Pharmacognosy Magazine Vol 4, Issue 17, Jan-Mar, 2009 Page 67-70
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 17/03/2008
Modified: 02/08/2008
Accepted: 02/11/2008

PHCOG MAG.: Research Article

Taxane Ditepenoids from the Needles of *Taxus baccata* L. Growing in Iran

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ABSTRACT
Chemical examination of the needles and young stems of *Taxus baccata* L. from Iran, resulted in the isolation of three taxane diterpenoids , two previously reported taxoids, taxupinanane L and taxupinanane G, and a new taxoid, 2,9-deacetyltaxinine J. The structures of these compounds were determined on the basis of spectroscopic data. These three taxoids were not previously encountered in *Taxus baccata* L. species.
KEY WORDS: *Taxus baccata* L., isolation, taxoids, Taxaceae.

INTRODUCTION

Genus *Taxus* (Taxaceae) , yew, is widely distributed in the northern hemisphere, and has recently attracted a great deal of attention as sources for an anticancer agent , paclitaxel (Taxol), a unique diterpene taxoid originally extracted from the bark of the pacific yew, *Taxus brevifolia* (1, 2, 3). Paclitaxel has been approved for the treatment of ovarian and breast cancers as well as Kaposi 's sarcoma and non-small-cel lung cancers. It is also under critical trial for treatment of several other cancers in combination with other chemotherapeutic agents (4,5).

However, due to the poisonous properties of yew, few records have been encountered as traditional medicine in the literature. Yew leaves are reported to be used in traditional medicine as abortifacient, antimalarial, antiheumatic and for bronchitis (6,7,8), while dried leaves and barks were used against asthma (9). It was also listed in Avicenna's cardiac drugs (10).

There are eight *Taxus* species and two hybrids in the world (11,12) and *Taxus baccata* L. (European yew) is the single representative in Iran (13). 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 (2,3). 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 three taxoids, taxupinanane L , taxupinanane G, and 2,9-deacetyltaxinine J. 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. The FAB-MS were obtained on a Keatosl MS9 spectrometer using glycerol as matrix. 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. A voucher specimen has been located at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

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 vacuo 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 (50g). 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 (900mg), 6 (750mg) and 7 (450mg) 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** (3mg), **2** (12mg) and **3** (4.8 mg) were obtained in pure form.

RESULTS AND DISCUSSION

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

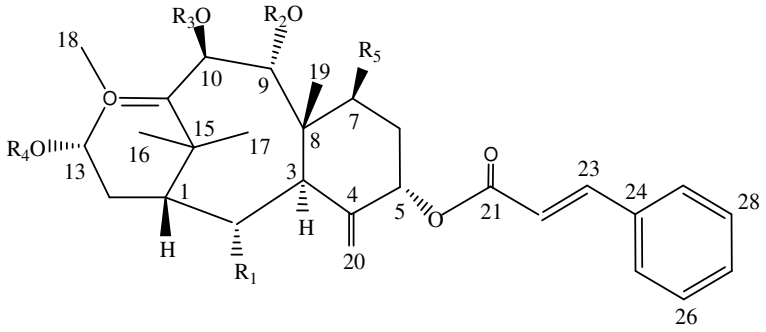
Compound **1** was isolated as a colorless amorphous solid in a 0.0001 % yield based on the dry material. A molecular formula of $C_{35}H_{44}O_9$ was established on the basis of ^{13}C NMR and FAB-MS spectrum. Analysis of the ^1H and ^{13}C NMR spectrum data suggested the presence of a 6/8/6–membered ring system while ^1H and ^{13}C NMR data of **1** resembled those of taxinine E. The ^{13}C NMR spectrum showed signals due to five oxygenated carbons, one tetrasubstituted olefin, one monosubstituted aromatic ring, seven methyl groups, one keto carbonyl group and three ester carbonyl groups. Three acetyl groups were observed at δ_H 1.77, 2.06 and 2.09 (each 3H, s), this being confirmed by the respective signals at δ_C 21.03 (q), 21.03 (q), 20.80 (q), and by the corresponding carbonyl carbons at δ_C 170.28 (s), 169.84 (s) and 170.70 (s). The olefin protons signals of a cinnamoyl group at C-5 appeared at δ_H 7.78 (1H, d ,J=16.0 Hz), 6.66 (1H, d, J=16.0 Hz) and 7.41-7.45 (5H, m) and a OH group attached at C-2, δ_H 1.53 (1H, brs). The presence of 4(20)-unsaturation in **1**, common in non-oxetane type taxoids (6), was also apparent from the ^1H and ^{13}C NMR spectrum data, δ_H at 5.57 (s) and 5.51 (s), δ_C at 119.40 (t) and 143.44 (s). As the ^1H and ^{13}C NMR spectrum data of the compound **1** was similar to those of taxupinanane L, the structure of compound **1** was assigned to be taxupinanane L.

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 FAB-MS spectrum revealed the molecular formula to be $C_{37}H_{46}O_{11}$. The ^{13}C NMR spectrum showed signals due to six oxygenated carbons, one tetrasubstituted olefin, one monosubstituted aromatic ring, eight methyl groups, one keto carbonyl group and four ester carbonyl groups. The ^1H and ^{13}C NMR spectrum had well-dispersed signals suggestive of a taxane derivative with a 6/8/6–membered ring system containing four acetate groups at δ_H 1.98, 2.03, 2.09 and 2.11 (each 3H,s), this being confirmed by the respective signals at δ_C 2.99(s), 21.3(s), 21.3(s) and 21.33(s), and by the corresponding carbonyl carbons at δ_C 170.00(s), 171.00(s), 170.00(s) and 171.00(s). In addition, The ^1H and ^{13}C NMR spectrum revealed signals due to one cinnamoyl group at δ_H 7.78 (1H, d, J=16.0 Hz), 6.66 (1H,d, J=16.0 Hz) and 7.40-7.49 (5H,m). Since the ^1H and ^{13}C NMR signals were very close to those of taxupinanane G, the structure of **2** was assigned to be taxupinanane G.

Compound **3** was isolated as a white amorphous solid in a 0.00016 % yield based on the dry material. By the combined analysis of FAB-MS, ^1H NMR and ^{13}C NMR spectrum data, the molecular formula was proposed as $C_{35}H_{44}O_{10}$. The ^1H NMR spectrum showed the four tertiary methyl groups at δ_H 0.98, 1.71, 1.19 and 2.26 (each 3H, s) assignable to the 8-CH_3, 15-$(CH_3)_2$ and 12-CH_3 groups, respectively. Three acetyl groups at δ_H 2.10, 2.16 and 2.18 (each 3H, s) at relatively low field, six oxy-bearing methane groups at δ_H 5.87 (d, J=10 Hz), 5.45 (d, J=10.4 Hz), 5.32 (m), 4.30 (d, J=9.6) and 4.13 (d, J=6.8 Hz), one tow substituted olefin at δ_H 5.38 (s) and 5.47 (s) were also involved. One tetra substituted olefin at δ_C 138.00 (s) and 136.00 (s), was deducted from the ^{13}C NMR spectrum. The ^1H NMR spectrum also suggested one cinnamoyl group at δ_H 7.65 (1H, d, J=16.0 Hz), 6.39 (1H, d, J=16 Hz), 7.42 (3H, m) and 7.45 (2H, m) and two OH groups attached at C-2 at δ_H 1.56 (brs) and C-9 at δ_H 2.05 (d, J=3.8 Hz). The ^1H NMR and ^{13}C NMR spectrum data closely resembled to those of taxupinanane G, with the exemption of one signal upfielded to δ_H 4.30 in ^1H NMR spectrum and an acetyl group disappeared both in ^1H NMR and ^{13}C NMR spectrum. We compared the chemical shifts of H_9 and H_{10} in several previously known structures of derivatives of taxinine such as

Table 1. ^{1}H NMR and ^{13}C NMR spectrum data of compound 1, 2 and 3 in $CDCl_3$

	1		**2**		**3**	
Position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	2.10 (d , 11.0)	51.15	2.16 (m)	51.27	2.67 (d, 6.4)	46.50
2	4.24 (d, 6.4)	70.25	4.22 (d, J=6.0)	70.25	4.13 (d, 6.8)	75.37
3	3.22 (d, 6.4)	41.54	3.21 (d, J=6.0)	44.93	3.39 (d, 6.8)	45.41
4		143.44		144.00		144.00
5	5.46 (s)	78.44	5.46 (m)	75.90	5.32 (m)	78.00
6a	1.91 (dd, 13.2, 1.0)	28.30	1.75 (dd, 7.2, 17.6)	37.06	2.06 (m)	28.71
6b	1.81 (dd, 9.9, 1.0)		2.05(m)		1.87 (dd, 10.4, 4.8)	
7a	1.68-1.80 (m)	26.78	5.83 (d, 10.0)	70.53	5.45(d, 10.4)	28.71
7b	1.68-1.80 (m)					
8		44.37		45.69		44.33
9	5.88 (d, 10.5)	76.98	5.77(d, 10.4)	78.70	4.30 (d, 9.6)	77.19
10	6.05 (d, 10.5)	72.32	6.22 (d, 10.4)	76.68	5.87 (d, 9.6)	75.00
11		133.49		134.22		134.00
12		136.52		135.93		136.00
13	5.84 (dd, 12.5, 12.5)	70.48	5.86 (d, 10.0)	70.67	5.77 (d, 10.0)	77.00
14a	2.65 (m)	29.13	2.65 (m)	28.29	2.71 (d, 6.4)	29.01
14b	1.32 (dd, 15.0, 8.1)		1.30 (m)	37.31	1.76 (dd, 6.4, 9.6)	
15		37.26		37.31		34.46
16	1.70 (s)	27.48	1.56 (s)	27.13	1.71 (s)	34.00
17	1.14 (s)	31.68	1.15 (s)	31.97	1.19 (s)	27.00
18	2.29 (s)	15.32	2.27 (s)	18.13	2.26 (s)	17.75
19	0.96 (s)	17.82	1.13 (s)	15.26	0.98 (s)	13.95
20a	5.57 (s)	119.40	5.50 (s)	119.40	5.38 (s)	117.00
20b	5.51 (s)		5.51 (s)		5.47 (s)	
21		166.31		166.00		166.00
22	6.65 (d, 16.0)	118.57	6.66 (d, 16.0)	119.71	6.39 (d, 16.0)	117.00
23	7.78 (d, 16.0)	145.53	7.78 (d, 16.0)	145.23	7.65 (d, 16.0)	146.00
24		134.18		133.93		134.00
25	7.50 (m)	129.00	7.49 (m)	128.01	7.45 (m)	128.00
26	7.41 (m)	128.05	7.40 (m)	128.01	7.42 (m)	130.00
27	7.41 (m)	130.55	7.40 (m)	130.51	7.42 (m)	130.00
28	7.41 (m)	128.01	7.40 (m)	128.01	7.42 (m)	130.00
29	7.50 (m)	129.00	7.49 (m)	128.01	7.45 (m)	128.00
7-OAc			1.98 (s)	170.00	2.10 (s)	166.00
				20.99		20.36
9-OAc	2.06 (s)	170.28	2.03 (s)	171.00		
		21.03		21.30		
10-OAc	2.02 (s)	169.84	2.09 (s)	170.00	2.16 (s)	170.00
		20.80		21.30		21.03
13-OAc	1.77 (s)	170.70	2.11 (s)	171.00	2.18 (s)	166.00
		21.03		21.30		21.17



1: R_1=OH, R_2=Ac, R_3=Ac, R_4=Ac, R_5=H
2: R_1=OH, R_2=Ac, R_3=Ac, R_4=Ac, R_5=OAc
3: R_1=OH, R_2=H, R_3=Ac, R_4=Ac, R_5=OAc

taxinine J, taxezopidine H, danataxusin B and so on to determine the structure of **3**. In addition, a dd-coupling for H_9 was observed in **3** that is related to the coupling of the hydroxyl group at δ_H 2.05 as dd with J=3.8 Hz, which could support the structure of **3**. Finally we concluded that the structure of **3** should be 2,9-deacetyltaxinine J.

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