Biological Activity and Chemistry of Taxoids from the Japanese Yew, *Taxus cuspidata*[⊥]

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Approximately 120 taxoids have been isolated to date from the Japanese yew, *Taxus cuspidata*. These taxoids possess various skeletons containing 5/7/6-, 6/10/6-, 6/5/5/6-, 6/8/6-, or 6/12-membered ring systems. Among the taxoids, some non-paclitaxel-type compounds have been shown to reduce $CaCl_2$-induced depolymerization of microtubules, increase cellular accumulation of vincristine in multidrug-resistant tumor cells, and exhibit potent cytotoxicity. Chemical derivatization of taxoids of *T. cuspidata* is also reviewed.

Intoduction

Yew trees of the genus *Taxus* (Taxaceae) are dioecious and evergreen plants mainly distributed in the northern hemisphere. According to the classific method of Krussmann, the genus *Taxus* may be divided into eight natural species and two artifact species: *T. baccata* L., *T. wallichiana* Zucc., *T. celebica* Li, *T. cuspidata* Sieb. et. Zucc., *T. brevifolia* Nutt., *T. globosa* Shlechtd., *T. floridana* Nutt., and *T. canadensis* Marsh., and *T.* x *media* Rehd. and *T.* x *hunnewelliana* Rehd.[1] As early as the first century B.C., initial interest in the constituents of yew trees was sparked by the known toxicity of *T. baccata* L.[2] The first chemical study of *Taxus* species was carried out by Lucas in 1856,[3] and an ill-defined mixture of alkaloids named "taxine" was isolated. The main constituents of yew trees are diterpenoids called taxoids, generally containing a 6/8/6-membered ring skeleton, which has been named the "taxane" skeleton. According to IUPAC nomenclature, the taxane skeleton is that of [9.3.1.0.[3,8]] pentadecene.[4] In the 1960s, Wall and Wani were searching for anticancer compounds in higher plants and detected cytotoxic activity in an extract of the bark of *T. brevifolia* and reported the isolation and structure elucidation of taxol.[5] Since the discovery of the promising anticancer activity of paclitaxel (taxol) (**53**) and some related compounds,[6,7] chemical studies on constituents of different yew trees have resulted in isolation of a large number of new taxoids and interesting and novel chemistry have been discovered.[8−12]

The Japanese yew *T. cuspidata* has often been used as a garden tree, and there is evidence of its previous use as a crude drug to treat diabetes, to promote diuresis, and as an emmenagogue. The Japanese yew is classified into two subspecies, *T. cuspidata* Sieb. et Zucc. and *T. cuspidata* Sieb. et Zucc. var. *nana* Rehder. Chemical studies on the constituents of the Japanese yew began in the 1960s, and taxinine (**37**),[13] taxine II (**41**),[14] and taxusin (**119**)[15] were isolated. Since that time, however, studies were rarely carried out until about 1993. Approximately 120 taxoids have been isolated from the Japanese yew during the past 10 years. Our studies on taxoids from the Japanese yew have been reviewed.[16] The present review describes the structures, biological activities, and chemistry of taxoids from the Japanese yew, *T. cuspidata*.

Structures of the Taxoids from Japanese Yew

Approximately 120 new and known taxoids have been isolated from *T. cuspidata*, and their structures were elucidated by spectroscopic data and chemical means.[17−75]

11(15→1)-*abeo*-Taxanes (5/7/6-Membered Ring System). Fourteen taxoids of this class have been isolated in recent years from *T. cuspidata*. Taxuspine A (**1**) was the first 11(15→1)-*abeo*-taxoid from the Japanese yew. Taxuspines J (**9**) and M (**12**) are 10-debenzoyl-10-acetyltaxuspine A and 10-debenzoyltaxuspine A, respectively, while taxuspines O (**14**) and Y (**24**) and taxuspinanane B (**105**) contain a carbonyl group at C-13. Taxuspine Q (**16**), taxchinin B (**62**), taxacustin (**67**), taxayuntin (**68**), and taxuspinanane F (**109**) are also 11(15→1)-*abeo*-taxanes with an oxetane ring at C-4 and C-5. The ^{1}H NMR spectra of these taxoids show broad peaks, indicating that these compounds possess a B-twist-chair/C-boat or B-twist-boat/C-chair configuration (Figure 2).

2(3→20)-*abeo*-Taxanes (6/10/6-Membered Ring Systems). Seven taxoids in this class have been isolated from *T. cuspidata*. Taxuspine B (**2**) possess a 6/10/6-membered ring system with a cinnamoyl, a carbonyl, and three acetyl groups. The relative stereochemistry of the functional groups and the ring conformation in **2** were elucidated on the basis of the NOESY data and from ^{1}H−^{1}H coupling constants (Figure 2). Taxuspine W (**22**) was assigned as 5-decinnamoyltaxuspine B, possessing a marked cage conformation with hydrogen bonding between the hydroxy group at C-5 and the acetyl group at C-13. The side chains at C-5 of 7-*O*-acetyltaxine A (**69**), compound **80**, and compound **93** are 2-hydroxy-3-*N*,*N*-(dimethylamino)-3-phenylpropanoic acid, 3-*N*,*N*-dimethyl-3-phenylpropanoic acid (Winterstein's acid), and a cinnamoyl group, respectively. Compound **88** is a rare taxoid containing a 9,10-diketone moiety.

3,11-Cyclotaxanes (6/5/5/6-Membered Ring System). Five 3,11-cyclotaxanes have been isolated from *T. cuspidata*. Taxuspine C (**3**) is composed of a 6/5/5/6-membered ring system containing a cinnamoyl, a carbonyl, and three acetyl groups. The relative stereochemistry of **3** was elucidated by the NOESY spectrum and from ^{1}H−^{1}H coupling constants (Figure 2). Taxuspine H (**8**) was elucidated as 5-decinnamoyl-5-(3-*N*,*N*-(dimethylamino)-3-phen-

[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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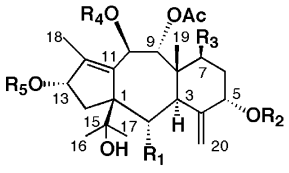
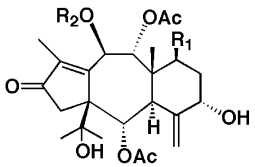
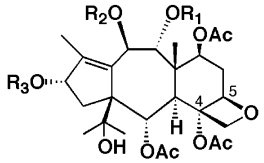
ylpropanoyl)taxuspine C by its NMR data and by a photochemical reaction from taxine II (**41**) into **8**. 3,11-Cyclotaxinine NN-2 (**75**) was assigned as 1-hydroxytaxuspine C. Taxinines K (**102**) and L (**103**) are the first 3,11-cyclotaxanes from *T. cuspidata*.

Taxanes (6/8/6-Membered Ring System). Most taxoids have a 6/8/6-membered ring system. Taxinine (**37**)[13] is a major taxoid of *T. cuspidata*. The structures of taxuspines F (**6**) and G (**7**) and taxezopidines C (**28**), D (**29**), and E (**30**) have a cyclohexenone (ring A) and a C-4(20)-exomethylene unit. Taxuspine D (**4**) was the first example of a taxoid involving an enol acetate moiety in ring A, while similar taxoids, taxuspine P (**15**), taxezopidine K (**35**), and compound **85**, were also isolated from *T. cuspidata*. The conformation of ring B in these compounds is different from those of other taxoids having a 6/8/6-membered ring system, judging from comparison of the $^1H-^1H$ coupling constants ($J_{9,10}$ = 4.4−4.8 Hz) of **4**, **15**, and **35** with those ($J_{9,10}$ = ca. 10 Hz) of other taxanes, such as taxinine (**37**) and **38**−**47** (Figure 2). Taxuspines L (**11**) and R (**17**) and compound **118** are rare examples of taxoids having a hydroxymethyl group at C-4. Taxuspine K (**10**) was the first example of a taxoid containing a 6/8/6-membered ring system with a tetrahydrofuran ring at C-2, C-3, C-4, and

5/7/6-Membered ring system





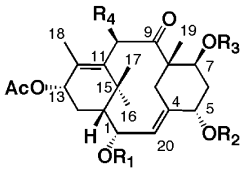


1 R_1=H, R_2=*trans*-COCH=CHPh, R_3=OAc, R_4=Bz, R_5=Ac
9 R_1=H, R_2=*trans*-COCH=CHPh, R_3=OAc, R_4=R_5=Ac
12 R_1=H, R_2=*trans*-COCH=CHPh, R_3=OAc, R_4=H, R_5=Ac
63 R_1=OAc, R_2=R_5=H, R_3=OAc, R_4=Bz
64 R_1=OAc, R_2=H, R_3=OAc, R_4=Bz, R_5=Ac
79 R_1=R_3=H, R_2=*trans*-COCH=CHPh, R_4=R_5=Ac

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

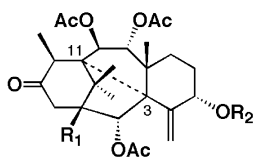
16 R_1=Ac, R_2=tigloyl, R_3=H
62 R_1=Ac, R_2=Bz, R_3=*trans*-COCH=CHPh
67 R_1=Ac, R_2= R_3=H
68 R_1=Ac, R_2=Bz, R_3=H
109 R_1=R_2=R_3=H

6/10/6-Membered ring system



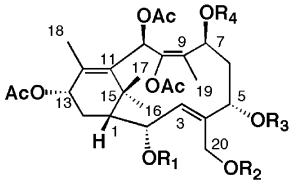
2 R_1=R_3=Ac, R_2=*trans*-COCH=CHPh, R_4=OH
22 R_1=R_3=Ac, R_2=H, R_4=OH
69 R_1=R_3=Ac, R_2=COCH(OH)CH(NMe_2)Ph, R_4=OH
80 R_1=R_3=Ac, R_2=$COCH_2$CH(NMe_2)Ph, R_4=OH
88 R_1=R_3=Ac, R_2=$COCH_2$CH(NMe_2)Ph, R_4==O
93 R_1=H, R_2=*trans*-COCH=CHPh, R_3=Ac, R_4=OH
111 R_1=Ac, R_2=*trans*-COCH=CHPh, R_3=H, R_4=OH

6/5/5/6-Membered ring system



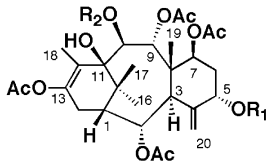
3 R_1=H, R_2=*trans*-COCH=CHPh
8 R_1=H, R_2=$COCH_2$CH(NMe_2)Ph
75 R_1=OH, R_2=*trans*-COCH=CHPh
102 R_1=R_2=H
103 R_1=H, R_2=Ac

6/12-Membered ring system

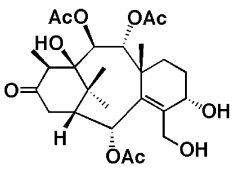


20 R_1=R_2=R_4=H, R_3=Ac
23 R_1=R_2=R_4=Ac, R_3=*trans*-COCH=CHPh
82 R_1=R_2=H, R_3=*trans*-COCH=CHPh, R_4=Ac
83 R_1=H, R_2=R_4=Ac, R_3=*trans*-COCH=CHPh
84 R_1=R_4=H, R_2=Ac, R_3=*trans*-COCH=CHPh

6/8/6-Membered ring system



4 R_1=*trans*-COCH=CHPh, R_2=Ac
15 R_1=$COCH_2$CH(NMe_2)Ph, R_2=Ac
35 R_1=*trans*-COCH=CHPh, R_2=H
85 R_1=H, R_2=Ac



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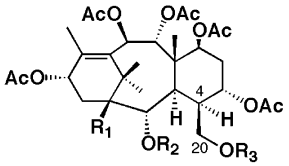
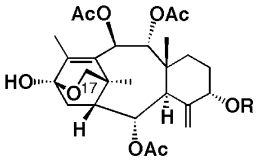
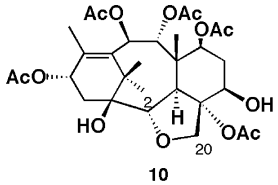


11 R_1=R_3=H, R_2=Ac
17 R_1=OH, R_2=H, R_3=Ac
118 R_1=R_2=H, R_3=Ac



26 R=H
34 R=*trans*-COCH=CHPh



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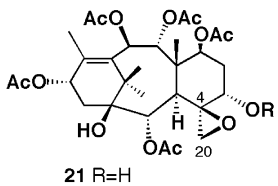
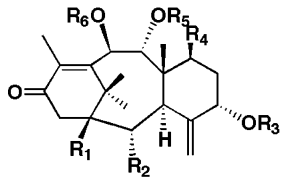


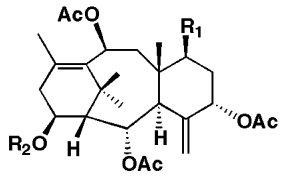
21 R=H
48 R=Ac
76 R=*trans*-COCH=CHPh

Figure 1. Structures of taxoids from Japanese yew, *Taxus cuspidata*.

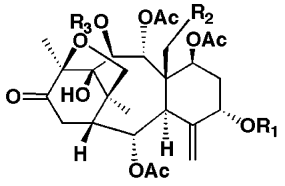
6/8/6-Membered ring system (continued)



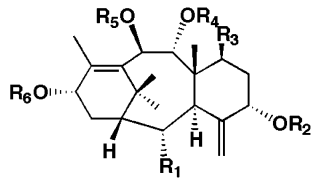
6 R_1=R_3=H, R_2=R_4=OAc, R_5=R_6=Ac
7 R_1=R_3=R_4=H, R_2=OH, R_5=R_6=Ac
28 R_1=R_3=R_4=R_6=H, R_2=OH, R_5=Ac
29 R_1=R_3=R_4=R_5=H, R_2=OH, R_6=Ac
30 R_1=R_5=H, R_3=COCH=CHPh (*trans*), R_2=OH, R_4=OH, R_6=Ac
37 R_1=R_4=H, R_5=R_6=Ac, R_2=OAc, R_3=COCH=CHPh (*trans*)
38 R_1=R_3=R_4=H, R_2=OAc, R_5=R_6=Ac
39 R_1=H, R_2=R_4=OAc, R_5=R_6=Ac, R_3=COCH=CHPh (*trans*)
40 R_1=OH, R_2=OAc, R_5=R_6=Ac, R_3=COCH=CHPh (*trans*), R_4=H
41 R_1=H, R_2=OAc, R_5=R_6=Ac, R_3=$COCH_2CH(NMe_2)Ph$, R_4=H
65 R_1=OH, R_2=OAc, R_5=R_6=Ac, R_3=$COCH_2CH(NMe_2)Ph$, R_4=H
72 R_1=OH, R_2=OAc, R_5=R_6=Ac, R_3=R_4=H
73 R_1=R_4=R_5=H, R_2=OAc, R_6=Ac, R_3=COCH=CHPh (*trans*)
78 R_1=R_4=H, R_2=OAc, R_5=Ac, R_6=H, R_3=COCH=CHPh (*trans*)
86 R_1=OH, R_2=R_4=OAc, R_5=R_6=Ac, R_3=COCH=CHPh (*trans*)
87 R_1=OH, R_2=OAc, R_5=R_6=Ac, R_3=COCH=CHPh (*trans*), R_4=OH
89 R_1=H, R_2=OAc, R_5=R_6=Ac, R_3=$COCH(OH)CH(NMe_2)Ph$
90 R_1=R_4=R_6=H, R_2=OAc, R_5=Ac, R_3=$COCH_2CH(NMe_2)Ph$
91 R_1=R_4=R_5=H, R_2=OAc, R_6=Ac, R_3=$COCH_2CH(NMe_2)Ph$
92 R_1=R_4=H, R_2=OH, R_3=$COCH_2CH(NMe_2)Ph$, R_5=R_6=Ac
96 R_1=R_4=H, R_2=OH, R_3=COCH=CHPh (*trans*), R_5=R_6=Ac
100 R_1=R_4=H, R_2=R_3=R_5=R_6=Ac
114 R_1=R_2=R_3=H, R_4=OAc, R_5=R_6=Ac



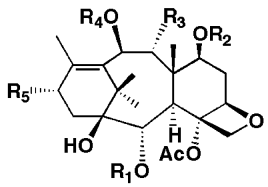
74 R_1=H, R_2=$COCH(Me)CH_2CH_3$
97 R_1=OH, R_2=Ac



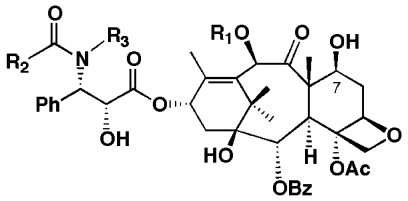
18 R_1=COCH=CHPh (*trans*), R_2=OH, R_3=Ac
19 R_1=COCH=CHPh (*trans*), R_2=OAc, R_3=H
36 R_1=COCH=CHPh (*trans*), R_2=OAc, R_3=Ac
49 R_1=COCH=CHPh (*trans*), R_2=H, R_3=Ac
50 R_1=COCH=CHPh (*trans*), R_2=OBz, R_3=Ac
51 R_1=H, R_2=H, R_3=Ac
52 R_1=H, R_2=OBz, R_3=Ac
66 R_1=H, R_2=OAc, R_3=Ac



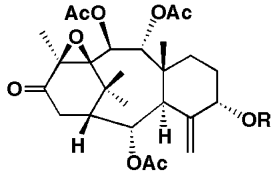
25 R_1=OH, R_2=$COCH_2CH(NMe_2)Ph$, R_3=H, R_4=R_5=R_6=Ac
31 R_1=R_3=OAc, R_4=R_5=Ac, R_2=R_6=H
32 R_1=OH, R_2=COCH=CHPh (*trans*), R_3=H, R_4=R_5=R_6=Ac
33 R_1=R_6=H, R_2=COCH=CHPh (*trans*), R_3=OAc, R_4=R_5=Ac
42 R_1=H, R_2=$COCH_2CH(NMe_2)Ph$, R_3=OAc, R_4=R_5=R_6=Ac
43 R_1=H, R_2=COCH=CHPh (*trans*), R_3=H, R_4=R_5=R_6=Ac
44 R_1=H, R_2=COCH=CHPh (*trans*), R_3=OAc, R_4=R_5=R_6=Ac
45 R_1=R_3=H, R_2=$COCH_2CH(NMe_2)Ph$, R_4=R_5=R_6=Ac
46 R_1=OAc, R_2=R_4=R_5=R_6=Ac, R_3=H
47 R_1=R_3=OAc, R_2=H, R_4=R_5=R_6=Ac
71 R_1=OH, R_2=H, R_3=OAc, R_4=R_5=R_6=Ac
77 R_1=OAc, R_2=R_3=H, R_4=R_5=R_6=Ac
94 R_1=OAc, R_2=R_4=H, R_3=OH, R_5=R_6=Ac
95 R_1=R_3=R_4=R_5=H, R_2=R_6=Ac
99 R_1=OAc, R_2=COCH=CHPh (*trans*), R_3=H, R_4=R_5=R_6=Ac
101 R_1=R_3=OAc, R_2=COCH=CHPh (*trans*), R_4=R_5=R_6=Ac
110 R_1=OH, R_2=COCH=CHPh (*trans*), R_3=OAc, R_4=R_5=R_6=Ac
119 R_1=R_3=H, R_2=R_4=R_5=R_6=Ac
120 R_1=H, R_2=R_4=R_5=R_6=Ac, R_3=OAc
121 R_1=R_2=H, R_3=OAc, R_4=R_5=R_6=Ac
122 R_1=R_2=H, R_3=OAc, R_4=R_5=R_6=Ac



5 R_1=Bz, R_2=Ac, R_4=H, R_3=R_5=OH
13 R_1=R_2=Ac, R_3=OAc, R_4=Bz, R_5=$OCOCH_2CH(NMe_2)Ph$
70 R_1=Bz, R_2=R_4=H, R_3==O, R_5=OH
106 R_1=Bz, R_2=R_4=H, R_3=OH, R_5==O
107 R_1=Bz, R_2=H, R_3=OH, R_4=Ac, R_5==O
113 R_1=Bz, R_2=H, R_3==O, R_4=Ac, R_5=OCOCH=CHPh (*trans*)
115 R_1=Bz, R_2=H, R_3==O, R_4=Ac, R_5=OH
116 R_1=Bz, R_2=H, R_3=OH, R_4=Ac, R_5=OAc
117 R_1=Bz, R_2=H, R_3=OH, R_4=H, R_5=OAc



53 R_1=Ac, R_2=Ph, R_3=H
54 R_1=H, R_2=Ph, R_3=H
55 R_1=Ac, R_2=C_4H_7, R_3=H
56 R_1=H, R_2=C_4H_7, R_3=H
57 R_1=Ac, R_2=*n*-C_5H_{11}, R_3=H
58 R_1=H, R_2=*n*-C_5H_{11}, R_3=H
59 R_1=Ac, R_2=*n*-C_3H_7, R_3=H
60 R_1=H, R_2=Ph, R_3=H, C-7 epimer
61 R_1=Ac, R_2=Ph, R_3=H, C-7 epimer
104 R_1=Ac, R_2=C_6H_{13}, R_3=H
108 R_1=H, R_2=*n*-C_5H_{11}, R_3=H, C-7 epimer
112 R_1=Ac, R_2=Ph, R_3=Me



81 R=H
98 R=COCH=CHPh (*trans*)

Figure 1. Continued.

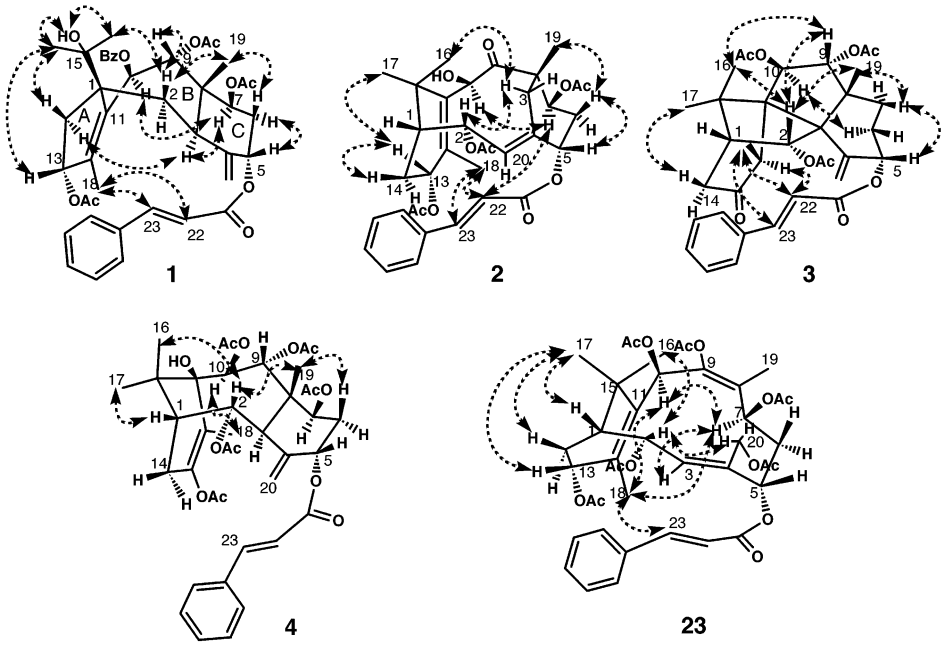
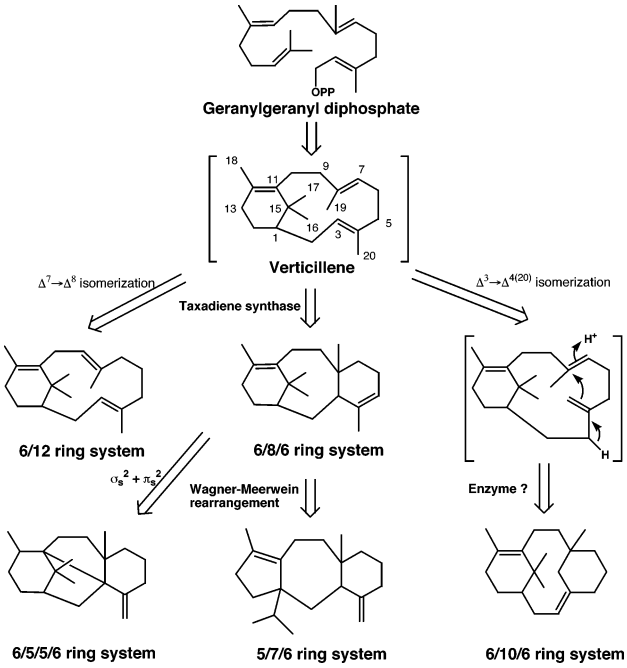


Figure 2. Stereostructures of taxuspines A−D (**1**−**4**) and X (**23**). (Dotted arrows denote NOESY correlations.)

C-20, although it has been reported that taxoids containing an oxetane ring at C-4 and C-5 can be converted into taxoids with a tetrahydrofuran ring at C-2, C-3, C-4, and C-20. A chair-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**), taxezopidine L (**36**), taxagifine (**49**), taxacin (**50**), 5α-decinnamoyltaxagifine (**51**), taxinine M (**52**), and 19-debenzoyl-19-acetyltaxinine M (**66**) have a tetrahydrofuran ring in ring A (C-11, C-12, C-15, C-17, and O-12). The conformation of ring B in **18**, **19**, and **36** is similar to those of taxuspines D (**4**) and P (**15**), judging from ^{1}H−^{1}H coupling constants ($J_{9,10}$ = 2.8 Hz for **18**, $J_{9,10}$ = 3.8 Hz for **19**, and $J_{9,10}$ = 3.2 Hz for **36**) and NOESY correlations. Taxuspines E (**5**) and N (**13**) possess an oxetane ring between C-4 and C-5, and their structures are similar to that of 10-deacetylbaccatin III (**70**), while **5** and **13** differ 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 (**70**)). Taxuspine N (**13**) was the first example of a taxoid containing a 6/8/6-membered ring system with a 3-*N*,*N*-(dimethylamino)-3-phenylpropanoyl group at C-13 from a *Taxus* species. The structure of taxuspine V was assigned as **21** by NMR data and acetylation of **21** into 1β-hydroxybaccatin I (**48**). Taxezopidines A (**26**) and J (**34**) are the only two examples of taxane diterpenes containing an oxabicyclo[2.2.2]octene moiety from yew trees. It was noted that **26** and **34** have a cage-like backbone conformation similar to usual taxoids consisting of a 6/8/6-membered ring system. Taxezopidine B (**27**) was the first taxoid with a double bond at C-3 and C-4. Compounds **81** and **98** are very rare examples of taxoids with an 11,12-epoxide group, while compound **74** is the first reported example of a taxoid from *T. cuspidata* to be oxidized at C-14, and this finding suggests the biosynthetic diversity of this plant.

Bicyclic Taxane-Related Diterpenes (6/12-Membered Ring System). Five bicyclic taxane related diterpenes have been isolated from *T. cuspidata*. Taxuspine U (**20**) was isolated from the stems of this species, and its relative stereochemistry was investigated by combination of NOESY data and molecular mechanics calculations using the Macromodel program. The structure of taxuspine X (**23**) is 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 possible to elucidate the relative stereochemistry of **23** by NOESY correlations (Figure 2). Compounds **82**−**84** were assigned as 2,20-dideacetyltaxuspine X, 2-deacetyltaxuspine X, and 2,7-dideacetyltaxuspine X, respectively, using spectroscopic techniques and by comparison with taxuspine X (**23**). The skeletons of these compounds and related taxoids are similar to that of verticillene (Scheme 1).

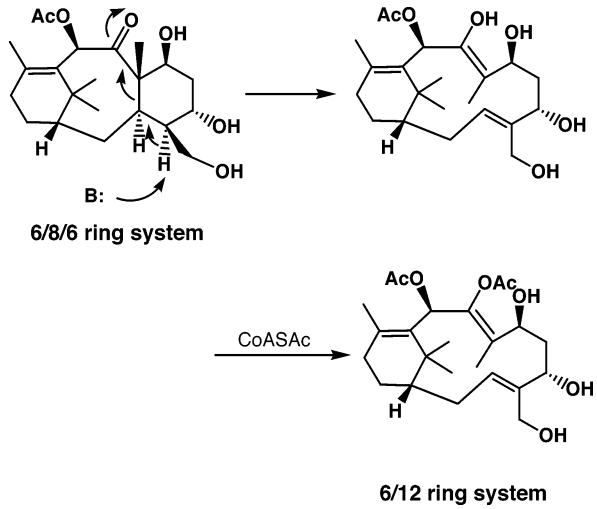
Scheme 1. Plausible Biogenesis of Taxoids



Biogenetic Considerations of Taxoids from *T. cuspidata*

Since the taxoid constituents of the Japanese yew, *T. cuspidata*, possess various skeletons, we wish to propose a plausible biogenesis of these taxoids. The biosynthesis of the taxane skeleton (6/8/6-membered ring system) has

Scheme 2. Plausible Biogenesis of Bicyclic Taxane-Related Diterpenes



recently been suggested to involve cyclization of geranylgeranyl diphosphate to taxa-4(5),11-diene by Croteau et al.,[76] while the pathway of the 3,11-cyclotaxanes (6/5/5/6-membered ring system) has been proposed to be derived from taxanes by a concerted $\sigma_s^2 + \pi_s^2$ route.[77] The 11-(15→1)-*abeo*-taxanes (5/7/6-membered ring system) may be generated biogenetically through a Wagner−Meerwein rearrangement of ring A in a 6/8/6-membered ring system.[78] On the other hand, the 2(3→20)-*abeo*-taxanes (6/10/6-membered ring system) seem to be derived from verticillene through the cyclization of $\Delta^{4(20),7}$-verticillene.[79] The bicyclic taxane-related compounds (6/12-membered ring system) might be derived from verticillene (Scheme 1).[80] An alternative plausible biogenesis of the 6/12-membered ring system is reported in Scheme 2, since it is unlikely that the enzymes responsible for hydroxylating the tricyclic taxoids skeleton would show a similar specificity for bicyclic taxoids.[10]

Bioactivities

Inhibitory Activity of Ca^{2+}-Induced Microtubule Depolymerization by Taxoids. Microtubules polymerized in the presence of paclitaxel (**53**) are resistant to depolymerization by Ca^{2+} ions.[81] The effect of taxoids (**1**−**73**) was examined against the $CaCl_2$-induced depolymerization of microtubules. Microtubule proteins were polymerized under normal polymerization conditions[82] in the absence and the presence of paclitaxel (**53**) or taxoids **1**−**52** and **54**-**73**, and after a 30-min incubation, $CaCl_2$ was added. Microtubule polymerization and depolymerization were monitored by the increase and the decrease in turbidity. The results are summarized in Figure 3 as the changes in the relative absorbance at 400 nm.

The $CaCl_2$-induced depolymerization of microtubules (shown as control) is completely inhibited by 10 μM paclitaxel (**53**). Among the tested taxoids, taxuspine D (**4**), taxezopidine K (**35**), taxezopidine L (**36**), and taxagifine (**49**) reduced the depolymerization process in a remarkable fashion, suggesting that these compounds have paclitaxel-like activity to microtubule systems. The potencies of **4**, **35**, **36**, and **49** in inhibiting the depolymerization process corresponded to half to one-third of that of paclitaxel (**53**). Compounds **2**, **34**, **39**, and **62** exhibited moderate activity, while the other compounds showed little or no effects. On the other hand, the paclitaxel-type compounds **54**−**61** inhibited the depolymerization process as potently as paclitaxel (**53**). It is noted that compounds **4**, **35**, **36**, and **49** lack both an oxetane ring and an *N*-acylphenylisoserine moiety but still exhibit potent activity. Since active compounds **4**, **35**, **36**, and **49** possess 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 paclitaxel (**53**). In addition, the acetoxy group at C-10 and the hydroxy group at C-11 in **4**, **35**, **36**, and **49** may be important for the inhibition of microtubule depolymerization like the oxetane moiety at C-4 and C-5 and the acetoxy group at C-4 in paclitaxel (**53**) (Figure 4). These results suggest that both functional groups and the conformation of **4**, **35**, **36**, and **49** may be important for the inhibition of microtubule depolymerization.[83]

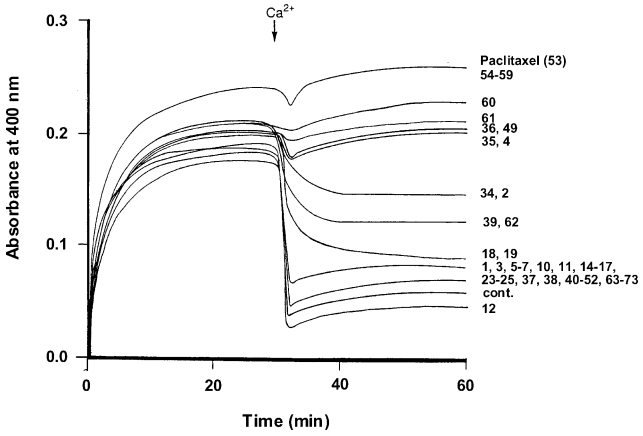


Figure 3. Effects of taxoids on Ca^{2+}-induced microtubule depolymerization. The temperature was held at 37 °C, and changes in turbidity were monitored at 400 nm. For the drug−protein studies, 10 μM of drug dissolved in DMSO was added to 1 mL of buffer solution containing 2 mg of microtubule protein. The final DMSO concentration was less than 1%. After a 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.

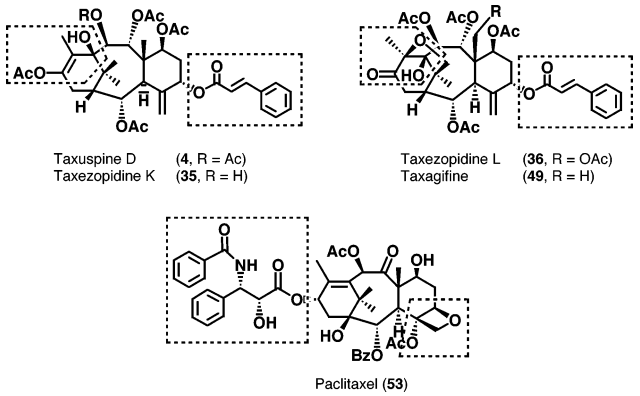


Figure 4. Taxoids inhibiting Ca^{2+}-induced microtubule depolymerization.

When fertilized sea urchin eggs were treated with 2.5 μg/mL of taxuspine D (**4**), the normal spindles were not seen at the metaphase, overstabilized spindles with high birefringence density were observed, and the subsequent egg divisions were completely suppressed. This mode of action was almost identical to that of paclitaxel (**53**) at 10 μg/mL.[28] Since taxuspine D (**4**) and paclitaxel (**53**) both induced overstabilized spindles with high birefringence density, they were shown to act on components of the mitotic apparatus and to inhibit cell division effectively.

Increased Cellular Accumulation of Vincristine in Multidrug-Resistant Cells by Taxoids. The cellular accumulation of vincristine (VCR) is reduced in multidrug-

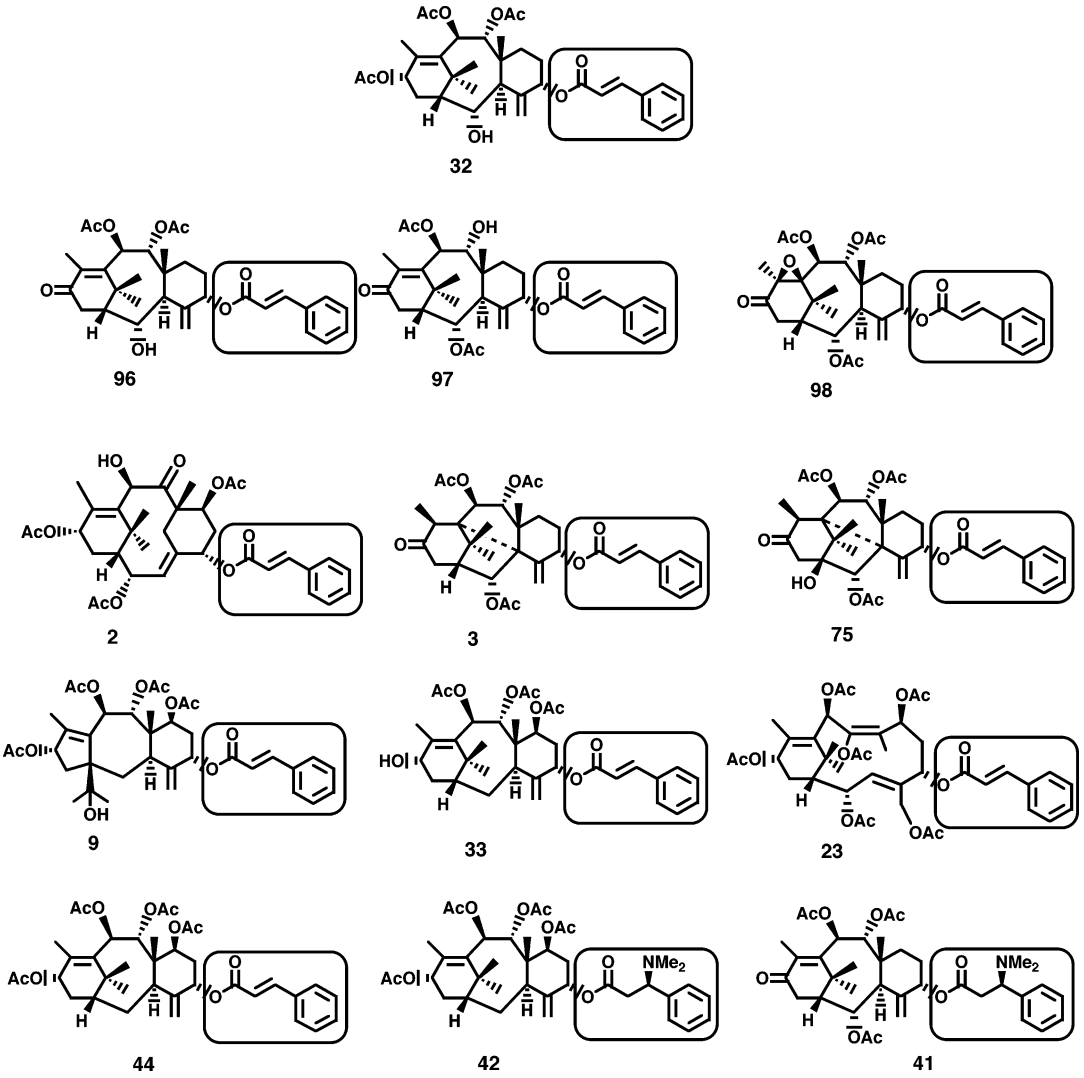


Figure 5. Bioactive taxoids from *Taxus cuspidata* inhibiting drug transport activity in MDR cells.

resistant (MDR) tumor cells as compared with parental cells.[84] MDR-reversing agents such as verapamil increase the reduced accumulation of antitumor agents in MDR cells and overcome multidrug resistance.[85,86] The effect of taxoids isolated from *T. cuspidata* on the cellular accumulation of VCR in multidrug-resistant human ovarian cancer 2780AD cells was examined, and the results were investigated. Taxinine NN-1 (taxezopidine G) (**32**) showed the strongest activity toward VCR accumulation in MDR tumor cells. The value of VCR accumulation with **32** was 323% of verapamil at 1 μg/mL.[87] Compounds **73** (185% at 10 μg/mL),[53] **75** (204% at 1 μg/mL),[66] **96** (191% at 10 μg/mL),[66] and **98** (142% at 1 μg/mL)[53] increased the VCR accumulation more than verapamil, while compounds **2** (96%, verapamil % at 10 μg/mL), **3** (104%), **9** (114%), **23** (105%), **25** (106%), **33** (106%), **41** (95%), **42** (114%), **44** (108%), and **45** (119%) increased the VCR accumulation as potently as verapamil.[83] It is noted that (i) the potently active compounds **2**, **3**, **9**, **23**, **25**, **32**, **33**, **41**, **42**, **44**, **45**, **75**, **96**, **97**, and **98** possess a cinnamoyl or 3-*N*,*N*-(dimethylamino)-3-phenypropanoyl group at C-5 and (ii) the various taxane skeletons do not appreciably affect VCR accumulation (Figure 5). These results suggest that many taxoids could be substrates of P-glycoprotein and some of them might be useful for overcoming multidrug resistance.

The above results have led to the examination of the structure−activity relationships of taxane derivatives from the Japanese yew for modulation of multidrug resistance in tumor cells. Compounds **123** (227%),[88] **124** (150%),[89] and **125** (139%),[89] which were derived from taxinine (**37**), and the 3,11-cyclotaxane derivative **126** (125%)[90] increased the VCR accumulation more potently than verapamil, and compounds **127** (98%), **128** (95%), **129** (87%), **130** (88%), **131** (87%), **132** (102%), **133** (101%), and **134** (91%) increased the VCR accumulation as potently as verapamil, while compounds **135**−**145** showed lower activities (28−38%).[89] In summary, some taxinine derivatives (**123**, **124**, **125**, **126**, **127**, **128**, **129**, **130**, **131**, **132**, **133**, and **134**), containing a phenylpropanoyloxy, a cinnamoyloxy, a benzoyloxy, a TES, or a BOM group at C-2, C-5, or C-13, effectively increase the cellular accumulation of VCR in MDR tumor cells, while other taxoids (**135**, **136**, **137**, **138**, **139**, and **140**) having cinnamoyloxy, benzoyloxy, or acetonide groups at both C-9 and C-10 showed considerable reduction of this type of activity. Since the 6/8/6-membered ring system of these taxinine derivatives commonly represent "cage"-like backbone structures, the presence of the bulky group at C-2, C-5, or C-13 oriented to the inside of the "cage" structure may be important for its effective binding to P-glycoprotein, whereas the presence of the bulky groups at C-9 and C-10 directed to the outside of the "cage" structure may result in less effective binding.

Compounds **2**, **3**, **41**, **42**, and **44** reduced binding of [^{3}H]-azidopine to P-glycoprotein that was present in adriamycin-resistant human leukemia K562/ADM cells more potently

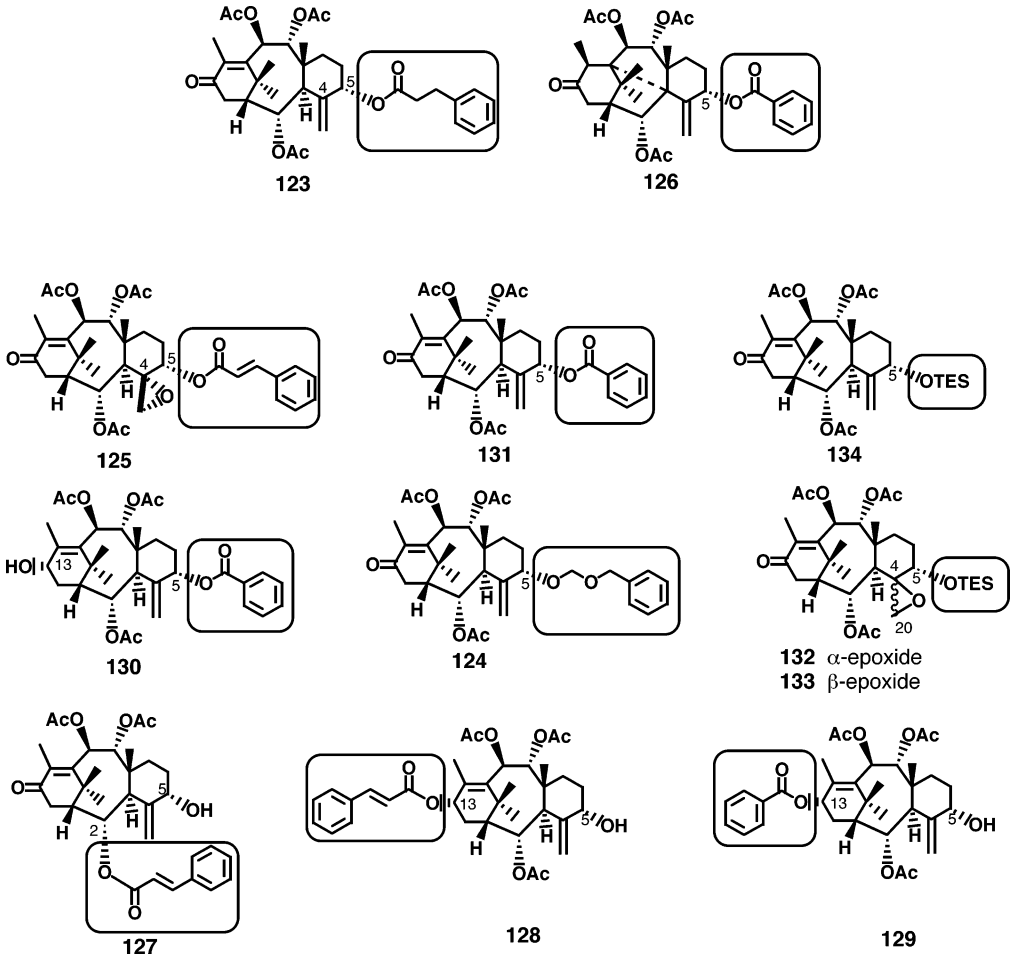


Figure 6. Taxinine derivatives (**123**−**134**) increasing the vincristine accumulation in MDR 2780AD cells.

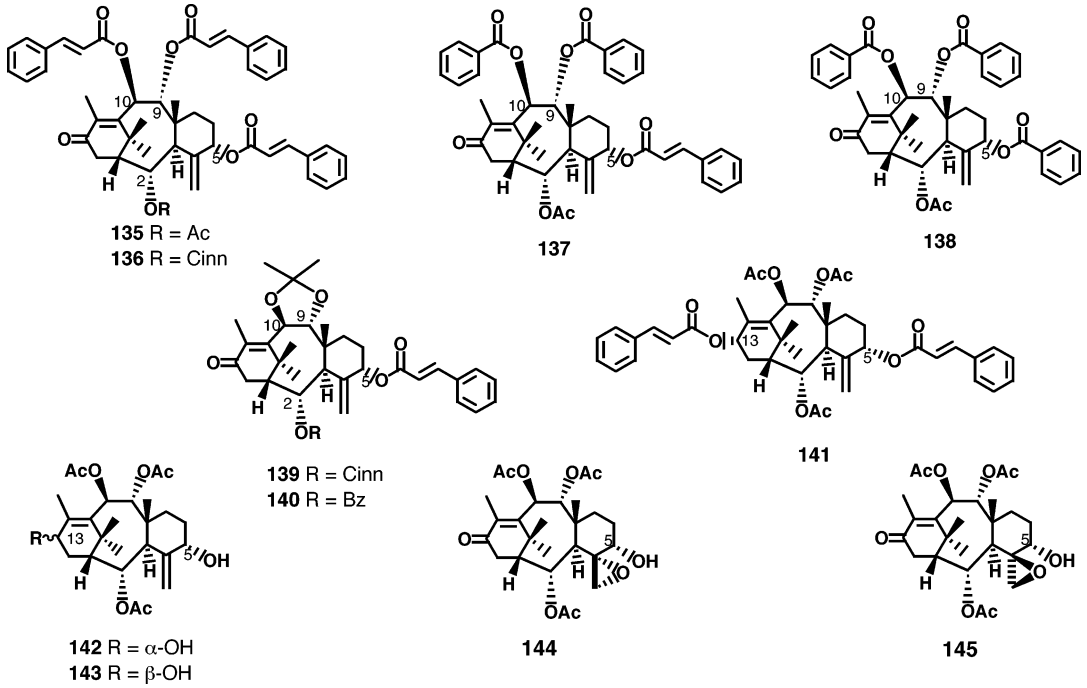
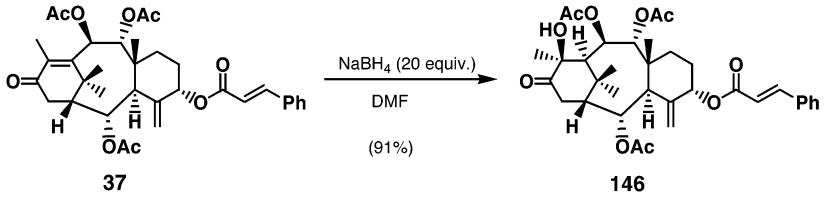


Figure 7. Taxinine derivatives (**135**−**145**) not affecting the vincristine accumulation in MDR 2780AD cells.

than verapamil. It is suggested that these taxoids bind to the same binding site on P-glycoprotein as that of azidopine.[83] Compounds **3**, **42**, and **44** at 10 μM completely reversed the resistance to colchicine, vincristine, and paclitaxel in KB-C2 cells overexpressing P-glycoprotein, which were originally isolated from human epidermoid carcinoma KB-3-1 cells.[91]

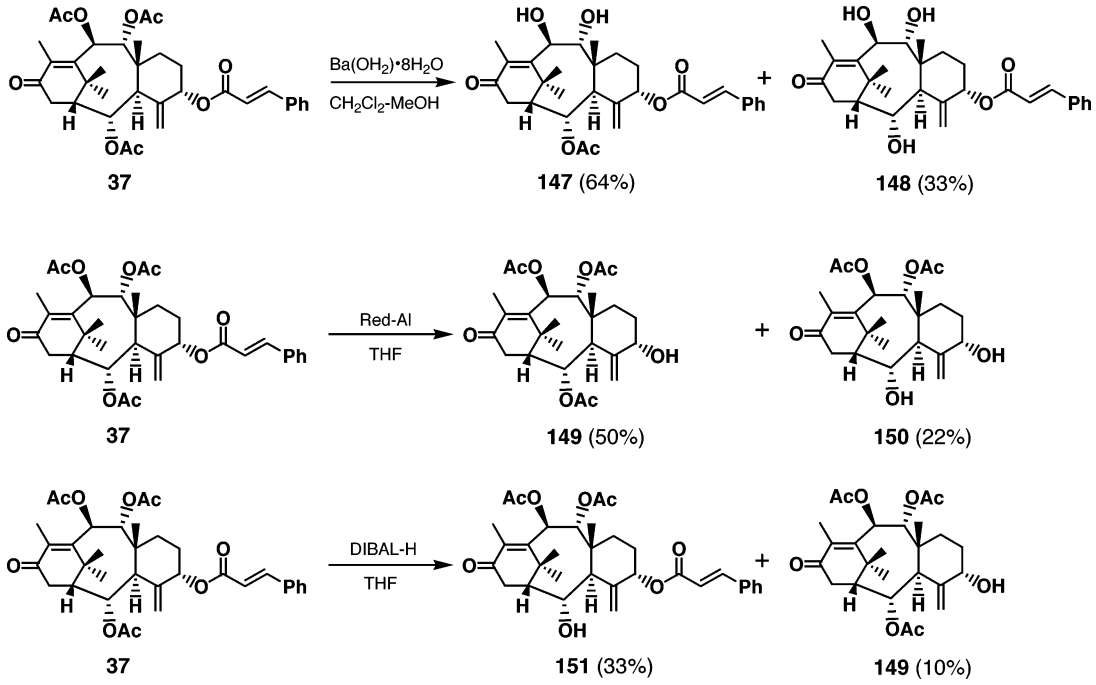
Combined Chemotherapeutic Effect of VCR and Taxuspine C (3) on P388/VCR-Bearing Mice. When taxuspine C (**3**, 200 mg/kg), together with VCR, was given

Scheme 3. Regio- and Stereoselective Hydration of **37** by $NaBH_4$



37 **146**

Scheme 4. Selective *O*-Deacylation of Taxoids



37 **147** (64%) **148** (33%)

37 **149** (50%) **150** (22%)

37 **151** (33%) **149** (10%)

Table 1. Effect of Taxuspine C (**3**) on Antitumor Activity of Vincristine (VCR) in P388/VCR-Bearing Mice

treatment[a]	n	median[b] (days)	range (days)	T/C (%)
control	5	11.4	10−15	100
VCR (0.2 mg/kg)	5	12.6	10−15	110
VCR (0.1 mg/kg)	5	10.6	10−11	92
taxuspine C (**3**) (200 mg/kg) + VCR (0.2 mg/kg)	5	15.8	12−20	138
taxuspine C (**3**) (200 mg/kg) + VCR (0.1 mg/kg)	5	12.0	10−15	105

[a] CDF_1 mice were given i.p. implants of 10^6 P388/VCR leukemia cells on day 0. Taxuspine C (**3**), together with VCR, was given i.p. daily for 5 days. [b] T/C value: median survival time of treated mice divided by that of control mice.

i.p. daily for 5 days, the life span of P388/VCR-bearing mice was increased. This result was observed at a taxuspine C (**3**) dose of 200 mg/kg given daily with 0.2 mg/kg VCR, wherein the T/C value was 138% (Table 1).[92]

Cytotoxicity Studies. The cytotoxic activity of all taxoids (**1**−**73**) was examined against murine leukemia L1210 cells and human epidermoid carcinoma KB cells. Paclitaxel (**53**) and the paclitaxel-type compounds **54**−**61** exhibited very potent cytotoxicity against KB cells (IC_{50} 0.0015−0.086 μg/mL). Non-paclitaxel-type compounds such as **5**, **16**, **18**, **19**, **22**, **49**, **63**, **72**, and **73** 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 the most potent (IC_{50} 0.08 μg/mL) among these non-paclitaxel-type taxoids. It was interesting to observe that compounds **16**, **18**, **19**, **22**, **49**, **63**, **72**, and **73**, without an oxetane ring and an *N*-acylphenylisoserine moiety, exhibited such cytotoxicity. The cytotoxic compounds **16**, **18**, **19**, **22**, **49**, **63**, **72**, and **73** possess an acetoxy group at C-2, while the other functional groups are different from one another. A 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 the southern part (C-1, C-2, C-4, and C-5) but not the northern part (C-7, C-9, and C-10) of the taxoids is intimately associated with their cytotoxicity.[16]

Chemical Reactions of Taxoids of *T. cuspidata*

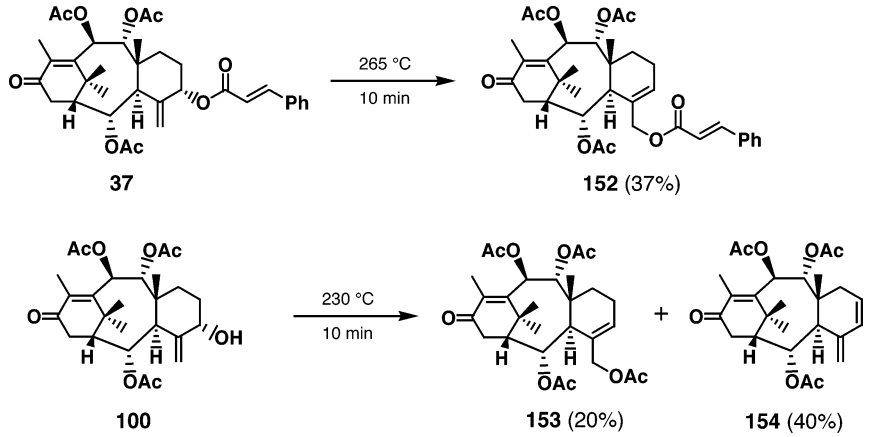
Regio- and Stereoselective Hydration of Taxoids. Treatment of taxinine (**37**) with a large excess of sodium borohydride ($NaBH_4$) in slightly hydrous *N*,*N*-dimethylformamide at ambient temperature resulted in regio- and stereoselective hydration at the C-11,C-12-double bond to give an isomeric taxuspine D derivative (**146**) (Scheme 3).[93]

Selective *O*-Deacylation of Taxoids. Selective *O*-deacylations of taxinine (**37**) at C-2, C-5, C-9, and C-10 have been accomplished by treatment with barium hydroxide octahydrate [$Ba(OH_2)$•$8H_2O$], sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al), or diisobutylaluminum hydride (DIBAL-H), under mild conditions, to give 9,10-di-*O*-deacetyltaxinine (**147**), 2,9,10-tri-*O*-deacetyltaxinine (**148**), 5-*O*-decinnamoyltaxinine (**149**), 2,5-di-*O*-deacyltaxinine (**150**), and 2-*O*-deacetyltaxinine (**151**), respectively (Scheme 4).[94]

Thermolysis of Taxoids. Thermolysis of taxinine (**37**) and taxinine H (**100**) resulted in the smooth formation of the corresponding 4-en-4-ylmethanol esters (**152** and **153**) and the 4(20),5-diene (**154**), by virtue of migration and elimination of the 5-*O*-ester moiety (Scheme 5).[95]

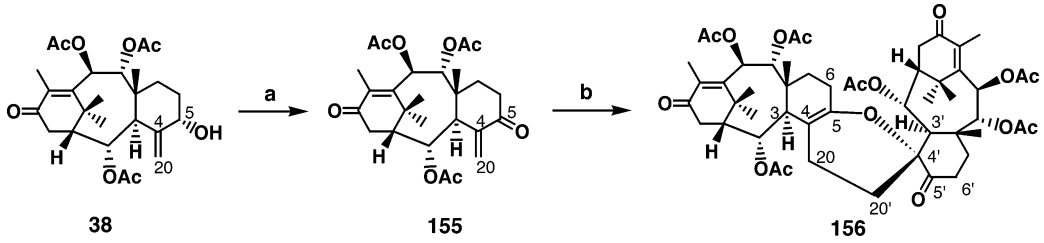
Generation of Dimeric Compounds of the Taxoids through Hetero-Diels−Alder Cycloaddition. Oxidation

Scheme 5. Thermolysis of Taxoids



37 **152** (37%) 100 **153** (20%) **154** (40%)

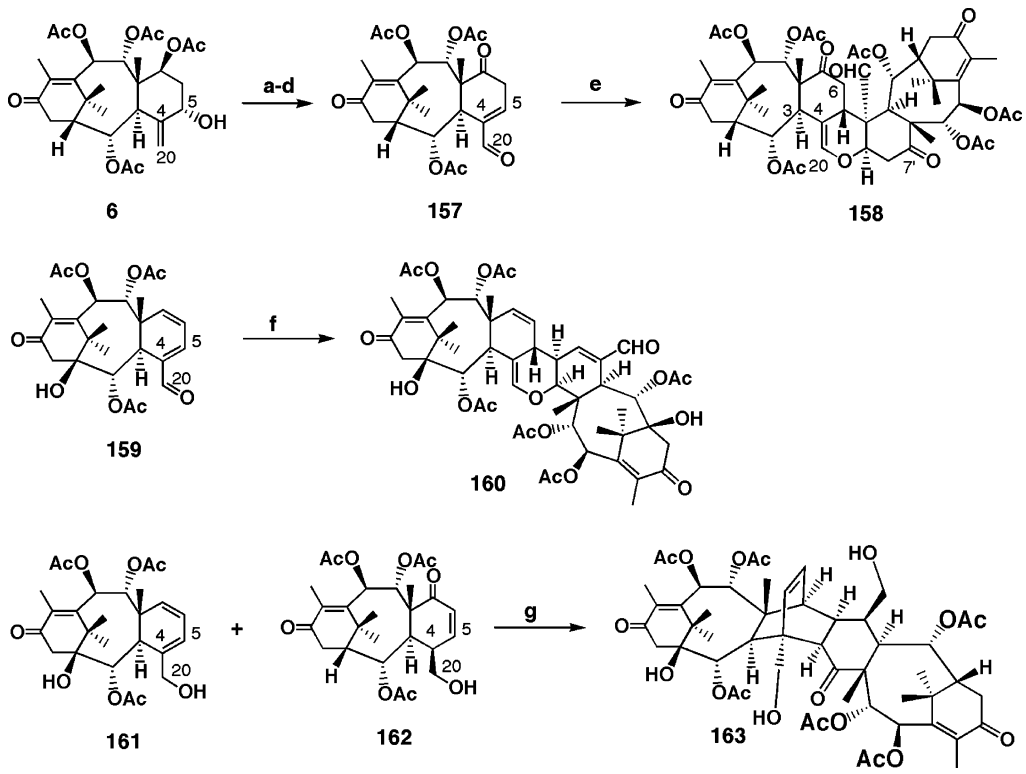
Scheme 6. Generation of Dimeric Compounds of Taxoids through Hetero-Diels−Alder Cycloaddition (1)[a]



38 155 156

[a] Reagents and conditions: (a) TPAP, 4-methylmorpholine *N*-oxide, CH_3CN, MS 4 Å, rt, 2 h, 80%; (b) neat, 80 °C, quant.

Scheme 7. Generation of Dimeric Compounds of Taxoids through Hetero-Diels−Alder Cycloaddition (2)[a]



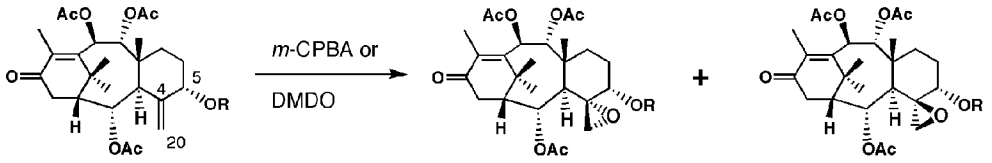
6 157 158 159 160 161 162 163

[a] Reactions and conditions: (a) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, rt, 3 h, 91%; (b) MsCl, Et_3N, CH_2Cl_2, 35−40 °C, 3 h, 68%; (c) $BF_3{\cdot}OEt_2$, 178 °C, 2 h, 62%; (d) TPAP, 4-methylmorpholine *N*-oxide, CH_3CN, MS 4 Å, rt, 3 h, 80%; (e) benzene, 80 °C, 8 h; (f) benzene, 80 °C, 7 h; (g) $Sc(OTf)_3$, CH_2Cl_2, rt, 12 h.

of taxinine A (**38**) with tetrapropylammonium perruthenate (TPAP) yielded 5-oxotaxinine A (**155**, 80%), which was allowed to stand at room temperature to form a new dimeric compound (**156**) (Scheme 6). Compound **156** was generated quantitatively from **155** without solvent at 80 °C. The relative stereostructure of **156** was established by X-ray analysis, which corresponded to that elucidated by NMR data. The formation of **156** from **155** is considered to be derived through regio- and stereospecific hetero-Diels−Alder cycloaddition between the enone (C-20, C-4, C-5, and O-5) of one molecule and the exomethylene (C-4′ and C-20′) of a second one, in which the exomethylene approaches the enone.[96]

In turn, oxidation of 20-hydroxy-4,5-en-7-oxotaxinine derived from taxuspine F (5-hydroxytaxinine B) (**6**) gave the corresponding aldehyde (**157**) via four steps, which

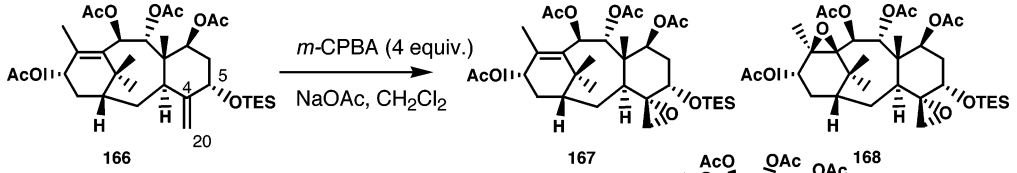
Scheme 8. Stereoselective Epoxidation of Taxoids (1)



37 R = COCH=CHPh (*trans*)
38 R = H
164 R = TBDMS
165 R = TES

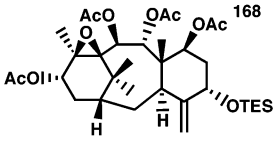
37a **38a** **164a** **165a** **37b** **38b** **164b** **165b**

Scheme 9. Stereoselective Epoxidation of Taxoids (2)



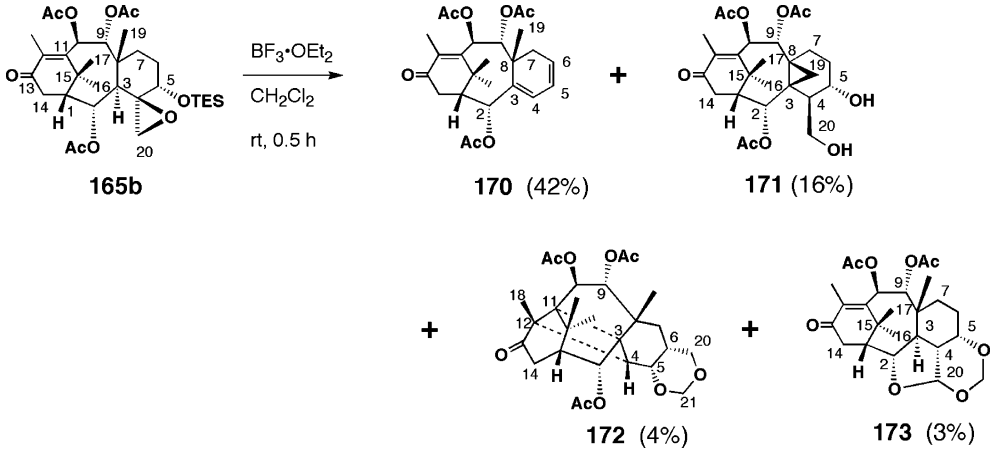
166 **167** **168**

	yield		
temperature	**167**	**168**	**169**
rt	80%	10%	none
0-10 °C	none	68%	25%



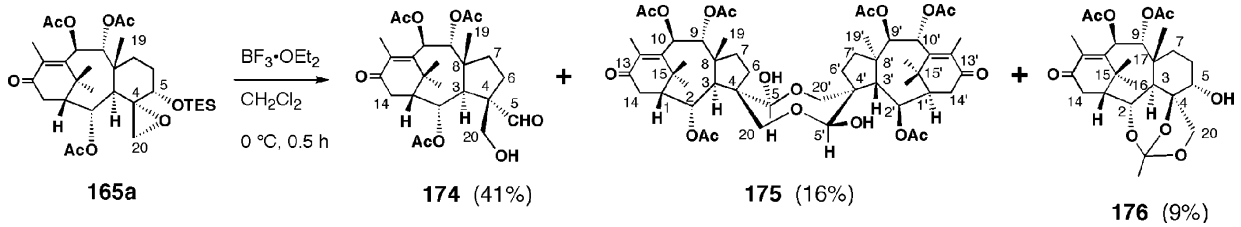
169

Scheme 10. Unusual Boron Trifluoride-Catalyzed Reactions of Taxoids with β-4(20)-Epoxides



165b **170** (42%) **171** (16%) **172** (4%) **173** (3%)

Scheme 11. Unusual Boron Trifluoride-Catalyzed Reactions of Taxoids with α-4(20)-Epoxides



165a **174** (41%) **175** (16%) **176** (9%)

afforded a new dimeric taxoid (**158**) through regio- and stereospecific hetero-Diels−Alder cycloaddition.[97] Similar hetero-Diels−Alder reaction of the aldehyde **159** gave a new dimeric taxoid (**160**), while Lewis acid-catalyzed Diels−Alder cycloaddition between **161** and **162** took place in the presence of $Sc(OTf)_3$ for 12 h, to give the exo-cycloadduct **163** (Scheme 7).

Stereoselective Epoxidation of Taxoids. Epoxidation of taxinine (**37**) and taxinine A (**38**) and the taxinine derivative **164** with *m*-CPBA afforded the α-4(20)-epoxides selectively (α:β = 99:1). However, recently it was found that epoxidation of taxinine derivatives **164** and **165** with dimethyldioxirane (DMDO) gave the β-4(20)-epoxides predominantly (α:β = 1:4−5). The β-selectivity of epoxidation of **164** and **165** with DMDO may be explained by the large steric hindrance between a silyl group at C-5 and DMDO (Scheme 8 and Table 2).[98]

Table 2. Epoxidation of C-4(20)-Exomethylene of Taxinine (**37**), Taxinine A (**38**), and the Related Compounds **164** and **165**

	m-CPBA		DMDO	
compound	α (**a**):β (**b**)	yield (%)[a]	α (**a**):β (**b**)	yield (%)[a]
37	99:1	99	99:1	77[b]
38	99:1	99	2:1	66
164	99:1	99	1:5	69
165	4:1	76	1:4	86

[a] Isolation yield. [b] The 22(23)-double bond was also epoxidized.

Epoxidation of the taxinine J derivative **166** with *m*-CPBA afforded a mixture of a 4α,20-epoxide (**167**), a 4α,20:11*β*,12*β*-diepoxide (**168**), and a 11*β*,12*β*-epoxide (**169**). The relative yields were dependent on temperature (**167**:**168** = 80:10 at room temperature; **168**:**169** = 68:25 at 0−10 °C) (Scheme 9).[99]

Unusual Boron Trifluoride-Catalyzed Reactions of Taxoids with α- and *β*-4(20)-Epoxides. Treatment of the *β*-4(20)-epoxy-5-*O*-TES-taxinine A (**165b**) with boron trifluoride diethyl etherate (BF_3·OEt_2, 2 equiv) in CH_2Cl_2 at room temperature for 0.5 h yielded the 3,5-diene (**170**, 42%), the 3,8-cyclopropane (**171**, 16%), the cyclobutane (**172**, 4%), and the dioxane (**173**, 3%) derivatives (Scheme 10), while reaction at 0 °C afforded the 3,5-diene (**170**, 47%), but the 3,8-cyclopropane (**171**) was not obtained. On the other hand, treatment of the α-4(20)-epoxide (**165a**) with BF_3·OEt_2 (2 equiv) in CH_2Cl_2 at 0 °C for 0.5 h afforded the cyclopentanecarbaldehyde (**174**, 41%), its hemiacetal dimer (**175**, 16%), and the ortho ester (**176**, 9%) derivative (Scheme 11). It is noted that the orientation of the 4(20)-epoxides results in different types of boron trifluoride-catalyzed reactions.[100,101]

Concluding Remarks

Approximately 120 taxoids have been isolated from the Japanese yew, *Taxus cuspidata* Sieb. et Zucc. (Taxaceae). These taxoids possess various skeletons containing 5/7/6, 6/10/6, 6/5/5/6, 6/8/6, or 6/12-membered ring systems. It is noted that the non-paclitaxel-type taxoids, taxuspine D (**4**), taxezopidines K (**35**) and L (**36**), and taxagifine (**49**), exhibit potent inhibitory activity against Ca^{2+}-induced depolymerization of microtubules, while taxuspine D (**4**) induced spindles with strong birefringence in the same manner as paclitaxel (**53**). Some natural taxoids (**2**, **3**, **9**, **23**, **32**, **33**, **41**, **42**, **44**, **45**, **75**, **96**, **97**, and **98**) and synthetic taxoids (**123**−**134**) increase VCR accumulation in MDR cells. Since these bioactive taxoids possess bulky groups at C-2, C-5, or C-13, the bulky group and "cage"-like backbone structures may be important for their effective binding to P-glycoprotein. Among these taxoids, compounds **2**, **3**, **41**, **42**, and **44** inhibited binding of azidopine to P-glycoprotein in adriamycin-resistant K562/ADM cells and increased the cellular accumulation of vincristine in multidrug-resistant 2780AD cells as potently as verapamil. It is noted that taxuspine C (**3**) given i.p. enhances the chemotherapeutic effect of vincristine in P388/VCR-bearing mice. These observations indicate that taxuspine C (**3**) interacts directly with P-glycoprotein and inhibits the efflux of antitumor agents, thus overcoming multidrug resistance in vivo, like verapamil. In addition these non-paclitaxel-type taxoids exhibited weak or no cytotoxicity. From these results, it is suggested that some taxoids might be good modulators of multidrug resistance in cancer chemotherapy. Chemical derivatization of taxoids of Japanese yew probably will be applicable to the synthesis of new taxoid analogues possessing such interesting biological activities.

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Note Added after ASAP: The version of this Review posted on Jan 29, 2004, was missing part of Figure 1. The complete Figure 1 appears in the version posted on Feb 9, 2004.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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