Chemistry of Natural Compounds, Vol. 46, No. 3, 2010

A NEW TAXANE DITERPENOID FROM *Taxus baccata* GROWING IN IRAN

M. R. Verdian-Rizi,[1] A. Hadjiakhoondi,[1] Sh. Rezazadeh,[1] R. Dowlatabadi,[2] and M. Pirali-Hamedani[2,3]*

UDC 547.913.6

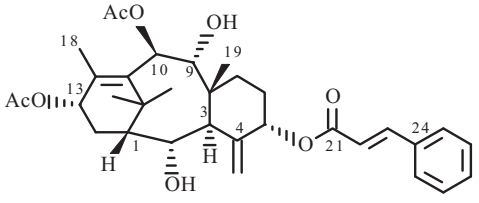
Chemical examination of the needles and young stems of the European yew Taxus baccata *L. growing in Iran resulted in the isolation of one new taxane diterpenoid, 5α-cinnamoyloxy-2α,9α-dihydroxy-10β, 13α-diacetoxy-4(20),11-diene. The structure of this taxoid has been elucidated on the basis of spectroscopic studies.*

Keywords: *Taxus baccata* L., isolation, taxane diterpenoids, Taxaceae.

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 order 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 [3–6].

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 one new taxane diterpenoid. In this paper, we describe the isolation and structure elucidation of this natural compound.

The methanolic extract of the needles and young stems of *Taxus baccata* L. was partitioned between hexane and water, and then the aqueous layer was extracted with CH_2Cl_2. The CH_2Cl_2-soluble portion was purified on a silica gel column followed by preparative thin layer chromatography (PTLC) to afford compound **1**.



1

1) Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; 2) Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, fax: +98 21 66461178, e-mail: mpirali@fdo.ir; 3) Food and Drug Lab. Research Centre, Ministry of Health (MOH), Tehran, Iran. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 318–320, May–June, 2010. Original article submitted December 17, 2008.

0009-3130/10/4603-0377

TABLE 1. ^1H and ^{13}C NMR Spectral Data of Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

Position	δ_H	δ_C	Position	δ_H	δ_C
1	2.14 m	51.02	18	2.27 s	18.13
2	4.13 (d, J = 6.0)	69.44	19	1.13 s	15.26
3	3.21 (d, J = 6.0)	44.93	20a	5.51 s	119.40
4		144.04	20b	5.50 s	
5	5.46 m	78.72	21		166.39
6	1.95 m	26.03	22	6.66 (d, J = 16.0)	118.71
7	1.65 m	29.46	23	7.78 (d, J = 16.0)	145.41
8		45.68	24		133.92
9	4.26 (d, J = 10.4)	75.89	25	7.49 m	128.00
10	5.86 (d, J = 10.4)	70.25	26	7.40 m	128.98
11		134.20	27	7.40 m	130.51
12		135.91	28	7.40 m	128.98
13	5.84 m	70.53	29	7.49 m	128.00
14a	2.65 m	28.30	9-OAc	2.09 s	21.09
14b	1.31 (dd, J = 15.5, 5.0)				170.38
15		37.31	10-OAc	2.11 s	21.30
16	1.56 s	27.08			171.77
17	1.15 s	31.60			

Compound **1** was isolated as a colorless gummy substance in a 0.00026% yield based on the dry material. The mass spectrum of compound **1** revealed a parent peak [M^+] at *m/z* 566. By the combined analysis of EI-MS, ^1H, and ^{13}C NMR spectral data, the molecular formula was suggested to be $C_{33}H_{42}O_8$. Analysis of the ^1H and ^{13}C NMR spectral data suggested the presence of a 6/8/6-membered ring system. The ^{13}C NMR spectrum showed signals due to five oxygenated carbons, one tetra-substituted olefin, one mono-substituted aromatic ring, six methyl groups, and three ester carbonyl groups (Table 1). The ^1H and ^{13}C NMR of compound **1** showed the presence of four methyl groups at δ_H 1.56, 1.15, 2.27, and 1.13 (each 3H, s) and δ_C 27.08, 31.60, 18.13, and 15.26, and one 4(20)-unsaturation, common in non-oxetane type taxoids, at δ_H 5.50 (s) and 5.51 (s) and δ_C 144.04 (s) and 119.40 (t). The olefin and aromatic proton signals of a cinnamoyl group at C-5 appeared at δ_H 7.78, 6.66 (each 1H, d, J = 16.0 Hz), 7.40 (3H, m) and 7.49 (2H, m). Two acetyl groups were observed at δ_H 2.09 and 2.11 (each 3H, s), this being confirmed by the respective signals at δ_C 21.09 (q) and 21.30 (q), and by the corresponding carbonyl carbons at δ_C 170.38 (s) and 171.77 (s). Comparative analysis between the observed NMR shifts of compound **1** with various taxanes with the same skeleton and having acetyls or hydoxyls at C-9 or C-10 was done to determine the structure of compound **1** [10–13]. It was observed that for C-9-hydroxyl taxanes, the H-9 chemical shift is in the range of δ_H 4.0–4.4. ^1H and ^{13}C NMR data of **1** resembled those of taxezopidine G [14], a taxane diterpenoid isolated from the Japanese yew *Taxus cuspidata*, except for H-9 (δ 4.26) which was shielded, indicating the presence of a hydroxyl group. As taxezopidine G possessed the molecular formula $C_{35}H_{44}O_9$, this suggests the absence of one acetyl group. The major difference between the ^{13}C NMR spectrums of the two compounds was the absence of a singlet in the ketone region between 165 and 175 ppm and a quartet between 15 and 25 ppm. The ^1H NMR spectrum of compound **1** shows a doublet at δ 5.86 (d, J = 10.4 Hz) for the allylic proton at C-10, which is comparable to the H-10 signal at δ 5.86 (d, J = 9.7 Hz) in 9-deacetyltaxinine E [15]. On the other hand, the doublet for H-9 in compound **1** shifted almost 1.75 ppm to higher field when compared to taxezopidine G. From these observations, the presence of a hydroxyl group at C-9 in compound **1** can be concluded. Thus the structure of compound **1** was established as 5α-cinnamoyloxy-2α,9α-dihydroxy-10β,13α-diacetoxy-4(20),11-diene.

EXPERIMENTAL

General Methods. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker AMX-500 spectrometer with TMS as an internal standard. Mass spectra were obtained using a Finnigan-MAT TSQ® 70. Column chromatography (CC) was performed by using silica gel (Kieselgel 60, 0.63–0.200 mm, Art. 7734, Merck). 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, 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 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 dichloromethane, and the combined dichloromethane extracts were evaporated under reduced pressure to give a residue (50 g). This residue was adsorbed with silica gel (50 g) and then fractionated by column chromatography (CC) over silica gel (1000 g). Elution was carried out with a hexane–ethyl acetate gradient system (1:0–0:1). Twelve fractions were obtained, and each was evaporated to dryness under reduced pressure. Fraction 5 (900 mg) was further separated by preparative thin layer chromatography (PTLC) repeatedly with different developing solvents (chloroform–methanol, hexane–ethyl acetate, hexane–acetone), and finally compound **1** (8 mg) was obtained in pure form.

5α-Cinnamoyloxy-2α,9α-dihydroxy-10β,13α-diacetoxy-4(20),11-diene (1). Colorless gummy substance; EI-MS *m/z*: 566 $[M]^+$, 506 $[M-AcOH]^+$, 446 $[M-2AcOH]^+$, 428 $[M-2AcOH-H_2O]^+$, 418 $[M-C_6H_5COOH]^+$, 408 $[M-2AcOH-2H_2O]^+$, 368, 340, 298, 280, 131, 43. ^1H and ^{13}C NMR of this compound are shown in Table 1.

ACKNOWLEDGMENT

We are grateful to the Faculty of Pharmacy, Tehran University of Medical Sciences, for financial support of this investigation.

REFERENCES

1. M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon, and A. T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).
2. E. Baloglu and D. G. I. Kingston, *J. Nat. Prod.*, **62**, 1448 (1999).
3. V. S. Parmar, A. Jha, K. S. Bisht, P. Taneja, S. K. Singh, A. Kumar, P. Raijni-Jain, and C. E. Olsen, *Phytochemistry*, **50**, 1267 (1999).
4. E. K. Rowinsky, *Annu. Rev. Med.*, **48**, 353 (1997).
5. E. A. Eisenhauer and J. B. Vermorken, *Drugs*, **55**, 5 (1998).
6. T. Bryan-Brown, *Quart. J. Pharm. Pharmacol.*, **5**,205 (1932).
7. G. Appendino, *Fitoterapia*, **64**, 5 (1993).
8. E. L. M. Van Rozendall, S. J. L. Kurstjens, T. A. Van Beek, and R. G. Van Den Berg, *Phytochemistry*, **52**, 427 (1999).
9. A. Zargari, *Iranian Medicinal Plants*, Vol. **4**, Tehran University Press, Tehran, Iran, 1995, pp. 103–104.
10. G. Appendino, P. Gariboldi, A. Pisetta, E. Bombardelli, and B. Gabetta, *Phytochemistry*, **31**, 4253 (1992).
11. M. K. Yeh, J. S. Wang, L. P. Liu, and F. C. Chen, *Phytochemistry*, **27**, 1534 (1988).
12. H. Morita, A. Gonda, L. Wei, Y. Yamamura, H. Wakabayashi, K. Takeya, and H. Itokawa, *Planta Med.*, **64**, 183 (1998).
13. M. Pirali-Hamedani, A. Hadjiakhoondi, Sh. Rezazadeh, R. Dowlatabadi, M. Amini, M. R. Verdian-Rizi, and M. Yarigar-Ravesh, *Chem. Nat. Comp*., **42**, 396 (2006).
14. X. Wang, H. Shigemori, and J. Kobayashi, *J. Nat. Prod.*, **61**, 474 (1998).
15. J. Zhang, F. Sauriol, O. Mamer, and L. O. Zamir, *J. Nat. Prod.*, **63**, 929 (2000).