, **51**, **54**, and **60**) increased moderately the accumulation, while taxol (**42**) and cephalomannine (**44**) decreased the VCR accumulation in 2780AD cells. It is noted that i) the potent active compounds (**2**, **3**, **9**, **23**, **24**, **30**, **31**, **33**, and **34**) possessed a cinnamoyl or 3-*N*,*N*-dimethylamino-3-phenypropanoyl group at C-5; ii) these compounds have neither an oxetane ring nor a phenylisoserine group at C-13. These results suggested that many taxoids could be substrates of P-glycoprotein and some of them might be useful for overcoming multidrug resistance.

3) Competitive Binding of [^{3}H]Azidopine and Taxoids to P-glycoprotein.

Many calcium channel blockers reverse the multidrug-resistant phenotype and inhibit active transport of cytotoxic drugs out of multidrug-resistant cells. Azidopine is a

Table 2. Effects of Taxoids on Accumulation of Vincristine (VCR) in Multidrug-Resistant 2780AD Cells.

Compound	VCR accumulation (% of control) with a taxoid concentration of		Compound	VCR accumulation (% of control) with a taxoid concentration of	
	1 μg/mL	10 μg/mL		1 μg/mL	10 μg/mL
1	142	246	**34**	250	882
2	219	713	**36**	207	363
3	246	768	**37**	119	135
4	133	325	**38**	194	242
6	141	257	**39**	169	537
7	139	271	**40**	225	213
8	174	567	**41**	115	169
9	258	842	**42 (taxol)**	83	56
10	167	234	**44**	88	93
11	177	264	**49**	192	218
12	176	584	**50**	132	138
14	151	191	**51**	219	639
15	133	358	**52**	143	192
16	145	206	**53**	158	313
17	147	208	**54**	175	466
18	136	190	**55**	142	198
19	196	308	**56**	147	235
23	158	774	**57**	171	240
24	173	784	**58**	119	298
25	106	550	**59**	153	271
26	195	571	**60**	205	439
27	153	461	**61**	152	216
28	116	438	**62**	173	372
29	167	436	**Verapamil**	254	739
30	177	704			
31	233	841			
32	102	517			
33	266	798			

a) The amounts of VCR accumulated in multidrug-resistant human ovarian cancer 2780AD cells were determined in the presence of 1 and 10 μg/mL of taxoids. The values represent means of triplicate determinations, and are expressed as the relative amounts of VCR accumulated in the cells as compared with the control experiment.

photoaffinity analog of the dihydropyridine class of calcium channel blockers that specially labels P-glycoprotein. The ability to inhibit photoaffinity labeling of P-glycoprotein by azidopine has frequently been used as an indicator of whether a particular compound is a P-glycoprotein "substrate".[26,27] In particular, it is believed that compounds which compete with azidopine for a common binding site on the multidrug transporter will be able to block photolabeling. As shown in Figure 6, [^{3}H]azidopine labeled P-glycoprotein (170 kDa) that was present in adriamycin-resistant human leukemia K562/ADM cells (lanes 1 and 10), and binding of [^{3}H]azidopine to P-glycoprotein was significantly reduced by the presence of verapamil (lanes 17 and 18). Compounds (**2**, **3**, **30**, **31**, and **33**) reduced remakably binding of [^{3}H]azidopine to P-glycoprotein more potent than verapamil (lanes 2 ~ 5, 8, 9, and 11 ~ 14), while taxinine

(**26**) did not show such activity (lanes 6 and 7) and taxol (**42**) showed quite weak reduction (lanes 15 and 16). Since compounds (**2**, **3**, **30**, **31**, **33**, and **34**) increased the VCR accumulation as potent as verapamil as described above, it is suggested that these taxoids bind to the same binding site on P-glycoprotein as that of azidopine.

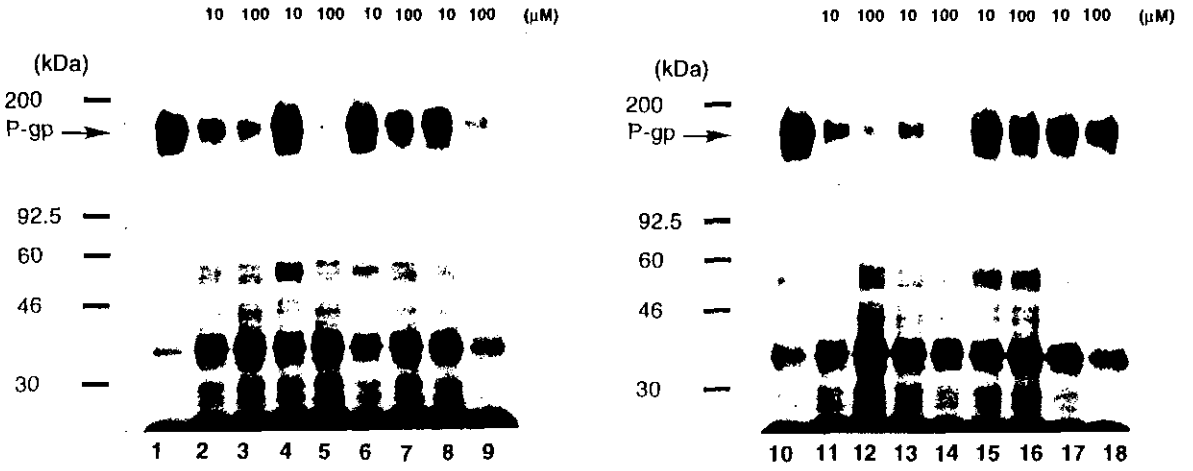


Figure 8. Effect of taxoids on [^{3}H]azidopine photoaffinity labeling of P-glycoprotein. Adriamycin-resistant human leukemia K562/ADM cell membrane vesicles (50 μg of protein) were incubated with [^{3}H]azidopine (200 nM) in the presence of of taxoids (10 μM and 100 μM), taxuspine B (**2**, lanes 2 and 3), taxuspine C (**3**, lanes 4 and 5), taxinine (**26**, lanes 6 and 7), 2-desacetoxyaustrospicatine (**31**, lanes 8 and 9), 2-desacetoxytaxinine J (**33**, lanes 11 and 12), taxine II (**30**, lanes 13 and 14), and taxol (**42**, lanes 15 and 16), and verapamil (lanes 17 and 18). After 1 h at room temperature, the samples were subsequently irradiated with UV light for 30 min. After separation by SDS-polyacrylamide gel electrophoresis, the intensity of P-glycoprotein photolabeling was detected by fluorography; the band with a molecular mass of 170 kDa is due to P-glycoprotein. Taxoid concentrations in μM are indicated along the top of each gel. The position of molecular mass standards in kDa are indicated at the left of gel.

4) Cytotoxicity Studies.

Cytotoxic activity of all the taxoids (**1** ~ **62**) against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells is shown in Table 2. Taxol (**42**) and taxol-type compounds (**43** ~ **50**) exhibited very potent cytotoxicity against KB cells (IC_{50} 0.0015 ~ 0.086 μg/mL). Compounds (**5**, **16**, **18**, **19**, **22**, **38**, **52**, **61**, and **62**) also showed potent cytotoxicity against KB cells (IC_{50} 0.08 ~ 0.86 μg/mL). Compound (**5**) possessing an oxetane ring but no *N*-acylphenylisoserine group was most potent (IC_{50} 0.08 μg/mL) among these taxoids. It was interesting that compounds (**16**, **18**, **19**, **22**,

Table 3. Cytotoxicity of Taxoids (1 ~ 62) against Murine Leukemia L1210 Cells and Human Epidermoid Carcinoma KB Cells

compound	L1210 IC_{50} (μg/mL)	KB IC_{50} (μg/mL)	compound	L1210 IC_{50} (μg/mL)	KB IC_{50} (μg/mL)
1	4.2	>10	**35**	>10	>10
2	>10	>10	**36**	>10	>10
3	5.8	>10	**37**	>10	>10
4	3.0	1.8	**38**	1.3	0.86
5	0.27	0.08	**39**	>10	>10
6	>10	>10	**40**	>10	>10
7	>10	>10	**41**	>10	9.4
8	>10	1.6	**42** (taxol)	0.33	0.0088
9	>10	>10	**43**	0.88	0.015
10	4.5	8.8	**44**	0.25	0.086
11	10.0	4.5	**45**	0.95	0.0048
12	1.2	5.8	**46**	0.21	0.0053
13	7.8	>10	**47**	0.24	0.0017
14	>10	>10	**48**	0.21	0.0016
15	2.5	2.6	**49**	0.71	0.013
16	4.0	0.3	**50**	0.026	0.0015
17	4.6	>10	**51**	3.8	>10
18	3.8	0.26	**52**	>10	0.4
19	1.0	0.06	**53**	>10	>10
22	8.0	0.35	**54**	2.7	>10
23	4.2	>10	**55**	>10	>10
24	5.4	>10	**56**	>10	>10
25	>10	6.2	**57**	>10	>10
26	>10	>10	**58**	7.1	>10
27	8.9	>10	**59**	>10	>10
28	>10	>10	**60**	10.0	7.4
29	4.6	6.9	**61**	6.2	0.60
30	>10	>10	**62**	1.1	0.17
31	7.2	>10			
32	9.5	8.2			
33	4.9	>10			
34	>10	>10			

38, **52**, **61**, and **62**) without an oxetane ring and an *N*-acylphenylisoserine moiety exhibited such cytotoxicity. These cytotoxic compounds (**16**, **18**, **19**, **22**, **38**, **52**, **61**, and **62**) possessed an acetoxy group at C-2, while the other functional groups were different from one another. Combination of the acetoxy group at C-2 and the other functional groups such as an oxetane ring may be important for cytotoxicity against KB cells, as many researchers have pointed out that no northern part (positions 7, 9, and 10) but the southern part (positions 1, 2, 4, and 5) of taxoids is intimately associated with its cytotoxicity.[1]

5) Biogenetical Consideration

Since new taxoids, taxuspines A ~ H and J ~ Z (**1** ~ **25**), from Japanese yew *T. cuspidata* possessed various skeletons, we propose the plausible biogenesis of these taxoids. The

biosynthesis of taxane skeleton (6/8/6-membered ring system) has recently been suggested to involve cyclization of geranylgeranyl diphosphate to taxa-4(5),11-diene by Croteau *et al.*[28] as described the next section, while the pathway of 3,11-cyclotaxanes (6/5/5/6-membered ring system) has been proposed to be derived from taxanes by concerted $\sigma_S^2 + \pi_S^2$ route.[14] The 11(15→1)-abeotaxanes (5/7/6-membered ring system) may be biogenetically generated through a Wagner-Meerwein rearrangement of ring A in 6/8/6-membered ring system.[29] On the other hand, the 2(3→20)-abeotaxanes (6/10/6-membered ring system) seem to be derived from verticillene through the cyclization of $\Delta^{4(20),7}$-verticillene.[30] The bicyclic taxane-related compounds (6/12-

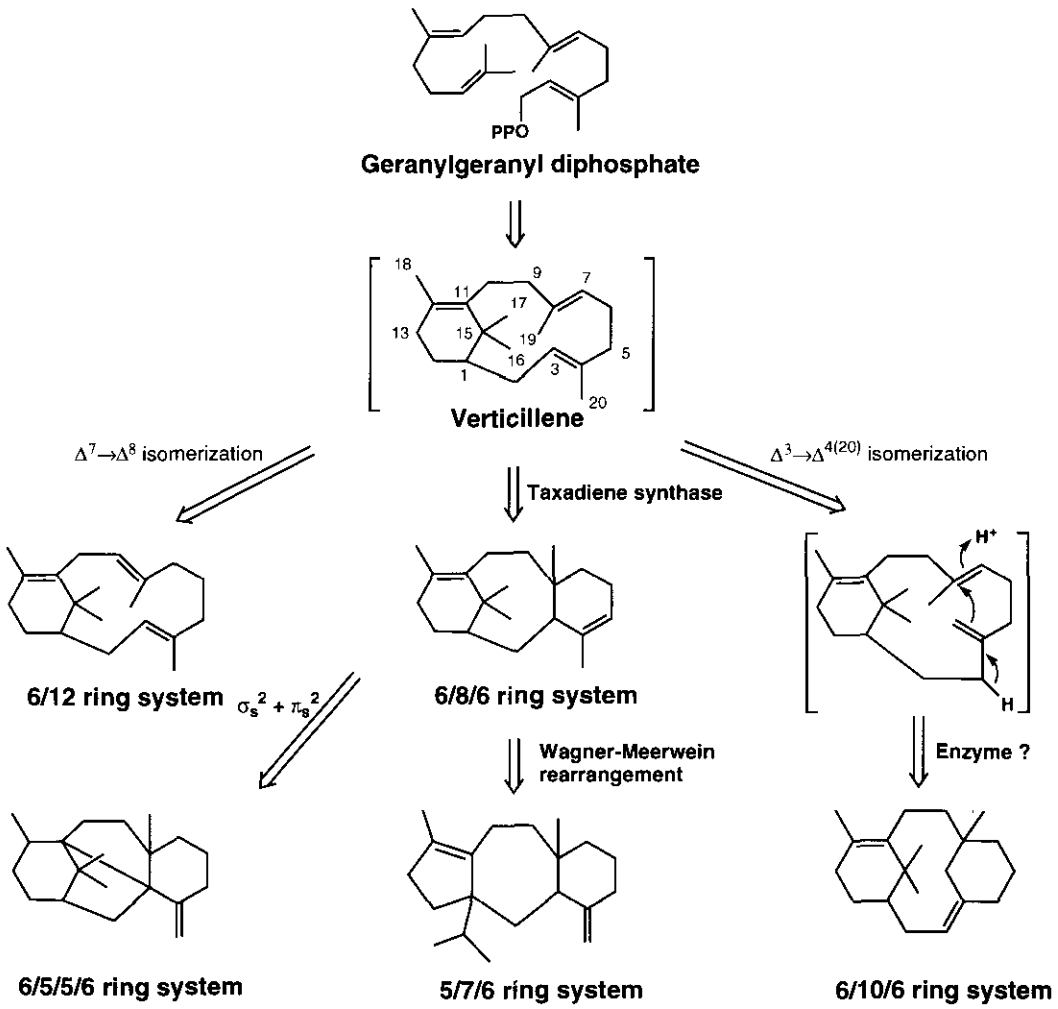


Figure 9. Plausible biogenesis of taxoids

membered ring system) might be derived from verticillene.[31]

6) Conclusive Remarks

Taxol (**42**) and taxol-type compounds (**43** ~ **50**) exhibited quite potent inhibition of the microtubule depolymerization and extremely potent cytotoxicity, while non-taxol-type compounds (**2**, **4**, **28**, **38**, and **51**) inhibiting microtubule depolymerization were not cytotoxic or not so cytotoxic as taxol. Some taxoids (**2**, **3**, **23**, **24**, **30**, **31**, and **33**) inhibiting binding of azidopine to P-glycoprotein in adriamycin-resistant K562/ADM cells increased the cellular accumulation of vincristine in multidrug-resistant 2780AD cells as potent as verapamil. In addition these taxoids exhibited weak or no cytotoxicity. From these results, it is suggested that some taxoids can be a good modifier of multidrug resistance in cancer chemotherapy.

4. BIOSYNTHESIS

The biosynthesis of taxol and related taxoids is thought to involve the cyclization of geranylgeranyl diphosphate to a taxadiene followed by extensive oxygenation of this diterpene olefin intermidiate. On the other hand, the biosynthetic pathway of the side chain at C-13 in taxol and related taxoids was suggested by the detailed feeding studies

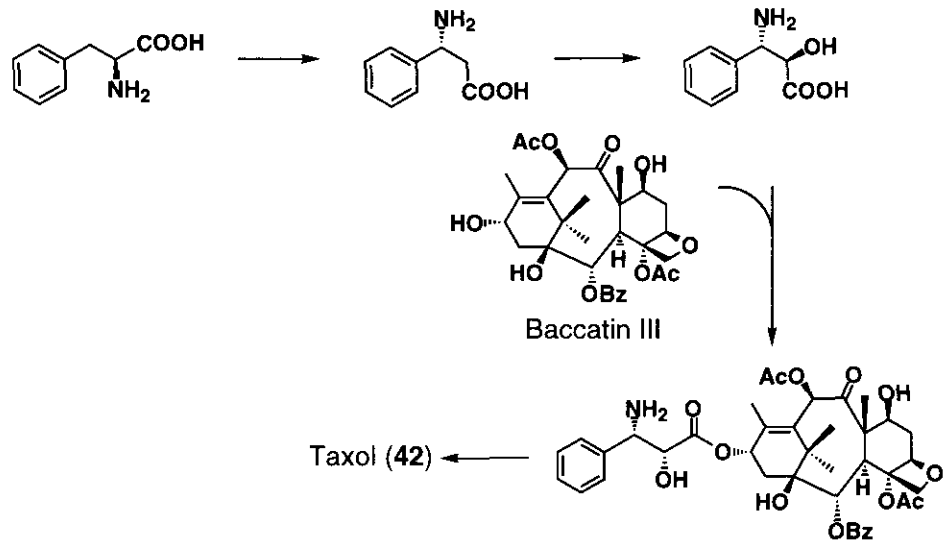


Figure 10

by Floss and colleagues with advanced metabolites that have demonstrated the origin and timing of assembly of the *N*-benzoyl phenylisoserine ester.[32] Thus baccatin III was shown to be a specific precursor of taxol with side chain added most likely as phenylisoserin (*via* phenylalanine) followed by *N*-benzoylation as the last step in taxol formation (Figure 10).

Recently, Croteau and collabolators found taxadiene synthase, a diterpene cyclase that catalyzed the first committed step of taxol biosynthesis.[28] A cell-free preparation from sapling yew stems catalyzed the conversion of [1-^{3}H]geranylgeranyl diphosphate to a cyclic diterpene olefin that, when incubated with stem sections, was converted in good radiochemical yield to several highly functionalized taxanes, including 10-deacetylbaccatin III and taxol itself. Addition of the labeled olefin to a yew bark extract, followed by radiochemically guided fractionation, provided sufficient product to establish the structure as taxa-4(5),11(12)-diene (**e**) by 2D NMR spectroscopic data. Therefore, the first dedicated step in taxol biosynthesis is the conversion of the universal diterpenoid precursor geranylgeranyl diphosphate to taxa-4(5),11(12)-diene (**e**), rather than to the 4(20),11(12)-diene isomer (**f**) previously suggested on the basis of the

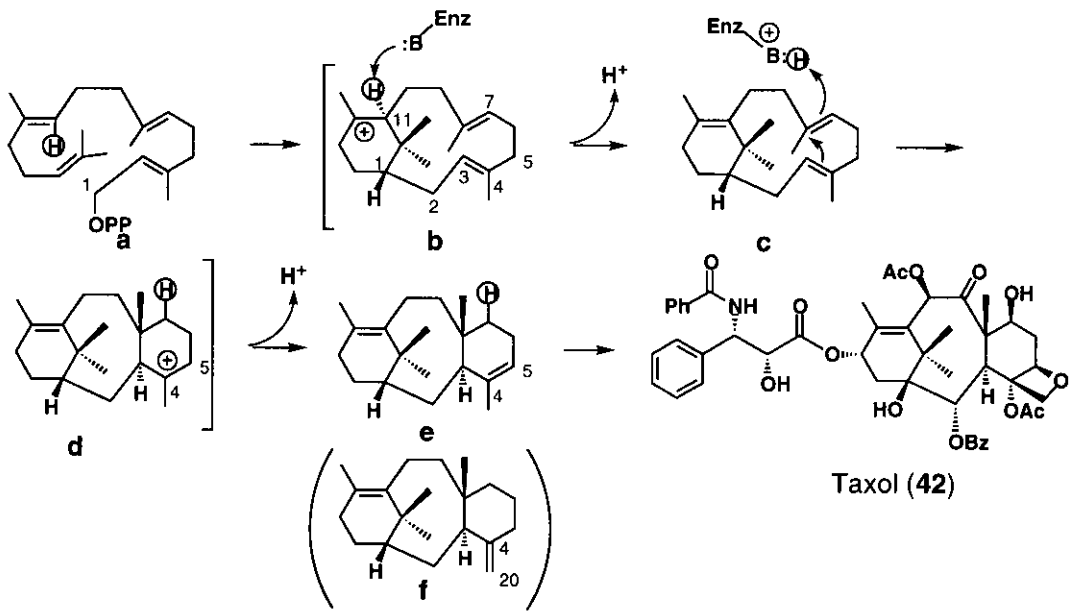


Figure 11

abundance of taxoids with double bonds in these positions (Figure 11).

In 1996, Zenk and collaborators proposed that the taxane carbon skeleton is not of mevalonoid origin.[33] A cell culture of *Taxus chinensis* was established to produce the diterpene taxayunanine C in 2.6% (dry weight) yield. The incorporation of [U-$^{13}C_6$]glucose, [1-^{13}C]glucose, and [1,2-$^{13}C_2$]acetate into taxayunanine C was analyzed by NMR spectroscopy. Label from [1,2-$^{13}C_2$]acetate was diverted to the four acetyl groups of taxuyunanine C (Figure 12A), but not to the [U-$^{13}C_6$]glucose was efficiently incorporated into both the taxane ring system and the acetyl groups (Figure 12B). The four isoprenoid moieties of the diterpene showed identical labeling patterns. The analysis of long-range ^{13}C-^{13}C couplings in taxuyunnanine C obtained from an experiment with [U-$^{13}C_6$]glucose documents the involvement of an intramolecular rearrangement in the biosynthesis of the isoprenoid precursor. The labeling patterns are inconsistent with mevalonate pathway.

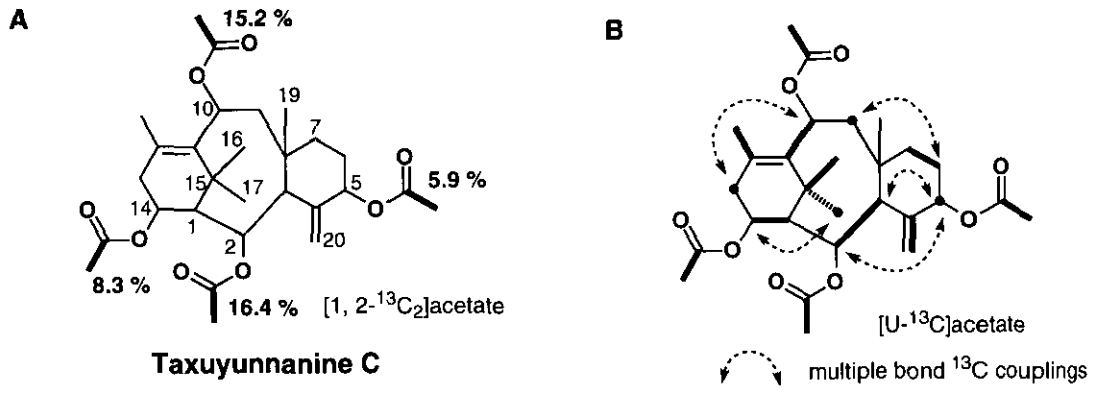
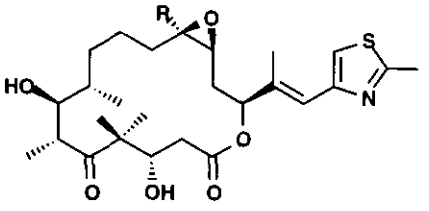


Figure 12

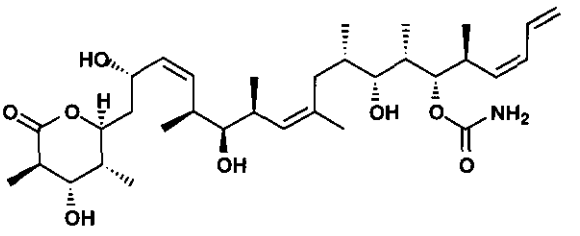
5. MICROTUBULE-STABILIZING AGENT WITH A TAXOL-LIKE MACHANISM OF ACTION

Tubulin polymerization into microtubules is a dynamic process, with the equilibrium between growth and shrinkage being essential for many cellular process. The antineoplastic agent taxol hyperstabilizes polymerized microtubules, leading to mitotic arrest and cytotoxicity in proliferrating cells. Recently, three non-taxoid compounds

have also been shown to stabilize microtubules. The macrolides, epothilones A and B (Figure 13),[34] from the bacterium *Sorangium celulosum*, and the polyhydroxylated alkatetraene lactone discordermolide (Figure 13), from the marine sponge *Discordermia dissoluta*, share with taxol the ability to arrest cells in mitosis, cause formation of bundles of intracellular microtubules in non-mitotic cells, and induce the formation of hyperstable tubulin polymer. Epothilones A and B are equipment and exhibit kinetics similar to taxol in inducing tubulin polymerization into microtubules *in vitro* (filtration, light scattering, sedimentation, and electron microscopy) and in producing enhanced microtubule stability and bundling in cultured cells. Furthermore, these 16-membered macrolides are competitive inhibitors of [^{3}H]taxol binding, exhibiting a 50% inhibitory concentration almost identical to that of taxol in displacement competition assays. Epothilones A and B also cause cell cycle arrest at the G_2-M transition leading to cytotoxicity, similar to taxol. In contrast to taxol, epothilones A and B retain a much greater toxicity against P-glycoprotein-expressing multiple drug resistant cells. In the taxol-sensitive human cell lines, epothilone B had greater antiproliferative activity than epothilone A or taxol, while epothilone A was usually less active than taxol.[35] After the X-Ray crystal structure of the epothilones was determined, organic chemsts began to pursue strategies for synthesis of the compounds. Several groups rushed into the total synthesis and it was a very close finish among three groups.[36]



Epothilone A (R = H)
Epothilone B (R = Me)



Discordermolide

Figure 13

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taxagifine (**38**), taxacin (**39**), decinnamoyltaxagifine (**40**), taxinine M (**41**), taxol (**42**), 10-deacetyltaxol (**43**), cephalomannine (**44**), 10-deacetylcephalomannine (**45**), taxol C (**46**), 10-deacetyltaxol C (**47**), taxol D (**48**), 7-*epi*-10-deacetyltaxol (**49**), 7-*epi*-taxol (**50**), taxchinin B (**51**), brevifoliol (**52**), 13-acetylbrevifoliol (**53**), diacyltaxinine B (**54**), 19-debenzoyl-19-acetyltaxinine M (**55**), taxacustin (**56**), taxayuntin (**57**), 7-*O*-acetyltaxine A (**58**), 10-deacetylbaccatin III (**59**), 2-deacetyl-5-decinnamoyltaxinine J (**60**), triacetyltaxicin I (**61**), and 2,9-dideacetyltaxinine (**62**).

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