Taxane Diterpenoids from *Taxus baccata* L. Growing in Iran

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Abstract

Background: There are eight *Taxus* species and two hybrids in the world and *Taxus baccata* L. (European yew) is the single representative in Iran. Until now, a large number of taxoids possessing different skeleton systems, as well as lignans, flavonoids, steroids and sugar derivatives have been isolated from various *Taxus* species. Taxoids are highly oxygenated diterpenes isolated from different species of yew trees (family Taxaceae). They have received considerable attention after the discovery of the clinical efficacy of paclitaxel against different cancers due to its remarkable inhibition of microtubules polymerization leading to apoptosis of cancer cells.

Objective: This study was conducted for extraction, purification and identification of taxoids occurring in the aerial parts of *Taxus baccata* L. growing in Iran.

Methods: The plant materials were extracted with organic solvent and after defatting, partitioning, column chromatography and thin layer chromatography steps, the obtained compounds were characterized on the basis of spectral data.

Results: Two taxane diterpenoids were isolated. The structures of these taxoids were established as 5-Cinnamoyl-10-acetyltaxicin-I and 2-Deacetyltaxinine E on the basis of spectral analysis.

Conclusion: These two taxoids were not previously encountered in *Taxus baccata* L. species.

Keywords: ***Taxus baccata* L., Isolation, Taxoids, Taxaceae**

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Introduction

The discovery of paclitaxel (Taxol®) as a potent anticancer drug from *Taxus brevifolia* has encouraged several groups all over the world to conduct research work on other *Taxus* species, in other to isolate potentially more effective paclitaxel derivatives for the treatment of various cancers or as starting materials for semi-synthesis [1, 2]. As a consequence, more than 350 taxane-type diterpenes have been isolated from various *Taxus* plants, and some of them were found to possess interesting anticancer activity [3, 4, 5, 6].

There are eight *Taxus* species and two hybrids in the world [7] and *Taxus baccata* L. (European yew) is the single representative in Iran. This plant is an evergreen tree commonly known as "Sorkhdar" and distributed mainly in the north of Iran [8]. Until now, a large number of taxoids possessing different skeleton systems, as well as lignans, flavonoids, steroids and sugar derivatives have been isolated from various *Taxus* species [9]. During our course of studies on the bioactive components, we have examined constituents of the needles and young stems of *Taxus baccata* L growing in Iran and isolated two taxoids, 5-Cinnamoyl-10-acetyltaxicin-I and 2-Deacetyltaxinine E. In this paper, we would like to describe the isolation and structure elucidation of these natural compounds.

Experimental

General

^{1}H and ^{13}C-NMR spectrum were recorded in $CDCl_3$ on a Bruker AMX-500 spectrometers with TMS as internal standard. Column chromatography (CC) was performed by using silica gel (Kieselgel 60, 0.63 - 0.200 mm, Art. 7734, Merck) and Kieselgel 60 F_{254} (0.5 mm thickness, Art. 5554, Merck) was used for preparative thin layer chromatography (PTLC). Analytical TLC was performed on precoated plates (Kieselgel 60 F_{254}, Art. 5554, Merck) and visualized under UV_{254} light, and then sprayed with anisaldehyde reagent and heated.

Plant material

The needles and young stems of *Taxus baccata* L. was collected from Sari, north of Iran, in November 2006. The plant materials were dried in the shadow and reduced to fine powder.

Extraction and Isolation

The air-dried and powdered needles and young stems (3 Kg) were extracted three times with methanol (MeOH) at room temperature. The methanolic extract was evaporated to dryness in vacuum and a reddish residue was obtained. The residue was diluted with distilled water and extracted three times with hexane to remove the major part of the neutral and lipid materials which were not investigated further. The resulting residue was extracted three times with CH_2Cl_2 and the combined CH_2Cl_2 extracts were evaporated under reduced pressure to give a residue (50 g). This residue was subjected on column chromatography (CC) eluted with hexane – ethyl acetate (2:1, 1:1, 1:2, 1:4). Twelve fractions were obtained and each was evaporated to dryness under reduced pressure. Fractions 5 (900 mg) and 6 (750 mg) were further separated by preparative thin layer chromatography (PTLC) repeatedly with different developing solvents ($CHCl_3$-MeOH, hexane-EtoAc, hexane-acetone) and finally compound **1** (3 mg) and **2** (12 mg) were obtained in pure form.

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Results and Disscusion

A methanolic extract of the needles and young stems of *Taxus baccata* L. was processed as described in the Experimental Section to provide two taxane diterpenoids.

Compound **1** was isolated as a colorless amorphous solid in a 0.0001 % yield based on the dry material. A molecular formula of $C_{31}H_{38}O_8$ was established on the basis of ^{13}C-NMR and MS spectrum. Analysis of the ^{1}H and ^{13}C-NMR spectrum data suggested the presence of a 6/8/6–membered ring system while ^{1}H and ^{13}C-NMR data of **1** resembled those of taxinine E (Table 1)**.** The ^{13}C NMR spectrum showed signals due to five oxygenated carbons, one tetra substituted olefin, one mono substituted aromatic ring, five methyl groups, one keto carbonyl group and one ester carbonyl groups.

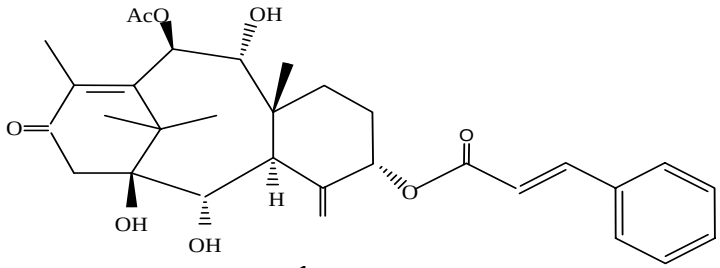

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Table 1: ^{1}H and ^{13}C NMR signal assignments of 1 in $CDCl_3$

Position	δ_H	δ_C	Position	δ_H	δ_C
1		77.79	18	2.26 (s)	13.75
2	4.02 (d, J=6.8 Hz)	71.38	19	1.19s)	17.75
3	3.39 (d, J=6.8 Hz)	46.50	20a	5.47 (s)	117.59
4		144.00	20b	5.38 (s)	
5	5.33 (t, J=2.8 Hz)	78.00	21		166.50
6	1.50-1.90 (m)	29.01	22	6.39 (d, J=16.0 Hz)	117.59
7	1.50-1.90 (m)	26.27	23	7.65 (d, J=16.0 Hz)	145.89
8		45.41	24		134.45
9	4.31(d, J=9.6 Hz)	75.50	25	7.75 (d, J=8.0 Hz)	128.45
10	5.87 (d, J= 9.6 Hz)	76.68	26	7.43 (m)	128.94
11		153.40	27	7.43 (m)	130.42
12		139.13	28	7.43 (m)	128.94
13		199.70	29	7.75 (d, J=8.0 Hz)	128.45
14a	2.71 (d, J=19.5 Hz)	44.33	1-OH	3.65 (brs)	
14b	2.64 (d, J= 19.5 Hz)		2-OH	2.40 (brs)	
15		42.30	9-OH	2.32 (brs)	
16	1.53 (s)	20.36	10-OAc	2.16 (s)	21.17
17	1.26 (s)	34.06			170.10

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One acetyl group was observed at δ_H 2.16 (3H, s), this being confirmed by the respective signal at δ_C 21.17 (q), and by the corresponding carbonyl carbon at δ_C 170.10 (s). The olefin protons signals of a cinnamoyl group at C-5 appeared at δ_H 7.75 (2H, d, J=8.0 Hz), 7.65 (1H, d, J=16.0Hz), 6.39 (1H, d, J=16.0 Hz) and 7.43 (3H, m) and three OH groups attached at C-1, C-2 and C-9, δ_H 3.65, 2.40 and 2.32 (each 1H, s) respectively. The presence of 4 (20)-unsaturation in **1**, common in non-oxetane type taxoids [6], was also apparent from the ^{1}H and ^{13}C-NMR spectrum data, δ_H at 5.38 (s) and 5.47 (s), δ_C at 117.59 (t) and 144.00 (s). As the ^{1}H and ^{13}C NMR spectrum data of the compound **1** was similar to those of 5-Cinnamoyl-10-acetyltaxicin-I, the structure of compound **1** was assigned to be 5-Cinnamoyl-10-acetyltaxicin-I [10].

Compound **2** was isolated as a colorless gummy substance in a yield of 0.0004 % based on the dry material. The ^{13}C-NMR and MS spectrum revealed the molecular formula to be $C_{35}H_{45}O_8$. The ^{13}C-NMR spectrum showed signals due to four oxygenated carbons, one tetra substituted olefin, one mono substituted aromatic ring, seven methyl groups, one keto carbonyl group and three ester carbonyl groups (Table 2). The ^{1}H and ^{13}C NMR spectrum had well-dispersed signals suggestive of a taxane derivative with a 6/8/6–membered ring system containing three acetate groups at δ_H 1.74, 2.02 and 2.07 (each 3H, s), this being confirmed by the respective signals at δ_C 20.85 (s), 20.96 (s) and 21.06 (s), and by the corresponding carbonyl carbons at δ_C 169.94 (s), 170.45 (s) and 170.70 (s). In addition,

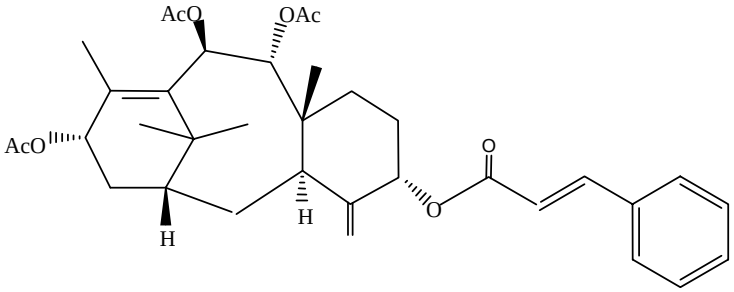

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Table 2: ^{1}H and ^{13}C NMR signal assignments of 2 in $CDCl_3$

Position	δ_H	δ_C	Position	δ_H	δ_C
1	1.75 (m)	40.13	18	2.25 (s)	15.28
2	1.80 (m)	27.36	19	0.78 (s)	17.78
3	1.80 (m)	37.90	20a	5.54 (s)	118.72
4	3.22 (d, J=6.8 Hz)	145.13	20b	4.91 (s)	
5	5.31 (m)	77.36	21		166.22
6	1.80 – 190 (m)	28.19	22	6.58 (d, J=16.0 Hz)	114.29
7	1.80 – 190 (m)	27.69	23	7.76 (d, J=16.0 Hz)	148.51
8		43.00	24		135.26
9	5.90 (d, J=11 Hz)	76.28	25	7.50 (m)	129.03

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Continuance Table 2: ^{1}H and ^{13}C NMR signal assignments of 2 in $CDCl_3$

Position	δ_H	δ_C	Position	δ_H	δ_C
10	6.11 (d, J=11 Hz)	72.52	26	7.40 (m)	127.95
11		134.25	27	7.40 (m)	130.50
12		136.90	28	7.40 (m)	127.95
13	5.76 (t, J=6.0 Hz)	70.62	29	7.50 (m)	129.03
14a	2.75 (m)	29.63	9-OAc	2.02 (s)	21.06
14b	1.10 (dd, J=6.0, 15.0 Hz)				170.45
15		39.15	10-OAc	2.07 (s)	20.85
16	1.62 (s)	26.97			169.94
17	1.08 (s)	31.27	13-OAc	1.74 (s)	20.96
					170.70

The ^{1}H and ^{13}C-NMR spectrum revealed signals due to one cinnamoyl group at δ_H 7.76 (1H, d, J=16.0 Hz), 6.58 (1H,d, J=16.0 Hz) ,7.40 (3H,m) and 7.50 (2H, m). Since the ^{1}H and ^{13}C-NMR signals were very close to those of 2,7-Deacetoxytaxinine J, the structure of **2** was assigned to be 2,7-Deacetoxytaxinine J [11].

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References

1. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. of American Chem. Soci.* 1971; 93: 2325 – 7.

2. Baloglu E, Kingston DGI. The taxane diterpenoids. *J. of Natural Products* 1999; 62: 1448 – 72.

3. Parmar VS, Jha A, Bisht KS, Taneja P, Singh SK, Kumar A, Raijni-Jain P,. Olsen CE. Constituents of yew trees. *Phytochem.* 1999; 50: 1267 – 304.

4. Rowinsky EK. The development and clinical utility of the Taxane class of antimicrotubule chemotherapy agents. *Annual Review of Medicine*, 1997; 48: 353 – 74.

5. Eisenhauer EA, Vermorken JB. The taxoids. *Drugs*, 1998; 55: 5 – 30.

6. Bryan-Brown T. The pharmacological actions of taxine. *Quaterly J. of Pharmacy and Pharmacol.* 1932; 5: 205 – 19.

7. Appendino G. Taxol (paclitaxel): historical and ecological aspects. *Fitoterapia*. 1993; 64: 5 – 25.

8. Van Rozendall ELM, Kurstjens SJL, Van Beek TA, Van Den Berg RG. Chemotaxonomy of *Taxus*. *Phytochem.* 1999; 52: 427 – 33.

9. Zargari A. *Iranian Medicinal Plants*. Tehran University Press, Tehran, Iran, 1995, Vol. 4, 103 - 4.

[DOR: 20.1001.1.2717204.2009.8.29.26.7]

10. Appendino G, Gariboldi P, Pisetta A, Bombardelli E, Gabetta B. Taxanes from *Taxus baccata*. *Phytochem.* 1992; 31: 4253 - 7.

11. Yeh MK, Wang JS, Liu LP, Chen FC. A new taxane derivative from the heartwood of *Taxus mairei*. *Phytochem.* 1988; 27: 1534 - 6.

[DOR: 20.1001.1.2717204.2009.8.29.26.7]