Pakistan Journal of Biological Sciences 7 (10): 1788-1792, 2004
ISSN 1028-8880

Isolation of Phenylisoserine Methyl Ester from the Bark of *Taxus mairei*

Sanro Tachibana, Etsuko Watanabe, [1]Yu Chang Su, [1]Jui Chung Shieh and [2]Hung Kuei Lee
Department of Applied Bioscience, Faculty of Agriculture,
Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
[1]Taiwan Forest Research Institute, Nan-Hai Road, Taipei, Taiwan
[2]Department of Chemical Engineering, Chinese Culture University, Hwa Kang, Yang Ming Shan, Taiwan

Abstract: From the methanolic extracts of bark of *Taxus mairei,* phenylisoserine methyl ester (2) was isolated along with paclitaxel (taxol) (1). This is the first time that phenylisoserine methyl ester (2) has been isolated from *T. mairei.* Compound 2 was also isolated from the ethanolic extracts of bark of *T. mairei.*

Key words: Phenylisoserine methyl ester, Paclitaxel (taxol), *Taxus mairei*, genus *Taxus*

INTRODUCTION

Trees contain many useful compounds, e.g. anticancer, anti-inflammatory and so on. Trees of the genus *Taxus* belong to *Taxaceae* contain paclitaxel (taxol), a very strong antitumor agent[1]. Wani *et al.*[2] isolated paclitaxel for the first time in 1971 from the bark of *Taxus brevifolia*. Since then, much research on paclitaxel and related taxane-type diterpenoids, taxoids, has been conducted in terms of chemistry, structure-activity relationships, clinical pharmacology and therapeutic potential[3-6].

In recent research on the isolation of compounds from Kyaraboku, the Japanese dwarf yew, *Taxus cuspidata* var. *nana*[7], and Ichii, Japanese yew, *T. cuspidata*[8], on the antifungal activities of these compounds and their derivatives against certain plant pathogenic fungi[9,10] and on the production of paclitaxel in tissue cultures of *T. cuspidata* var. *nana*[11-13], we isolated phenylisoserine methyl ester, a compound related to paclitaxel from *Taxus cuspidata* var. *nana* in trees of the genus *Taxus* for the first time[14]. More than one hundred paclitaxel derivatives have been isolated from trees of the genus *Taxus*[5]. However, there is only one report about the isolation of phenylisoserine methyl ester from trees of this genus[14]. Therefore, it is worthwhile to investigate whether phenylisoserine methyl ester exists in other trees of the genus *Taxus*.

T. mairei is an evergreen tree with dark and linear foliage and distributed over mountainous regions of the north and central parts of Taiwan[15]. Much research on the isolation of taxoids from *T. mairei* has been conducted[16-20]. However, the isolation of phenylisoserine methyl ester from *T. mairei* has not been reported. In the present study we describe for the first time the isolation of phenylisoserine methyl ester from the bark of *Taxus mairei.*

MATERIALS AND METHODS

This research project was carried out in the Faculty of Agriculture, Ehime University, Japan during 1995-2001 in an effort to isolate toxoid and related compounds from *Taxus mairei*. Authentic paclitaxel was purchased from Sigma Chemical Company as well as isolated from the leaves of *T. cuspidata* var. *nana*[7]. Authentic phenylisoserine methyl ester was synthesized from benzaldehyde by the method of Guo *et al.*[21] as well as isolated from the leaves of *T. cuspidata* var. *nana*[14]. Melting points (°C) were determined on a Yanaco micro melting point apparatus (Yanaco Co. Ltd., Kyoto, Japan) and were uncorrected. Ultraviolet (UV) spectra, λ_{max} (nm), were measured on a Shimadzu UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan); Mass spectra were recorded on a JMS-700 (JEOL, Ltd., Tokyo, Japan). ^{1}H-NMR spectra were recorded on a JEOL JNM-EX400 (JEOL, Ltd., Tokyo, Japan) and a JEOL JNM-CMX300 (JEOL, Ltd., Tokyo, Japan) at 400 MHZ and 270 MHZ, respectively. ^{13}C-NMR spectra were recorded on a JEOL JNM-EX400 (JEOL, Ltd., Tokyo, Japan) at 100 MHZ. Chemical shift was expressed as δ in ppm and TMS was used as an internal standard. Coupling constants (J) were recorded in Hz.

Plant materials: Fresh bark of *T. mairei* was collected in May 1995, on the outskirts of Taipei City, Taiwan,

Corresponding Author: Sanro Tachibana, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan Tel: +81 89 946 9864 E-mail: tatibana@agr.ehime-u.ac.jp

Republic of China by Dr. Y. Chang Su, J. Chung Shieh and H. Kuei Lee.

Extraction from bark of *T. mairei*: Fresh bark of *T. mairei* (370 g, to dried bark) was extracted twice for one week with methanol at room temperature. The methanol solution was concentrated to give methanolic extracts (31.8 g). The extracts were suspended with water and then successively extracted with n-hexane, chloroform and ethyl acetate to give an n-hexane-soluble fraction (0.48 g), chloroform-soluble fraction (2.10 g) and ethyl acetate-soluble fraction (4.47 g), respectively.

Isolation of compounds 1 and 2 from the extracts: The chloroform-soluble fraction (2.05 g) was separated into five fractions (Fr. 1-5) by silica gel column chromatography with chloroform-methanol as the solvent gradient.

Fr. 2 (0.68 g) was chromatographed on a silica gel column using n-hexane-acetone as the solvent gradient. Two compounds, 1 and 2, were isolated from this fraction. The numbering of the compounds reflects the order in which they were eluted.

Compound 1: The second eluate of Fr. 2 containing paclitaxel (29.7 mg) was rechromatographed on a preparative TLC plate with chloroform-methanol (10:1 v/v). This yielded paclitaxel (8.9 mg) as a colorless needle after recrystallization from methanol and water, mp. 213-215°C. (lit mp. 213-216°C) [2]. UV (ultraviolet) λ_{max}^{MeOH} nm (log ε): 227 (4.47), 273 (3.23). [lit UV λ_{max}^{MeOH} nm (log ε): 217 (4.24)] [2]. FD-MS (field desorption mass spectrum) m/z: 854 (M^++H), 853 (M^+), 568, 210 (100%), 105, 43. The UV spectrum of compound 1 was identical to that of authentic paclitaxel isolated from *T. brevifolia*[2]. The mixed-melting-point of compound 1 with authentic sample isolated from the leaves of *T. cuspidata* var. *nana*[7] was not depressed.

Isolation of compound 2 from fraction 2: The waxy solid obtained from the third eluate (117 mg) of fraction 2 was rechromatographed on a silica gel column using n-hexane-ethyl acetate as the solvent gradient and yielded a fraction containing phenylisoserine methyl ester (23.7 mg). This fraction was rechromatographed on a silica gel column using n-hexane-ethyl acetate as the solvent gradient. Phenylisoserine methyl ester (2) (7.6 mg) was obtained as colorless crystals after recrystallization from chloroform and methanol, mp. 182-184°C. (lit mp. 183-185°C)[2]. Uv λ_{max}^{MeOH} nm (log ε): 219 (4.23). [lit UV λ_{max}^{MeOH} nm (log ε): 217 (4.24)][2]. $[a]^{23}_{D}$ = -45 (C = 0.2, MeOH) (lit $[a]^{20}_{D}$ = -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: 300.1215 (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). The ^{13}C-NMR (carbon 13 nuclear magnetic resonance) data are shown in Table 1. Compound 2 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.*[21] and isolated from *Taxus cuspidata* var. *nana*[14], respectively. The mixed-melting-point of compound 2 with authentic samples showed no depression.

Preparation of compound 2: Phenylisoserine methyl ester (2), mp 182-184°C, was synthesized from benzaldehyde through 8 steps by the method of Guo *et al.*[21] in a total yield of 1.7% as described in a previous study[14]. 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 (2) in the ethanolic extracts from the bark of *T. mairei*: The fresh bark of *T. mairei* (350 g, dried bark) was extracted twice for one week with ethanol at room temperature. The ethanol solution was concentrated to give ethanolic extracts (30.9 g). The extracts were suspended with water and then successively extracted with n-hexane, chloroform and ethyl acetate, respectively in the same manner as described above. The n-hexane-soluble fraction (0.45 g), chloroform-soluble fraction (2.28 g) and ethyl acetate-soluble fraction (4.23 g) were obtained, respectively, after evaporation of the solvent.

Isolation of compound 2 from fraction 2: The chloroform-soluble fraction (2.25 g) was separated into five fractions (Fr. 1-5) by silica gel column chromatography with chloroform-methanol (9:1 v/v) in a similar manner as described above.

The second fraction (Fr. 2) (0.72 g) was chromatographed on a silica gel column using n-hexane-acetone as the solvent gradient in a similar manner as

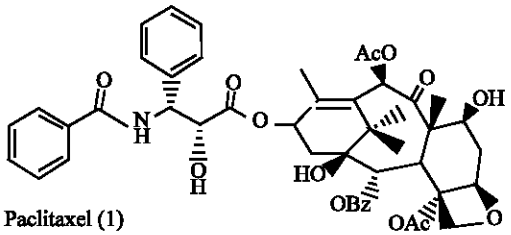
described above. The waxy solid obtained from the third eluate (125 mg) was rechromatographed on a silica gel column using n-hexane-ethyl acetate as the solvent gradient as described above and yielded a fraction containing phenylisoserine methyl ester (25.2 mg). The fraction was rechromatographed on a silica gel column using n-hexane-ethyl acetate as the solvent gradient. Phenylisoserine methyl ester (2) (7.1 mg) was obtained as colorless crystals after recrystallization from chloroform and methanol, mp 182-184°C, as described above. (lit mp. 183-185°C)[2], FAB-MS m/z: 300 (M^++H) (100%), 222, 210, 122, 105. ^{1}H-NMR (400 MHZ, $CDCl_3$) d: 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 was identical to that of compound 2 isolated from the methanolic extracts of *T. mairei* bark. Compound 2 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.*[21] and isolated from the bark of *T. cuspidate* var. *nana*[14], respectively. The mixed-melting-point of compound 2 with authentic samples showed no depression.

RESULTS AND DISCUSSION

Isolation of compounds 1 and 2 from the bark of *Taxus mairei*: Two compounds, namely paclitaxel (1) and phenylisoserine methyl ester (2), were isolated from the chloroform-soluble fraction of the methanolic extracts of bark of *T. mairei* in a yield of 0.0024 and 0.0021% dry weight, respectively. This is the first time that compound 2 have been found in bark. The chemical structures of compounds 1 and 2 isolated from *T. mairei* are shown in Fig. 1. Their structures were determined by UV (ultraviolet), ^{1}H-NMR (proton nuclear magnetic resonance), ^{13}C-NMR (carbon thirteen nuclear magnetic resonance) and MS (mass spectroscopy).

Compound 1, $C_{47}H_{51}NO_{14}$ (M^+) [(molecular ion peak)=853], mp 213-215°C, was composed of colorless needles. The UV spectrum of compound 1 showed a UV absorption spectrum characteristic of paclitaxel [2]. The mass spectrum of compound 1 was well consistent with that of authentic paclitaxel isolated from *T. brevifolia* bark[2]. The mixed-melting-point of compound 1 with the authentic sample isolated from *T. cuspidata* var. nana[7] was undepressed. Therefore, compound 1 was identified as paclitaxel.

The isolation of paclitaxel (1) from the twigs of *T. mairei* was reported by Yang *et al.*[20]. Paclitaxel has strong anti-leukemic and tumor-inhibiting activities[3-6]. It



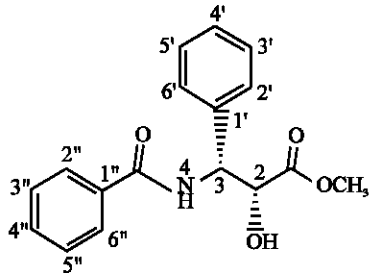


Fig. 1: Chemical structures of compounds 1 and 2

is said that paclitaxel is present in leaves, roots, stems, seeds, shoot and bark of trees of the genus Taxus[22]. However, the supply crisis for paclitaxel and its biogenetic precursor, 10-deacetylbaccatin III, which is the starting compound for the semisynthesis of paclitaxel[23], has stirred interest in producing these compounds using cell cultures. The commercial scale production of these natural products by cell suspension cultures has been reported by Ma *et al.*[24] and Yukumune *et al.*[25], respectively.

Compound 2, $C_{17}H_{17}O_4N$, (M^+ = 299), mp 182-184°C, was composed of colorless crystals. The FAB (fast atom bombardment) mass spectrum of compound 2 showed $[M+H]^+$ (molecular plus proton ion peak) at 300. The high-resolution mass spectrum of compound 2 showed $[M+H]^+$ = 300.1215. ($C_{17}H_{18}O_4N$: theoretical: 300.1231). The molecular formula of compound 2 was confirmed as $C_{17}H_{17}O_4N$ by high-resolution mass spectrometry. The UV spectrum of compound 2 coincided with that of phenylisoserine methyl ester reported by Tachibana *et al.*[14].

In the ^{1}H-NMR spectrum of compound 2, signals from two monosubstituted rings (10 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 one methoxy group at 3.85 ppm were observed. From the results obtained above, compound 2 was suggested to be phenylisoserine methyl ester. The structure was supported by the COSY spectrum. MS and ^{1}H-NMR spectra of compound 2 were consistent with the data reported by Wani *et al.* [2] and Tachibana *et al.*[14]. The ^{13}C-NMR spectrum well explained the structure of compound 2. The ^{13}C-NMR assignments for compound 2 are shown in Table 1. To confirm the

Table 1: ^{13}C-NMR assignments for compound 2 in deuteriochloroform solution (100 MHZ, TMS as internal standard).[a]

Carbon[b]	Compound 2
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) ^{13}C Chemical shifts (δ) are in ppm from TMS tetramethyl silane. [b]For numbering of carbons in compound 2, refer to Fig. 1

Table 2: Content of phenylisoserine methyl ester and paclitaxel in the bark of *Taxus mairei*.

	Content (%, of dry weight)	
Sample	Phenylisoserine methyl ester	Paclitaxel
Bark	0.0021	0.0024

chemical structure of compound 2, a mixed-melting point test of compound 2 with the synthesized compound and the isolated compound from *T. cuspidata* var. *nana*[14] was conducted. There was no depression in the mixed-melting point of compound 2 and the authentic samples. From the results obtained here, compound 2 was identified as phenylisoserine methyl ester.

It was considered that phenylisoserine methyl ester (2) may be obtained as an artifact derived from phenylisoserine and methanol by methylation during the extraction of *T. mairei bark* with methanol. However, even when the extraction solvent was changed from methanol to ethanol, phenylisoserine methyl ester (2) was isolated in the extracts from the bark of *T. mairei* in a yield of 0.0020% dry weight. Therefore, phenylisoserine methyl ester (2) was not an artifact. Phenylisoserine methyl ester (2) was obtained as a product of the methanolysis of paclitaxel by Wani *et al.*[2] and isolated from the leaves of *T. cuspidata* var. *nana* by Tachibana *et al.*[14]. However, there is no report about the isolation of phenylisoserine methyl ester from bark of *T. mairei*. This is the first report of the isolation of phenylisoserine methyl ester (2) from *T. mairei*.

Paclitaxel is present in the leaves, roots, seeds, stems, shoot and bark of trees of the genus *Taxus*[22]. Therefore, phenylisoserine methyl ester (2) is thought to be present in the leaves, roots, seeds, stems, shoot and bark of *T. mairei* because compound 2 is a side chain of the paclitaxel. However, we could not attempt to detect the compound by HPLC as we could not obtain the samples. The detection of the ester will be conducted later.

Content of paclitaxel and phenylisoserine methyl ester in the bark of *T. mairei*: The content of paclitaxel and phenylisoserine methyl ester in the bark of *T. mairei* is shown in Table 2. The amount of these compounds in the bark was almost the same. In the report, we found that paclitaxel was contained in the bark of *T. mairei*. The amount of paclitaxel in the bark of *T. mairei* was a little less than that in the bark of *T. brevifolia*[2] and *T. cuspidata* var. *nana*[7]. However, phenylisoserine in the extract was not detected here on a TLC plate. Compound 2 is considered to be related to the biosynthesis of paclitaxel because paclitaxel has phenylisoserine as a side chain.

Fleming *et al.*[26,27] reported the biosynthesis of phenylisoserine, a side chain of paclitaxel. They found that phenylalanine was first converted to phenylisoserine via phenylalanine, phenylisoserine was then incorporated into baccatin III to produce debenzoyltaxol and finally paclitaxel (taxol) was produced after N-benzoylation[26,27]. However, many questions regarding the biosynthesis of paclitaxel are still unanswered[28].

Tachibana *et al.*[14] suggested that the isolation of phenylisoserine methyl ester may indicate the existence of another biosynthetic route for paclitaxel. Phenylisoserine methyl ester may be incorporated into paclitaxel after hydrolysis of the ester with a lipase. In general, the isolation of lipases from higher plants is difficult because of problems with purification[29]. However, Aizono *et al.*[29] reported that methyl butylate was hydrolyzed to butyric acid with three lipases present in rice bran. From their results, it is suggested that phenylisoserine methyl ester in *T. mairei* is hydrolyzed by lipases such as those present in rice bran. If so, phenylisoserine could exist in the extracts from bark of *T. mairei*. However, phenylisoserine was not detected in the extracts here on the TLC plate. Therefore, this hypothesis may be ruled out. However, Muranaka *et al.*[13] reported that the amount of paclitaxel produced increased several fold when phenylisoserine was added to cell suspension cultures of *T. cuspidata* var. *nana*. This result suggests that phenylisoserine acts as a precursor of paclitaxel and the amount of paclitaxel produced increases because part of the phenylisoserine is incorporated into the paclitaxel molecule. It is not clear why phenylisoserine methyl ester exists in the bark of *T. cuspidata* var. *nana* and *T. mairei*. It may be necessary to investigate whether phenylisoserine methyl ester exists in other trees of the genus *Taxus*. The significance of phenylisoserine methyl ester in *taxus* trees may be clarified in the near future.

ACKNOWLEDGMENT

The authors thank the Advanced Instrumentation Center for Chemical Analysis, Ehime University for measuring NMR and MS spectra.

REFERENCES

1. Tachibana, S., 1995. Utilization of biologically active substances in trees. Mokuzai Gakkaishi, 41: 967-977.
2. Wani, M.C., H.L. Taylor and M.E. Wall, 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, M.D., 1997. Paclitaxel (Taxol®) A new natural product with major anticancer activity. Phytomedicine, 4: 273-282.
4. Eisenhauer, E.A. and J.B. Vermorken, 1998. The taxoids comparative clinical pharmacology and therapeutic potential. Drugs, 55: 5-30.
5. Baloglu, E. and D.G.I. Kingston, 1999. The taxane diterpenoids. J. Nat. Prod., 62: 1448-1472.
6. Gu ritte, F., 2001. General and recent aspects of the chemistry and structure-activity relationships of taxoids. Current Pharmaceutical Design, 7: 1229-1249.
7. Tachibana, S., A. Matsuo, K. Itoh and T. Oki, 1994. Extractives in the leaves and bark of *Taxus cuspidata* Sieb. et. Zucc. var. *nana* Rehder. Mokuzai Gakkaishi, 40: 1008-1013.
8. Kurose, K., K. Itoh, S. Tachibana K. Irie and T. Oki, 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., J. Ueno, K. Itoh, I. Matsumoto and S. Tachibana, 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*. Bull. Ehime Univ. Forest, 35: 45-53.
10. Muranaka, T., K. Kurose, K. Itoh and S. Tachibana, 1999. Utilization of extractives from genus *taxus* tree. I. Antifungal activities of flavonoids, taxinine and its derivatives against *Cochiliobolus miyabeanus* and *Altenaria kikuchiana*. Mokuzai Gakkaishi, 45: 42-50.
11. Tachibana, S., E. Watanabe, K. Itoh and T. Oki, 1994. Formation of taxol in *Taxus cuspidata* Sieb. et *Zucc.* var. *nana* Rehder callus cultures. Mokuzai Gakkaishi, 40: 1254-1258.
12. Yoshida, M., T. Muranaka, K. Kurose, K. Itoh and S. Tachibana, 2002. Stimulation of the production of taxol by oligosaccharides in *Taxus cuspidata* variety nana callus cultures. Pak. J. Biol. Soc., 5: 461-465.
13. Muranaka, T., M. Yoshida, K. Itoh and S. Tachibana, 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 callus cultures. Pak. J. Biol. Sci., 7: 399-405.
14. Tachibana, S., E. Watanabe, J. Ueno, K. Tokubuchi and K. Itoh, 2004. Isolation of phenylisoserine methyl ester from the leaves of *Taxus cuspidata* var. *nana*. J. Wood Sci., (In Press).
15. Li, H. L., T.S. Huang, T. Koyama and C.E. DeVol, 1981. Flora of Taiwan, Vol.1. Epoch Publishing Co. Ltd., Taipei, pp: 499-501.
16. Yang, S.J., J.M. Fang and Y.S. Cheng, 1996. Taxanes from *Taxus mairei*. Phytochemistry, 43: 839-842.
17. Shen, Y.C. and C.Y. Chen, 1997. Taxanes from the roots of *Taxus mairei*. Phytochemistry, 44: 1527-1533.
18. Shen, Y.C., C.Y. Chen and Y.H. Kuo, 1998. A new taxane diterpenoid from *Taxus mairei*. J. Nat. Prod., 61: 838-840.
19. Shen, Y.C., C.Y. Chen and Y.J. Chen, 1999. Taxumairol M: A new bicyclic taxoid from seeds of *Taxus mairei*. Planta Med., 65: 582-584.
20. Yang, S.J., J.M. Fang and Y.S. Cheng, 1999. *Abeo*-taxanes from *Taxus mairei*. Phytochemistry, 50: 127-130.
21. Guo, D.M., Y.C. Liu and C.S. Chen, 1993. A partial chemoenzymatic synthesis of the taxol C-13 side chain n-Benzoyl-(2R, 3S)-3-phenylisoserine. J. Org. Chem., 58: 1287-1299.
22. Edward, M. and Jr. Croom, 1995. *Taxus* for Taxol and Taxoids, Section II Supply of Taxol Chapter 3. In: Suffness, M., (Ed.). TAXOL® Science and Applications. CRC Press, Inc., New York, pp: 37-70.
23. Suffness, M. and M.E. Wall, 1995. Discovery and Development of Taxol, Section I Introduction Chapter 1. In: Suffness, M., (Ed.). TAXOL® Science and Applications. CRC Press, Inc., New York, pp: 3-25.
24. Ma, W., G.L. Park, G.A. Gomez, M.H. Nieder, T.L. Adams, J.S. Aynsley, O.P. Sahai, R.J. Smith, R.W. Stahlhut and P.J. Hylands, 1994. New bioactive taxoids from cell cultures of taxus baccata. J. Nat. Prod., 57: 116-122.
25. Yukumune, Y., H. Tabata, Y. Higashi and Y. Hara, 1996. Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in Taxus cell suspension cultures. Nat. Biotechnol., 14: 1129-1232.
26. Fleming, P.E., U. Mocek and H.G. Floss, 1993. Biosynthesis of taxoids. Mode of formation of the taxol side chain. J. Am. Chem. Soc., 115: 805-807.
27. Fleming, P.E., A.R. Knaggs, X.G. He, U. Mocek and H.G. Floss, 1994. Biosynthesis of taxoids. Mode of attachment of the taxol side chain. J. Am. Chem. Soc., 116: 4137-4138.
28. Rohr, J., 1997. Biosynthesis of taxol. Angew. Chem. Intl. Ed. Engl., 36: 2190-2195.
29. Aizon, Y., Y. Fujiki and M. Funatsu, 1976. Purification of rice bran lipase and its multiple form, Section III A guide for purification of plant enzymes and proteins Chapter 6. In: Morita, Y., M. Shin, K. Asada S. Ido, (Eds.). Shokubutsu koso tanpakushitsu kenkyuho. Kyoritsu Publisher Inc., Tokyo, pp: 209-216.