

## ON THE STRUCTURES OF SIX NEW DITERPENOIDS, TAXCHININS E, H, I, J, K AND TAXCHIN B

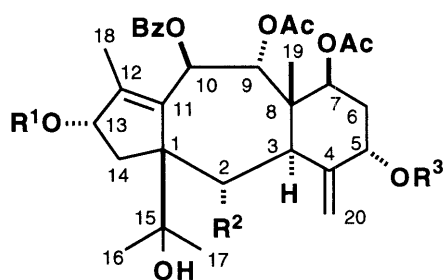
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The structures of taxchinin E, H, I, J, K and taxchin B, isolated from *Taxus chinensis*, have been elucidated by means of NMR spectroscopy. The former five possess a rearranged taxoid skeleton, and the latter has an ordinary taxane framework.

**KEYWORDS** structural elucidation; *taxus chinensis*; taxane; diterpenoid; NMR; long range CH-COSY

Taxol is now recognized as a promising anticancer drug of plant source, and extensive chemical as well as biological investigations on this and related terpenoids have been done for the purpose of ample and efficient material supply.<sup>1)</sup> In spite of the brilliant success in making taxol from scratch,<sup>2,3)</sup> new taxoids from natural sources are still required for developing a new therapeutic agent of this category, and over two hundred taxoids have been isolated to date.<sup>4)</sup>

In the course of our investigation on new taxanes of antitumor activity as well as on a plant source of sufficient and useful constituents, we have isolated thirteen new diterpenoids, taxchinins A-K and taxchin A and B, from stems and needles of *Taxus chinensis*.<sup>5,6)</sup> Here we describe the structures of taxchinin E (1), H (2), I (3), J (4), K (5) and taxchin B (6) based on the NMR measurement.<sup>7)</sup> The long-range CH-COSY (LR-CH-COSY) and NOESY spectra were found to be an extremely effective tool for determining the position of the ester linkage and relative stereochemistry, respectively.

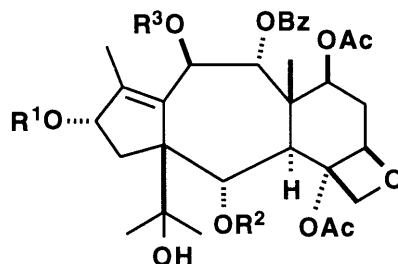


taxchinin E (1):

$R^1 = \text{cinnamoyl}$ ,  $R^2 = \text{OAc}$ ,  
 $R^3 = \text{H}$

taxchinin H (2):

$R^1 = R^2 = \text{H}$ ,  $R^3 = \text{cinnamoyl}$



taxchinin I (3):

$R^1 = \text{Ac}$ ,  $R^2 = \text{Bz}$ ,  $R^3 = \text{H}$

taxchinin J (4):

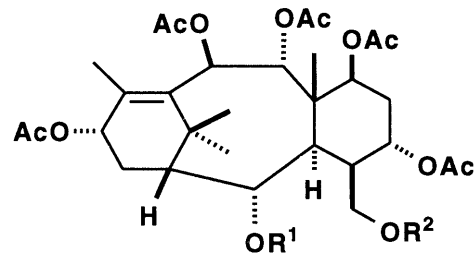
$R^1 = \text{cinnamoyl}$ ,  $R^2 = \text{Ac}$ ,  $R^3 = \text{H}$

taxchinin K (5):

$R^1 = R^2 = \text{Ac}$ ,  $R^3 = \text{Bz}$

taxchinin C (7):

$R^1 = \text{Ac}$ ,  $R^2 = R^3 = \text{Bz}$



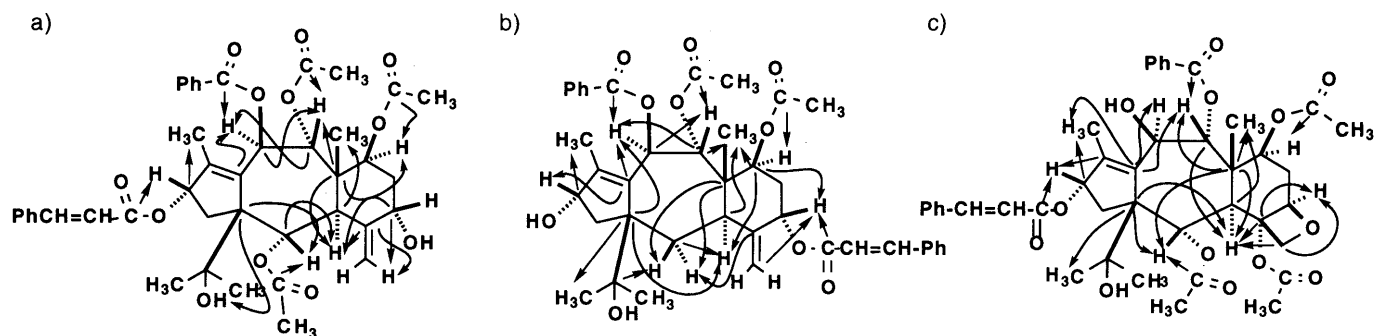
taxchin B (6):

$R^1 = \text{Ac}$ ,  $R^2 = \text{cinnamoyl}$

taxchin A (8):

$R^1 = \text{H}$ ,  $R^2 = \text{Ac}$

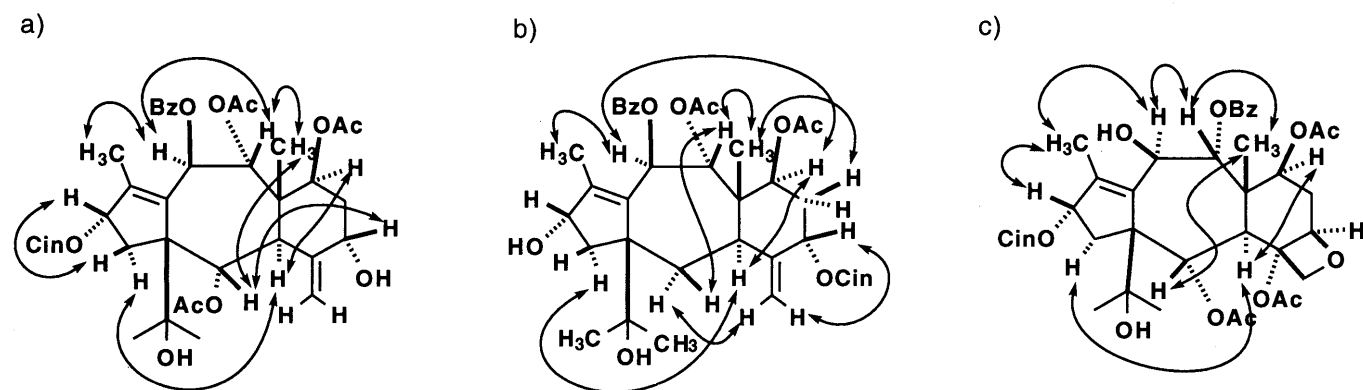
Taxchinin E (1),  $\text{C}_{42}\text{H}_{48}\text{O}_{12}$ , mp 134–136°C,  $[\alpha]_{\text{D}}^{19} -17.49$  (c 0.12,  $\text{CHCl}_3$ ), was isolated as amorphous powder in  $1.8 \times 10^{-4}\%$  yield, and its broadened  $^1\text{H}$  NMR spectrum in  $\text{CDCl}_3$  at an ambient temperature suggested slow conformational change on the NMR time scale. Both  $^1\text{H}$ - and  $^{13}\text{C}$  NMR spectra of taxchinin E at 263 K revealed two sets of signals corresponding to each conformer (major:minor 0.58:0.42). From HH-COSY experiment, it became clear that they derived from the chair/boat and boat/chair ( $J_{\text{H-9,H-10}} = 3.5$  and  $10.5$  Hz) for B/C ring, respectively.<sup>6)</sup> Unusual downfield shift of C-15 ( $\delta$  75.4 for the major and  $\delta$  75.3 for the minor conformer) indicated the rearranged taxane skeleton,<sup>8)</sup> tricyclo[9.3.0.0<sup>3,8</sup>]tetradecane, already found in taxchinins A-D.<sup>5,6)</sup> Detailed investigation on LR-CH-COSY (Fig. 1) and NOESY (Fig. 2) spectra of each conformer led to the conclusion that taxchinin E possesses the formula 1.



**Fig. 1.** Principal Long Range  $^{13}\text{C}$ - $^1\text{H}$  COSY Observed for a) Taxchinin E (1, Major Conformer), b) Taxchinin H (2), c) Taxchinin J (4, Major Conformer)

Taxchinin H (2),  $\text{C}_{40}\text{H}_{46}\text{O}_{10}$ , mp 115-118°C,  $[\alpha]_{\text{D}}^{19}$  -65.29 (c 0.17,  $\text{CHCl}_3$ ), was isolated as amorphous powder in  $1.1 \times 10^{-3}\%$  yield. Interestingly, taxchinin H exists as a single conformer in  $\text{CDCl}_3$  at an ambient temperature despite its 5/7/6-membered ring system. For this diterpene, COSY and NOESY (Fig. 2) as well as LR-CH-COSY (Fig. 1) spectra were found to be of diagnostic importance for deduction of the structure depicted as formula 2. The coupling constant between H-9 and H-10 ( $J = 10.5$  Hz) suggested that the B/C ring in 2 adopts boat/chair conformation in solution. Judging from the  $^{13}\text{C}$  NMR ( $\delta$  29.3) and DEPT ( $\text{CH}_2$ ) data, taxchinin H has no oxygen functionality on C-2, but methylene protons are geminally coupled with  $J = 14.1$  Hz. This compound belongs to the group with rearranged taxane skeleton bearing no functionality at C-2.<sup>9</sup>

Both taxchinin I (3),  $\text{C}_{40}\text{H}_{46}\text{O}_{13}$ , mp 235-237°C (colorless needles from ether),  $[\alpha]_{\text{D}}^{19}$  -6.08 (c 0.12,  $\text{CHCl}_3$ ),  $6.1 \times 10^{-4}\%$  yield, and taxchinin J (4),  $\text{C}_{42}\text{H}_{48}\text{O}_{13}$ , mp 238-240°C (colorless needles from ether),  $[\alpha]_{\text{D}}^{19}$  +23.36 (c 0.11,  $\text{CHCl}_3$ ),  $3.4 \times 10^{-4}\%$  yield, exhibited their signals in  $^1\text{H}$ - and  $^{13}\text{C}$  NMR spectra at 263 K in  $\text{CDCl}_3$  or  $\text{CDCl}_3$ -benzene- $d_6$  (conformer ratio, 0.6:0.4 for taxchinin I, 0.54:0.46 for taxchinin J) as a mixture of two conformational isomers due to the ring flip of the central 7-membered ring. Taxchinin I turned out to be debenzoyl-taxchinin C, since benzylation of taxchinin I with benzoyl chloride in pyridine gave an identical compound with taxchinin C (7), whose structure had already been established.<sup>5</sup> The position and configuration of the free OH group at C-10 was confirmed by the experiments of COSY, NOESY and LR-CH-COSY. Besides a hydroxyl at C-10, taxchinin J (4) has a cinnamoyl and whose structure including stereochemistry was elucidated as 4 in a way similar to that described above.



**Fig. 2.** Crucial NOe's Observed for a) Taxchinin E (1, Major Conformer: Chair/Boat for B/C ring), b) Taxchinin H (2), c) Taxchinin J (4, Major Conformer: Chair/Boat for B/C ring)

Taxchinin K (5),  $\text{C}_{42}\text{H}_{48}\text{O}_{14}$ , mp 217-219°C (colorless needles from ether-hexane),  $[\alpha]_{\text{D}}^{18}$  -30.00 (c 0.05,  $\text{CHCl}_3$ ),  $4.0 \times 10^{-5}\%$  yield, revealed its  $^1\text{H}$  NMR signals as a single conformer in  $\text{CDCl}_3$  and whose coupling constant between H-9 and H-10 ( $J = 11$  Hz) was consistent with that of the boat-like conformation of ring B. The  $^1\text{H}$ - and  $^{13}\text{C}$  NMR spectra of 5 were very similar to those of taxchinin C (7) and suggested the presence of two benzoyls and four acetyls. The downfield shift of C-9, H-9 and H-10, compared with the chemical shift of the related diterpenoid having C-8 OAc group,<sup>5</sup> indicated the location of another benzoyl at C-9.

Taxchin B (**6**),  $C_{41}H_{52}O_{14}$ , mp 124-126°C (colorless needles from ether-hexane),  $[\alpha]_D^{19} +39.74$  (c 0.15,  $CHCl_3$ ),  $8.3 \times 10^{-5}\%$  yield, has a normal taxane skeleton, and its NMR spectra were quite similar to those of taxchin A (**8**);<sup>6)</sup> the difference between the two diterpenes is the presence of an acetoxy at C-2 and a cinnamate at C-20 in the former, whereas the latter lacks the ester and has an acetoxy at corresponding positions. The structure and relative stereochemistry depicted were supported by LR-CH-COSY and NOESY experiments (Fig. 3) This terpene also belongs to a taxoid group bearing neither oxygen functionality nor  $sp^2$ -hybridized carbon at C-4.

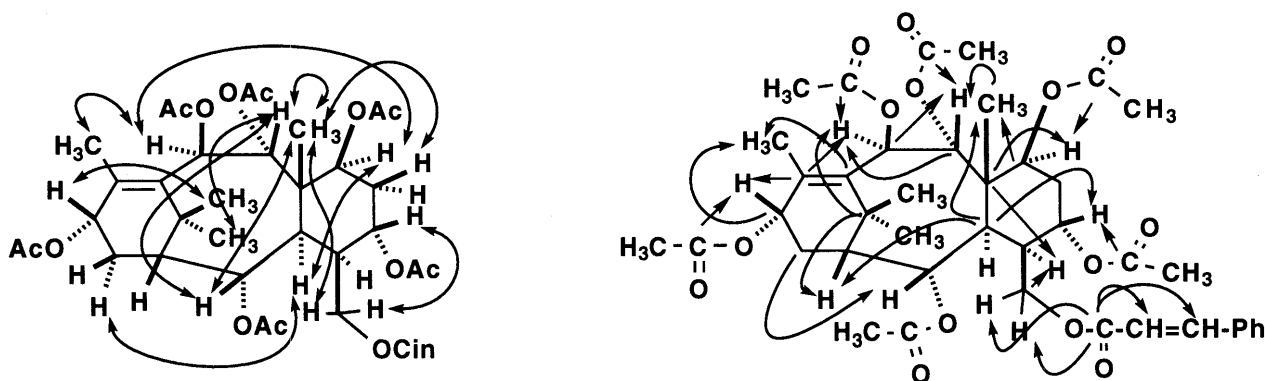


Fig. 3. Key NOe's (Left) and Long Range  $^{13}C$ - $^1H$  COSY (Right) Observed for Taxchin B (**6**)

Evaluation of the biological activity of the new diterpenoids described above toward tubulin depolymerization assay is currently under way.

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