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NOTE

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Isolation of phenylisoserine methyl ester from the leaves of *Taxus cuspidata* var. *nana*

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Abstract From methanolic extracts of leaves of kyaraboku, *Taxus cuspidata* var. *nana*, phenylisoserine methyl ester (**3**) was isolated along with taxinine (**1**), taxol (**2**), sciadopitysin (**4**), ginkgetin (**5**), isorhamnetin (**6**), and quercetin (**7**). This is the first time that phenylisoserine methyl ester has been isolated from *T. cuspidata* var. *nana.* Compound **3** was also isolated from the ethanolic extracts of leaves of *T. cuspidata* var. *nana.* Furthermore, compound **3** was identified in methanolic extracts from the bark of this tree.

Key words Phenylisoserine methyl ester · *Taxus cuspidata* var. *nana* · Taxol · Genus *taxus*

Introduction

Trees of the genus *Taxus* belonging to *Taxaceae* contain many biologically active substances including antitumor compounds, antifungicides, and others; however, only taxol has been utilized to date.[1] A very strong antitumor agent, taxol was first isolated from the bark of *Taxus brevifolia* by Wani et al.[2] in 1971. Since then, taxol and related taxane-type diterpenoids, known as taxoids, have been studied intensively in terms of their chemistry, structure–activity relationships, clinical pharmacology, and therapeutic potential.[3–6]

In continuation of our recent work on the isolation of compounds from Kyaraboku, *Taxus cuspidata* var. *nana*,[7] and Ichii, *Taxus cuspidata*,[8] on the antifungal activities of these compounds and their derivatives against certain plant pathogenic fungi[9,10] and on the production of taxol in tissue cultures of *T. cuspidata* var. *nana*,[11,12] we isolated phenylisoserine methyl ester, a compound related to taxol. The biosynthesis of taxol, especially the biosynthesis of phenylisoserine, the taxol side chain, and its incorporation into the taxol molecule, has been studied by Fleming et al.[13,14] More than 100 taxol derivatives have been isolated from trees of the genus *Taxus*,[5] however, there is no report about the isolation of phenylisoserine and/or its derivatives from this genus. In the present report, we describe about the isolation of phenylisoserine methyl ester from the leaves of *T. cuspidata* var. *nana*. This is the first report of the isolation of phenylisoserine methyl ester from the genus *Taxus*.

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Materials and methods

Plant material

Fresh leaves of *Taxus cuspidata* var. *nana* were collected in July 2000, on the outskirts of Matsuyama City, Ehime Prefecture, Japan.

Extraction from leaves of *Taxus cuspidata* var. *nana*

Fresh leaves of *T. cuspidata* var. *nana* (800 g) were extracted twice for 1 week with methanol at room temperature. The methanol solution was concentrated to give methanolic extracts (84.6 g). The extracts were suspended with water and then successively extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. The chloroform-soluble fraction (7.42 g) and the ethyl acetate-soluble fraction (15.64 g) gave positive color reactions for flavonoids with magnesium and hydrochloric acid (Mg-HCl). Terpenoids of the taxane type were also identified after reaction with potassium dichromate in 40% sulfuric acid on a thin-layer chromatography (TLC) plate.[2] The two fractions were found to contain almost the same compounds by TLC, so they were combined and called fraction 1.

Isolation of compounds **1–3** from fraction 1

Fraction 1 (23.0 g) was separated by silica gel column chromatography into two fractions that contained terpenoids and flavonoids by eluting with chloroform–methanol with a solvent gradient as described in the previous report.[7]

The terpenoid fraction (12.54 g) was chromatographed on a silica gel column using an *n*-hexane–acetone solvent gradient as described previously.[7] Three compounds, **1–3**, were isolated from this fraction. The numbering of the compounds reflects the order in which they were eluted.

*Taxinine (**1**) and taxol (**2**)*

Taxinine (**1**) (244.7 mg), m.p. 266°–268°C (lit m.p. 266°–268°C)[15] and taxol (**2**) (5.4 mg), m.p. 213°–215°C (lit m.p. 213°–216°C)[2] were isolated from the first and second eluates of the terpenoid fraction, respectively, as described previously.[7]

*Isolation of compound **3** from fraction 1*

The waxy solid obtained from the third eluate (359 mg) was rechromatographed on a silica gel column using an *n*-hexane–ethyl acetate solvent gradient and yielded the fraction containing phenylisoserine methyl ester (24.1 mg). The fraction was rechromatographed on a silica gel column using *n*-hexane–ethyl acetate as the eluting solvent. Phenylisoserine methyl ester (**3**) (4.4 mg) was obtained as colorless crystals after recrystallization from chloroform and methanol, m.p. 182°–184°C (lit m.p. 183°–185°C).[2] UV (ultraviolet) λ_{max}^{MeOH} nm (loge): 219 (4.23). [lit λ_{max}^{MeOH} nm (loge): 217 (4.24)].[2] $[\alpha]_D^{23} = -45$ ($C = 0.2$, MeOH) [lit $[\alpha]_D^{20} = -49.6$ ($C = 1.0$, MeOH)].[2] FAB-MS (fast atom bombardment mass spectrum) *m/z*: 300 $(M + H)^+$ (100%), 222, 210, 122, 105. HR/FAB-MS (high-resolution fast atom bombardment mass spectrum) *m/z*: 300.1218 $(M + H)^+$. ^{1}H-NMR (proton nuclear magnetic resonance) (400 MHz, $CDCl_3$) δ 3.30 (1H, br s, OH), 3.85 (3H, s, OCH_3), 4.64 (1H, d, $J = 2.2$ Hz, 2-H), 5.75 (1H, dd, $J = 9.3$, 2 Hz, 3-H), 6.98 (1H, d, $J = 8.8$ Hz, NH), 7.31–7.52 (8H, m, aromatic protons), 7.76–7.78 (2H, m, aromatic protons). ^{13}C-NMR data are shown in Table 1. Compound **3** was identified as phenylisoserine methyl ester by comparison of the NMR and mass spectra with authentic sample synthesized by the method of Guo et al.[16] The mixed melting point of compound **3** with an authentic sample was not depressed.

Isolation of compounds **4–7** from fraction 1

The flavonoid fraction (9.12 g) was chromatographed on a silica gel column using chloroform–methanol as described in the previous report.[7] Four compounds, **4–7**, were isolated from the flavonoid fraction and were numbered according to the order of their elution.

Sciadopitysin (**4**) (94.8 mg), m.p. 294°–296°C (lit m.p. 295°–297°C),[17] ginkgetin (**5**) (5.7 mg), m.p. >300°C (lit m.p. 336°C),[18] isorhamnetin (**6**) (9.6 mg), m.p. 303°–305°C (lit m.p. 304°–305°C),[19] and quercetin (**7**) (30.1 mg), m.p. 312°–314°C (lit m.p. 313°–315°C)[20] were isolated from the first to forth eluates of the flavonoid fraction, respectively, as described previously.[7]

Table 1. ^{13}C-NMR assignments for compound 3

Carbon position[a]	Chemical shift (ppm)[b]
C-1	166.8
C-2	73.2
C-3	54.8
C-5	173.4
OCH_3	53.3
1′	134.0
2′, 6′	128.7
3′, 5′	128.8
4′	128.0
1″	138.7
2″, 6″	126.9
3″, 5″	127.0
4″	131.8

[a] For numbering of carbons in compound **3**, refer to Fig. 1
[b] 100 MHz, $CDCl_3$. Chemical shifts are in ppm from tetramethylsilane

Preparation of compound **3**

Phenylisoserine methyl ester (**3**), m.p. 182°–184°C, was synthesized from benzaldehyde through eight steps by the method of Guo et al.[16] in a total yield of 1.7%. FAB-MS *m/z*: 300 $(M + H)^+$ (100%), 222, 210, 122, 105. ^{1}H-NMR (270 MHz, $CDCl_3$) δ 3.26 (1H, s, OH), 3.85 (3H, s, OCH_3), 4.64 (1H, d, $J = 1.8$ Hz, 2-H), 5.74 (1H, dd, $J = 8.8$, 1.9 Hz, 3-H), 6.98 (1H, d, $J = 8.6$ Hz, NH), 7.31–7.55 (8H, m, aromatic protons), 7.75–7.79 (2H, m, aromatic protons). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 53.3 (OCH_3), 54.8 (C-3), 73.2 (C-2), 126.9 (2″ and 6″), 127.0 (3″ and 5″), 128.0 (4′), 128.7 (2′ and 6′), 128.8 (3′ and 5′), 131.8 (4″), 134.1 (1′), 138.7 (1″), 166.8 (C-1), 173.4 (C-5).

Isolation of phenylisoserine methyl ester **3** in the ethanolic extracts from the leaves of *Taxus cuspidata* var. *nana*

The fresh leaves of *T. cuspidata* var. *nana* (650 g) were extracted twice for 1 week with ethanol at room temperature. The ethanol solution was concentrated to give ethanolic extracts (74.1 g). The extracts were suspended with water and then sucessively extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol in the same manner as described above. The chloroform-soluble fraction (6.82 g) and the ethyl acetate-soluble fraction (12.07 g) contained almost the same compounds by TLC, so the two fractions were combined and named fraction A.

Isolation of compound **3** from fraction A

Fraction A (18.80 g) was roughly separated by silica gel column chromatography into two fractions that contained

terpenoids and flavonoids by eluting with chloroform–methanol (9:1 v/v) in a manner similar to that described above.

The terpenoid fraction (10.49 g) was chromatographed on a silica gel column using *n*-hexane–acetone in the manner as described above. The waxy solid obtained from the third eluate (268 mg) was rechromatographed on a silica gel column using *n*-hexane–ethyl acetate as eluting solvent and yielded the fraction containing phenylisoserine methyl ester (18.6 mg). The fraction was rechromatographed on a silica gel column using *n*-hexane–ethyl acetate as eluting solvent. Phenylisoserine methyl ester (**3**) (3.2 mg) was obtained as colorless crystals after recrystallization from chloroform and methanol, m.p. 182°–184°C (lit m.p. 183°–185°C).[2] FAB-MS *m/z*: 300 $(M + H)^+$ (100%), 222, 210, 122, 105. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.30 (1H, br s, OH), 3.85 (3H, s, OCH_3), 4.64 (1H, d, J = 2.2 Hz, 2-H), 5.75 (1H, dd, J = 9.3, 2 Hz, 3-H), 6.98 (1H, d, J = 8.8 Hz, NH), 7.31–7.52 (8H, m, aromatic protons), 7.76–7.78 (2H, m, aromatic protons). The ^{13}C-NMR spectrum was identical with that of compound **3** isolated from the methanolic extracts of *T. cuspidata* var. *nana* leaves. Compound **3** was identified as phenylisoserine methyl ester by comparison of the NMR and mass spectra with authentic samples synthesized by the method of Guo et al.[16] and isolated from *T. cuspidata* var. *nana* leaves. The mixed melting point of compound **3** with authentic sample was unchanged.

Measurement of phenylisoserine methyl ester and taxol content in the leaves and bark of *Taxus cuspidata* var. *nana*

Fresh leaves and bark of *T. cuspidata* var. *nana* (30 g) were extracted twice with methanol at room temperature. The methanolic solutions were concentrated to give methanolic extracts. The extracts were suspended in dichloromethane–water (1:1) using the method of Witherup et al.[21] The organic solvent evaporated to dryness under reduced pressure. The residue was dissolved in methanol and the solution diluted to 5 ml with methanol. The amount of taxol and phenylisoserine methyl ester in the solution was determined by high-performance liquid chromatography (HPLC) performed on a reverse-phase column (Supelco sil TMLCF) in a Shimadzu LC 10A liquid chromatograph equipped with a UV detector (wavelength 227 nm) by isocratic elution with acetonitrile–tetrahydrofuran–water (17:28:5 v/v) as the mobile phase. The flow rate was 1.5 ml/min, and all chromatograms were plotted at the absorbance maximum of taxol (227 nm). A 10-μl aliquot of the solution was injected into the column. The taxol and phenylisoserine methyl ester in the solution was identified by comparing the retention times with those of authentic taxol and phenylisoserine methyl ester isolated from *T. cuspidata* var. *nana* and by adding authentic taxol and phenylisoserine methyl ester. A calibration curve was obtained using the authentic taxol and phenylisoserine methyl ester. The results are summarized in Table 2.

Table 2. Content of phenylisoserine methyl ester and taxol in the leaves and bark of *Taxus cuspidata* var. *nana*

Sample	Phenylisoserine methyl ester	Taxol
Leaves	0.0023	0.0029
Bark	0.0034	0.0042

Results given as percent on dry weight

Results and discussion

Isolation of compounds **1–3** from the leaves of *Taxus cuspidata* var. *nana*

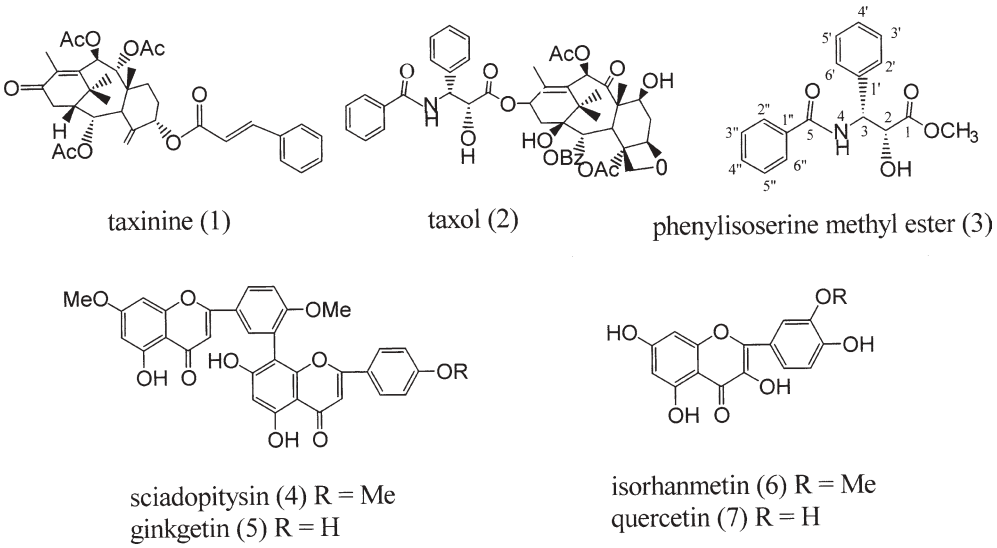
Compounds **1**–**3** were isolated from the terpenoid fraction of the methanolic extracts of leaves of *Taxus cuspidata* var. *nana* in yields of 0.0651%, 0.0014%, and 0.0012%, respectively, from the dried leaves. Compound **3** was isolated from the leaves of *T. cuspidata* var. *nana* for the first time. The isolation of taxinine (**1**) and taxol (**2**) from the leaves of *T. cuspidata* var. *nana* was previously reported by Tachibana et al.[7] The chemical structures of compounds **1**–**3** isolated from *T. cuspidata* var. *nana* are shown in Fig. 1. In addition, taxinine B, isolated previously from the leaves of *T. cuspidata* var. *nana* by Tachibana et al.,[7] was not isolated in the present study. However, one spot having the positive color reaction as taxinine B with potassium dichromate in 40% sulfuric acid[2] and with a similar Rf value was detected on a TLC plate. Therefore, it is considered that taxinine B exists in the leaves of *T. cuspidata* var. *nana*. The isolation of taxinine B will be conducted later.

Compound **3**, $C_{17}H_{17}O_4N$, (M^+ = 299), m.p. 182°–184°C, was composed of colorless crystals. The FAB mass spectrum of compound **3** showed $[M + H]^+$ at 300. The high-resolution mass spectrum of compound **3** showed $[M + H]^+$ at 300.1218 ($C_{17}H_{18}O_4N$ requires 300.1231). The molecular formula of compound **3** was confirmed as $C_{17}H_{17}O_4N$ by high-resolution mass spectrometry. The UV spectrum of compound **3** coincided with that of phenylisoserine methyl ester reported by Witherup et al.[21]

In the ^{1}H-NMR spectrum of compound **3**, signals from two monosubstituted rings (ten aromatic protons) at 7.31–7.78 ppm, one amine proton at 6.98 ppm, one benzyl proton at 5.76 ppm, one proton attached to a secondary carbon atom at 4.78 ppm, and of one methoxy group at 3.85 ppm were observed. From the results obtained above, compound **3** was suggested to be phenylisoserine methyl ester. The structure was also supported by the 2D-COSY spectrum. Mass spectra and the ^{1}H-NMR spectrum of compound **3** were in good agreement with the data reported by Wani et al.[2] and Denis et al.[22] The ^{13}C-NMR spectrum well explained the structure of compound **3**. The ^{13}C-NMR assignments for compound **3** are shown in Table 1.

To confirm the chemical structure of compound **3**, phenylisoserine methyl ester was synthesized from benzaldehyde through eight steps in a yield of 1.7% by the method of Guo et al.[16] The NMR and MS spectra of compound **3** were consistent with those of the authentic sample synthe-

Fig. 1. Chemical structures of compounds **1**–**7**



sized by the method of Guo et al.[16] Also, there was no depression in the mixed melting point test of compound **3** and the synthesized compound. From the results obtained here, compound **3** was identified as phenylisoserine methyl ester.

It was considered that phenylisoserine methyl ester may be obtained as an artifact by methylation of phenylisoserine during the methanolic extraction of kyarboku leaves. However, even when the extraction solvent was changed from methanol to ethanol, phenylisoserine methyl ester was isolated in the extracts from kyaraboku leaves in a yield of 0.0011% from dried leaves. Therefore, phenylisoserine methyl ester was not an artifact. Phenylisoserine methyl ester was obtained as a product of the methanolysis of taxol by Wani et al.;[2] however, there is no report about the isolation of phenylisoserine methyl ester from trees of the genus *Taxus*. This is the first report of the isolation of phenylisoserine methyl ester from *Taxus* trees. Taxol is present in the leaves, root, shoot, and bark of trees of the genus *Taxus*.[23] Therefore, phenylisoserine methyl ester is thought to be present in the root, wood, and bark of *T. cuspidata* var. *nana.* Compound **3** in the bark of *T. cuspidata* var. *nana* was detected by HPLC. Isolation of the compound in the root and wood will be conducted later. Furthermore, phenylisoserine methyl ester was also isolated from the bark of *Taxus chinensis*. The isolation of the ester will be published elsewhere.

Isolation of compounds **4**–**7** from the leaves of *Taxus cuspidata* var. *nana*

Compounds **4**–**7** were isolated from the flavonoid fraction of the methanolic extracts of leaves of *T. cuspidata* var. *nana* in a yield of 0.0252%, 0.0015%, 0.0026%, and 0.0080%, respectively, from the dried leaves. The isolation of sciadopitysin (**4**), ginkgetin (**5**), isorhamnetin (**6**), and quercetin (**7**) from the leaves of *T. cuspidata* var. *nana* was reported by Tachibana et al.[7] and Kurose et al.[8] The chemical structures of compounds **4**–**7** isolated from *T. cuspidata* var. *nana* are shown in Fig. 1.

Content of taxol and phenylisoserine methyl ester in the leaves and bark of *Taxus cuspidata* var. *nana*

The content (%, dry weight) of taxol and phenylisoserine methyl ester in the leaves and bark of *T. cuspidata* var. *nana* was 0.0023% and 0.0029%, and 0.0034% and 0.0042%, respectively, as determined by HPLC (see Table 2). The amounts of these compounds in the leaves and bark was almost the same. However, phenylisoserine was not detected in either extract by TLC. It is not clear why phenylisoserine methyl ester exists in the leaves and bark of *T. cuspidata* var. *nana*. Compound **3** is considered to be related to the biosynthesis of taxol because taxol has phenylisoserine as a side chain.

The biosynthesis of taxol,[24] and especially the biosynthesis of phenylisoserine, a side chain of taxol, and its incorporation into the taxol molecule, has been studied by Fleming et al.[13,14] According to Fleming et al.,[13,14] phenylalanine was converted to phenylisoserine via β-phenylalanine and phenylisoserine was then incorporated into baccatin III to produce debenzoyltaxol. Finally, *N*-benzoylation of debenzoyltaxol produced taxol. An alternative route of biosynthesis of phenylisoserine via phenylalanine, cinnamic acid, its isomerization, epoxidation, and final ring opening with concurrent amination was ruled out because of negative incorporation by Fleming et al.[13,14] However, many questions regarding the biosynthesis of taxol are still unanswered.

The isolation of phenylisoserine methyl ester may suggest the existence of another biosynthetic route for taxol. Phenylisoserine methyl ester may be incorporated into taxol after hydrolysis of the ester with a lipase. It is known that lipases are difficult to isolate from higher plants because of difficulties in purification.[25] However, Aizono et al.[25] reported that methyl butylate was hydrolyzed to butylic

acid with three lipases present in rice bran. Therefore, it is suggested that phenylisoserine methyl ester in *T. cuspidata* var. *nana* may be hydrolyzed with a lipase such as the lipases present in rice bran. If so, phenylisoserine could exist in extracts from both leaves and bark of *T. cuspidata* var. *nana*. However, phenylisoserine was not detected in either extract in this study. Therefore, this hypothesis may be ruled out. However, Muranaka et al.[26] reported that the amount of taxol production increased several-fold when phenylisoserine was added to cell suspension cultures of *T. cuspidata* var. *nana.* This result suggests that phenylisoserine is a precursor of taxol and the amount of taxol produced increases because part of the phenylisoserine is incorporated into the taxol molecule. It is not clear why phenylisoserine methyl ester exists in the leaves and bark of trees of the genus *Taxus*. The significance of phenylisoserine methyl ester in *Taxus* trees may be clarified in the near future.

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References

1. Tachibana S (1995) Utilization of biologically active substances in trees (in Japanese). Mokuzai Gakkaishi 41:967–977
2. Wani MC, Taylor HL, Wall ME (1971) Plant antitumor agents. VI. The isolation and structure of taxol, a novel antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93:2325–2327
3. De Furia MD (1997) Paclitaxel (taxol): a new natural product with major anticancer activity. Phytomedicine 4:273–282.
4. Eisenhauer EA, Vermorken JB (1998) The taxoids comparative clinical pharmacology and therapeutic potential. Drugs 55:5–30
5. Baloglu E, Kingston DGI (1999) The taxane diterpenoids. J Nat Prod 62:1448–1472
6. Guéritte F (2001) General and recent aspects of the chemistry and structure–activity relationships of taxoids. Curr Pharm Des 7:1229–1249
7. Tachibana S, Matsuo A, Itoh K, Oki T (1994) Extractives in the leaves and bark of *Taxus cuspidata* Sieb. et. Zucc. var. *nana* Rehder. Mokuzai Gakkaishi 40:1008–1013
8. Kurose K, Itoh K, Tachibana S, Irie K, Oki T (1995) Extractives of *Taxus cuspidata* Sieb. et. Zucc. leaves and chemotaxonomy of trees of genus *taxus.* Bull Ehime Univ Forest 33:1–16
9. Muranaka T, Ueno J, Itoh K, Matsumoto I, Tachibana S (1997) Studies on utilization of extractives from the genus *taxus* tree (2). Antifungal activities of taxinine and its derivatives against *Colletotrichum fragariae*, *Botrytis cinerea* and *Corynespora cassiicola* (in Japanese). Bull Ehime Univ Forest 35:45–53
10. Muranaka T, Kurose K, Itoh K, Tachibana S (1999) Utilization of extractives from genus *taxus* tree I. Antifungal activities of flavonoids, taxinine, and its derivatives against *Cochiliobolus miyabeanus* and *Altenaria kikuchiana* (in Japanese). Mokuzai Gakkaishi 45:42–50
11. Tachibana S, Watanabe E, Itoh K, Oki T (1994) Formation of taxol in *Taxus cuspidata* Sieb. et Zucc. var. *nana* Rehder callus cultures. Mokuzai Gakkaishi 40:1254–1258
12. Yoshida M, Muranaka T, Kurose K, Itoh K, Tachibana S (2002) Stimulatiuon of the production of taxol by oligosaccharides in *Taxus cuspidata* variety nana callus cultures. Pak J Biol Soc 5:461–465
13. Fleming PE, Mocek U, Floss HG (1993) Biosynthesis of taxoids. Mode of formation of the taxol side chain. J Am Chem Soc 115:805–807
14. Fleming PE, Knaggs AR, He X-G, Mocek U, Floss HG (1994) Biosynthesis of taxoids. Mode of attachment of the taxol side chain. J Am Chem Soc 116:4137–4138
15. Yoshizaki F, Madarame M, Takahashi C (1986) Principal constituents of the sheeds of Japanese yew (*Taxus cuspidata*). Shoyakugaku Zasshi 40:429–431
16. Guo DM, Liu YC, Chen CS (1993) A partial chemoenzymatic synthesis of the taxol C-13 side chain *N*-benzoyl-(2R,3S)-3-phenylisoserine. J Org Chem 58:1287–1299
17. Kariyone T, Sawada T (1958) Studies on flavonoids in the leaves of coniferae and allied plant. III. On the flavonoid from the leaves of *Taxus cuspidata* Sieb. et Zucc. and relation between ginkgetin, kayaflavone, sciadopitysin, and sotetsuflavone (in Japanese). Yakugaku Zasshi 78:1023–1027
18. Wilson BA, Finch CM, Ollis WD, Robinson KW (1963) The structures of the naturally occurring bisflavonyls. J Chem Soc 1477–1490
19. Kostanecki St V, Lampe V (1904) Syntheses des 2-oxyflavonols. Chem Ber 37:1402–1405
20. Horhammer L, Wager H, Kr mer H, Farkas L (1967) Isolierung und konstitution aufklrung neuer glykoside von *Brassica napus* L. und *Sinapis avensis* L. Chem Ber 100:2301–2305
21. Witherup KM, Look SA, Stasko MW, Ghiorzi TJ, Muschik GM (1990) *Taxus* spp. needles contain amounts of taxol comparable to the bark of *Taxus brevifolia*: analysis and isolation. J Nat Prod 53:1249–1255
22. Denis JN, Greene AW, Serra AA, Luche MJ (1986) An efficient, enantioselective synthesis of the taxol side chain. J Org Chem 51:46–50
23. Edward M, Croom Jr (1995) *Taxus* for taxol and taxoids, section II, supply of taxol. Chapter 3. In: Suffness M (ed) Taxol science and applications. CRC, New York, pp 37–70
24. Rohr J (1997) Biosynthesis of taxol. Angew Chem Int Ed Engl 36:2190–2195
25. Aizon Y, Fujiki Y, Funatsu M (1976) Purification of rice bran lipase and its multiple form, section III. A guide for purification of plant enzymes and proteins. In: Morita Y, Shin M, Asada K, Ido S (eds) Shokubutsu koso tanpakushitsu kenkyuho. Kyoritsu, Tokyo, pp 206–216
26. Muranaka T, Yosida M, Itoh K, Tachibana S (2004) Effect of culture medium, elicitors, a plant growth regulator and a biogenetic precursor on taxol production in cell suspension cultures of *Taxus cuspidata* variety nana. Pak J Biol Sci 7:399–405