Pergamon

0031-9422(94)00751-9

Phytochemistry, Vol. 38, No. 3, pp. 671–674, 1995

0031-9422/95 $9.50 + 0.00

A TAXOID FROM NEEDLES OF HIMALAYAN *TAXUS BACCATA**†

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(*Received in revised form* 25 *May* 1994)

Key Word Index—*Taxus baccata*; Taxaceae; taxoid; 13-acetyl-13-decinnamoyltaxchinin B.

Abstract—13-Acetyl-13-decinnamoyltaxchinin B, a taxoid having a rearranged 11(15 → 1)*abeo*-taxane skeleton, has been isolated from the needles of the Himalayan yew, *Taxus baccata*. The compound has not previously been encountered in nature. Its structure was established by 1- and 2D NMR techniques including DQF-COSY, HETCOR and HMBC experiments. The conversion of 13-decinnamoyltaxchinin B, a known taxoid, to 13-acetyl 13-decinnamoyltaxchinin **B** confirmed the structure of the latter.

INTRODUCTION

Chemical investigation of different yew trees has resulted in the isolation of a large number of taxoids. Recent structural investigations [1–3] on these compounds has proved that some of them possess a rearranged 11(15 → 1) *abeo*-taxane skeleton and not a taxane skeleton as originally proposed. Brevifoliol was the first reported naturally occurring taxoid (isolated from *T. brevifolia* [4]) having such a rearranged skeleton. Its structure was originally proposed as **1** [4] but as a result of the re-interpretation of its NMR spectra (mainly HMBC spectrum) and X-ray studies the structure was revised to **2** [1–3]. Two other taxoids, taxchinin A (2α-acetoxybrevifoliol) (**3**), isolated from *T. chinensis* [5], and 13-decinnamoyltaxchinin B (**4**), isolated from *T. wallichiana* [1, 6, 7], have also been proved by X-ray crystallographic analysis to possess the 11(15 → 1)*abeo*-taxane skeleton. The latter was originally reported [6] as a derivative of baccatin VI (**5**) [8]. Consequently, a number of taxoids whose basic structure was previously deduced from the correlation of their spectroscopic data to those of the originally proposed structure of brevifoliol with the normal taxane skeleton or by considering them as baccatin **VI** derivatives (though the spectral correlation was not accurate) have now been shown to have an *abeo*-taxane skeleton [1–3].

In a continuation of our recent investigation [9] on the Himalayan *Taxus baccata* L. as a source of taxol, a promising antitumour agent, and related bioactive compounds, we have isolated a taxoid, 13-acetyl-13-decinnamoyltaxchinin B (**6**), which possesses the rearranged 11(15 → 1)*abeo*-taxane skeleton. This is the first report on the isolation of the compound from a natural source.

RESULTS AND DISCUSSION

13-Acetyl-13-decinnamoyltaxchinin B (**6**) was isolated as crystals. Its structure was settled by means of extensive NMR spectroscopic studies (Table 1). The ^{1}H NMR spectrum showed the presence of one benzoyl and five acetyl groups and an oxetane ring [6] along with four methyls typical of taxoids [6]. The aromatic protons were more upshielded than those of the taxoids containing a benzoate group at C-2 [7, 10]. However, the signals were located in a similar region to those observed in the taxoids with the benzoate group at C-10 [7]. The connectivities of protons in **6** were determined by a DQF-COSY experiment (Table 1). The ^{13}C NMR spectrum showed the signals for all the carbons present in the molecule. A DEPT experiment was used to characterize the nature of the carbons and HETCOR analysis to assign carbon signals for all proton-bearing carbons (Table 1). Heteronuclear multiple bond correlation (HMBC) experiments were then used to assign quaternary carbons and attachment of various ester functionalities (Table 1). A correlation of the signal due to the benzoyl carbonyl at δ163.9 with that of H-10 at δ6.58 and the aromatic proton at δ7.86 *ortho* to the carbonyl group suggested the location of the benzoate group at C-10. The five acetate groups were then reasonably placed at C-2, C-4, C-7, C-9 and C-13. The C-15, bearing germinal dimethyl groups, was unusually shifted downfield (δ75.7) as compared to that of conventional taxane diterpenoids (δ*ca* 43) [11, 12]. This indicates that a hydroxyl group is located at C-15 [7]. The other quaternary carbons at C-1, C-4 and C-8

*IICT Communication No.: 3343.

†Part 5 in the series 'Studies on phytochemicals'. For Part 4, see Das, B., Takhi, M., Srinivas, K. V. N. S. and Yadav, J. S. (1994) *Phytochemistry* **36**, 1031.

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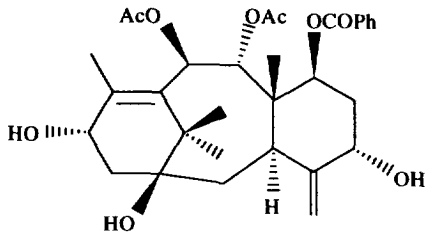
Table 1. NMR spectral data of compound **6***

Position	δ_C	DEPT	HMBC (for quaternary carbons)	δ_H†	DQF-COSY
1	68.5	C	H-10, Me-16, Me-17		
2	67.8	CH		6.17 (*d*, 7.9)	H-3
3	44.7	CH		3.01 (*d*, 7.9)	H-2
4	79.2	C	H-3, H-5, H-20β		
5	84.6	CH		4.99 (*d*, 7.6)	H-6α
6	34.7	CH_2		2.60 (H-α), (*m*)	H-5, H-6β, H-7
				1.91 (H-β) (*dd*, 15.4, 8.3)	H-6α, H-7
7	70.6	CH		5.59 (*t*, 8.2)	H-6α, β
8	43.5	C	H-2, H-3, Me-19		
9	76.3	CH		6.21 (*d*, 10.9)	H-10
10	68.8	CH		6.58 (*d*, 10.9)	H-9
11	135.7	C	H-13, Me-18		
12	147.7	C	Me-18		
13	78.7	CH		5.62 (*t*, 7.7)	H-14α, β
14	36.7	CH_2		2.50 (H-α) (*dd*, 15.0, 7.5)	H-13, H-14β
				2.00 (H-β) (*m*)	H-13, H-14α
15	75.7	C	H-2, Me-16, Me-17		
16	27.7	Me		1.15 (*s*)	
17	25.4	Me		1.07 (*s*)	
18	11.9	Me		2.01 (*s*)	
19	12.4	Me		1.68 (*s*)	
20	74.5	CH_2		4.50 (H-α) (*d*, 7.7)	H-20β
				4.41 (H-β) (*d*, 7.7)	H-20α
OAc-2					
CO	170.4	C	H-2, Me (2-OAc)		
Me	21.6	Me		2.02 (*s*)	
OAc-4					
CO	168.9	C	Me (4-OAc)		
Me	22.0	Me		2.02 (*s*)	
OAc-7					
CO	170.2	C	H-7, Me (7-OAc)		
Me	21.3	Me		2.08 (*s*)	
OAc-9					
CO	169.7	C	H-9, Me (9-OAc)		
Me	20.6	Me		1.74 (*s*)	
OAc-13					
CO	169.0	C	H-13, Me (13-OAc)		
Me	21.4	Me		2.12 (*s*)	
OBz-10					
CO	163.9	C	H-10, *o*-Ph (OBz)		
Ar-1	129.0	C			
Ar-2 & 6	129.4	CH		7.86 (*d*, 7.8)	Ar-3 & 5
Ar-3 & 5	128.6	CH		7.43 (*t*, 7.8)	Ar-2,4 & 6
Ar-4	133.3	CH		7.55 (*t*, 7.8)	Ar-3 & 5

*^{1}H NMR, HMBC and DQF-COSY (400 MHz, $CDCl_3$); ^{13}C NMR and HETCOR (100 MHz, $CDCl_3$); δ in ppm; Assignments for δ_C and δ_H were supported by HETCOR experiment.

†Multiplicities and J values in Hz in parentheses.



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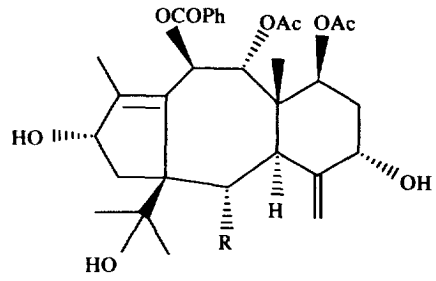
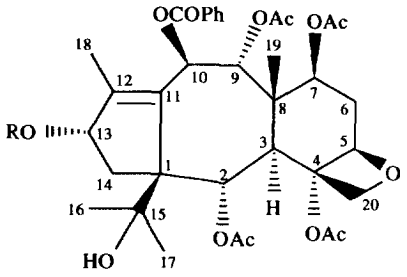
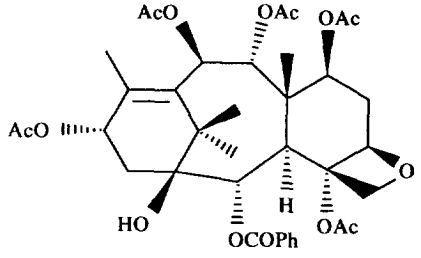


R
2 H
3 OAc



R
4 H
6 Ac



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resonated at δ68.5, 79.2 and 43.5. No three bond correlation between H_3-16 or H_3-17 and C-11 (HMBC experiment) was observed. All these data (Table 1) clearly suggested the rearranged 11(15 → 1)*abeo*-taxane skeleton for the taxoid **6**.

A direct comparison of the ^1H and ^{13}C NMR spectra of 13-acetyl-13-decinnamoyltaxchinin B (**6**) with those of the known taxoid, 13-decinnamoyltaxchinin B (**4**) [1, 6, 7] isolated by us from the needles of the title plant led to the conclusion that both possess the same skeleton, but that the former differed from **4** in having one more acetate group. Compounds **6** and **4** were shown to contain five and four acetate groups, respectively, in their molecules. In fact acetylation of **4** afforded a pentaacytylated taxoid which was similar to 13-acetyl-13-decinnamoyltaxchinin B in all respects and thus confirmed the structure **6** proposed for the latter.

EXPERIMENTAL

General. Mps: uncorr; CC: silica gel (100–200 mesh); TLC: silica gel G, spots were visualized by spraying the plates with 10% methanolic H_2SO_4.

Plant material. Needles of *Taxus baccata* were collected from the Himalaya region in May 1992. A voucher specimen (Tb-N) is deposited in our laboratory.

Extraction and isolation of taxoids **4** *and* **6**. Needles (1 kg) were dried, powdered and then extracted with CH_2Cl_2–MeOH (1:1, 3 × 2 l) at room temp. The suspension was filtered and the solvent removed in a rotavapour to yield a dark green residue (81 g). The residue was partitioned between H_2O (1 l) and CH_2Cl_2(1 l). The CH_2Cl_2 solubles were dried, filtered and concd to afford a green gum (21 g). This was chromatographed on silica gel eluted with solvents of increasing polarity (hexane, C_6H_6 and EtOAc) to give 72 frs. Frs 25–28 and 33–37 were combined separately and concd to afford a residue in each case. The two residues were further purified separately by repeated CC. The residue from frs 25–28 was purified to produce 13-acetyl-13-decinnamoyltaxchinin B (**6**), 18 mg, mp 243–244° (Me_2CO–hexane). $[\alpha]_D^{25}$ − 54° ($CHCl_3$; *c* 0.3219). CI-MS *m/z* (rel. int.): 732 $[M + NH_4]^+$ (82), 672 (100), 612 (71), 610 (38); ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Table 1.

Purification of the residue from frs 33–37 yielded 13-decinnamoyltaxchinin-B (**4**) [1, 6, 7], 14 mg, mp 227–228° (Me_2CO–hexane). $[\alpha]_D^{25}$ − 40° (MeOH; *c* 4825) lit. [6] mp 225–226° (MeOAc–hexane). $[\alpha]_D^{20}$ − 38° (MeOH; *c* 1.01).

Acetylation of 13-*decinnamoyltaxchinin B* (**4**). A mixture of **4** (10 mg), Ac_2O (0.5 ml) and pyridine (0.2 ml) was warmed at 40° for 1 hr and left at room temp. overnight. The usual work-up afforded a product (10 mg) identical to naturally occurring 13-acetyl-13-decinnamoyltaxchinin B (**6**) in all respects.

Acknowledgements—The authors thank Dr A. V. Rama Rao (Director) for his keen interest and valuable suggestions and Dr R. S. Kapil (RRL, Jammu, India) for the

supply of plant material. One of the authors (R.D.) is grateful to the Principal, Gurudas College, for his encouragement.

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