Pergamon

0031-9422(95)00672-9

Phytochemistry, Vol. 41, No. 3, pp. 863–866, 1996

0031-9422/96 $15.00 + 0.00

TAXANES FROM THE BARK OF *TAXUS BREVIFOLIA**

KOPPAKA V. RAO,†‡ RAJENDRA S. BHAKUNI,‡ JAMPANI. B. HANUMAN,† RICHARD DAVIES‡ and JAMES JOHNSON‡

†The Yew of Florida Project and the ‡Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Box J-100485, Gainesville, FL 32610, U.S.A.

(*Received in revised form* 1 *August* 1995)

Key Word Index—*Taxus brevifolia*; Taxaceae; yew bark; taxanes; paclitaxel; abeotaxane; 9-dihydrobaccatin III; baccatin IV; panosterone A.

Abstract—Examination of the extracts of the bark of *Taxus brevifolia* showed the presence of two new crystalline taxanes: 10β-benzoyloxy-2α,4α-diacetoxy-5β,20-epoxy-1β,7β,9α,13α-tetrahydroxy-11(15 → 1)-abeotaxane and 7,9-deacetyl baccatin IV, along with ponasterone A and three other crystalline taxanes: baccatin IV, 9-dihydro-13-acetyl baccatin III and 1β,7β-dihydroxy-4β,20-epoxy-2α,5α,9α,10β,13α-penta-acetoxy-tax-11-ene, isolated earlier from other species of *Taxus*.

INTRODUCTION

The diterpene ester paclitaxel (**1**), first isolated from the bark of *Taxus brevifolia* by Wani *et al.* [1] in 1971, has been shown to be a promising anti-tumour drug [2–4]. During the course of our development of a new large-scale process for the isolation of **1** and several related taxanes [5, 6], a number of other taxanes have been recognized and isolated, and this paper describes their characterization.

RESULTS AND DISCUSSION

Source of the compounds

As described in detail earlier [5, 6], the concentrated methanoloic extract of the bark of *T. brevifolia* (100 kg) was partitioned between chloroform and water, and the solvent extract was concentrated to a solid (2.6 kg) and applied to a reverse phase (C-18 bonded silica, 12.5 kg) column, using 25% acetonitrile in water as eluent. Elution with a step gradient of 35–50% acetonitrile in water gave successively, 10-deacetyl baccatin III, 10-deacetyl cephalomannine-7-xyloside, 10-deacetyl paclitaxel-7-xyloside, 10-deacetyl paclitaxel-C-7-xyloside, paclitaxel-7-xyloside, 10-deacetyl paclitaxel, cephalomannine and paclitaxel, all of which crystallized out of the fractions and were purified further by recrystallization and/or a short column. The filtrates from the region between 10-deacetyl baccatin III and 10-deacetyl paclitaxel were concentrated to a syrup (400 g). An aliquot (20 g) was applied to a normal phase silica column (150 g) in dichloromethane, with the elution sequence: 2–5% acetone, 2–5% methanol in dichloromethane. Further purification by rechromatography and/or preparative TLC led to the compounds **2**–**7** described below.

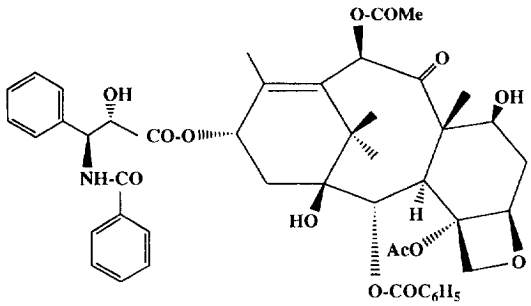
Compounds **2**–**7**

Compound **2** has the molecular formula of $C_{31}H_{40}O_{11}$, and the ^1H and ^{13}C NMR spectra suggested a taxane-type skeleton: four methyls, two acetates and one benzoate group. Assignment of these and other groups on the taxane skeleton was made by an analysis of the COSY, APT, HETCOR and HMBC spectra.

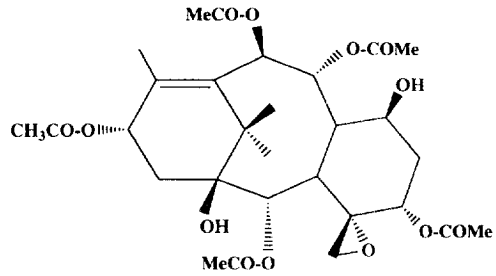
Starting with H-3α (δ2.82 *d*), its coupling pattern with H-2 (δ5.92 *d*, J = 7.5 Hz) established H-2 as β. Similarly, H-5α (δ4.93 *d*) was correlated with the one-proton multiplet of H-6α (δ2.48 *m*), which also shared a geminal coupling with the one proton multiplet of H-6β (δ1.78 *m*). Both H-6α and β shared a cross-peak with the triplet at δ4.27 (J = 8.3 Hz), assigned to H-7α. The doublet at δ6.27, assigned for H-10α was correlated with H-9β (δ4.51 *dd*, collapsible to a *d* with D_2O) and the latter showed a cross-peak with the doublet at δ6.10 (D_2O-exchangeable), which was assigned to 9α-OH. The vicinal coupling between H-9β and H-10α, with the coupling constants of J = 10 and 11.2 Hz indicated their *trans* orientation in the molecule. The doublet of doublets at δ1.66 and the multiplet at δ2.12 were designed to the H-14α and H-14β, respectively, on the basis of their geminal coupling (J = 14.4 Hz) and coupling with the H-13β (δ4.45 *t*, J = 8 Hz).

All of the proton-bearing carbons were assigned by an analysis of the HETCOR spectrum. Seven oxygen-containing carbons (C-2, C-5, C-7, C-9, C-10, C-13 and C-20) were correlated with their corresponding proton signals. The signal at δ24.4 and 26.7 corresponding to C-16 and C-17 (methyl groups), respectively, showed a cross-peak

*For part II of the series see ref. [6].



1



5

with a six-proton singlet at δ1.14, indicating that both methyl groups were resonating at the same frequency.

An analysis of the HMBC spectrum permitted an unambiguous assignment of the C-18 and C-19 signals, the ester functions and quaternary carbons. The signal at δ74.3 (C-9) showed a three-bond coupling with the C-19 methyl group (δ1.71) and at the same time H-3α (δ2.82) and H-9β signals displayed a cross-peak with an up-field signal at δ10.5 (assigned to C-19) and with the resonance at δ40.7 (assigned to C-8). The H-9β signal did not show a cross-peak with a carbonyl carbon, and this, along with its up-field position (δ4.51), strongly indicated a free hydroxyl at C-9.

Proton resonances at δ7.95, 2.14 and 1.99, assigned for the *ortho* protons of the benzoate, and the acetate protons, showed three-bond coupling with the carbon signals at δ163.7, 168.5 and 169.5, respectively, thus indicating that these resonances were due to the C=O carbons of the benzoate and the two acetate groups. At the same time, resonances δ163.7 (benzoate C=O) and 168.5 displayed cross-peaks with the proton signals at δ6.27 (H-10α) and 5.92 (H-2β), respectively, and strongly indicated an attachment of the benzoate function at C-10 and one acetate (δ2.14 corresponding to the acetate methyl signal) at C-2. The second acetate carbonyl carbon signal did not show a cross-peak with any proton except with the singlet at δ1.99, and this indicated that the second acetate function was located at the C-4 carbon. The signal for the C-4 carbon was assigned by its correlation with H-3α, H-5α and H-20 (α,β) signals.

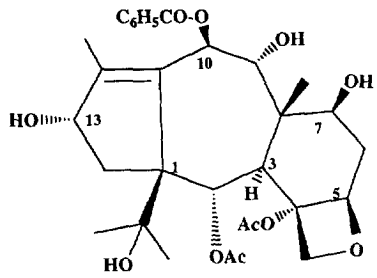
The H-10α signal showed cross-peaks with resonances at δ131.6, 148.8 and 65.7, which were assigned for C-11, C-12 and C-1. The C-11 and C-12 carbon signals showed a cross-peak with the H-14β resonance, which indicated that both C-11 and C-12 are three bonds apart from H-14β. This meant that the A ring is a cyclopentene as in an 11(15 → 1)-abeotaxane structure [7]. The carbon signal at δ76.3, assigned to the hydroxyl-bearing C-15, displayed a cross-peak with C-16 and C-17 methyl resonances at δ1.14. The C-1 signal (δ65.7), apart from H-10α, also showed three-bond coupling with H-3α and C-16, C-17 methyl signals. That no cross-peak was observed between C-16, C-17 (methyl) signals and the C-11 olefinic carbon in the HMBC spectrum [8] further supported the 11(15 → 1)-abeotaxane skeleton for **2**.

The C-11 and C-12 carbon signals also showed coupling with a three-proton resonance at δ1.82, assigned to the C-18 methyl. Since the carbon signal at δ10.5 was assigned to the C-19 methyl, the remaining carbon signal in the methyl region at δ10.7 must be assigned to C-18 methyl. Thus, the structure **2** is shown to be 10β-benzoyloxy-2α,4α-diacetoxy-5β,20-epoxy-1β,7β,9α,13α-tetrahydroxy-11(15 → 1)-abeotaxane.

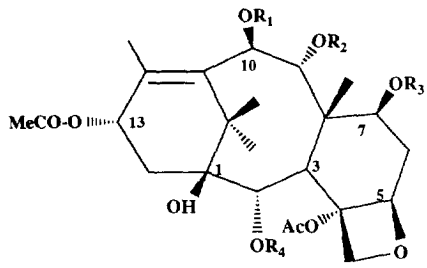
Compound **3** has a molecular formula of $C_{28}H_{40}O_{12}$, and the ^1H and ^{13}C NMR spectra displayed the presence of four methyls, four acetates and an oxetane function, typical of a taxane skeleton. The proton assignments on the taxane skeleton were based on COSY spectral analysis. Three acetate groups were located at C-2, C-10 and C-13, based on their down-field methine proton signals at δ5.46 (H-2β), 6.13 (H-10α) and 6.11 (H-13β) and correlation with the vicinal methine proton signals. The fourth acetate function, which did not show any association with a CH or CH_2 group, was assigned at the C-4 position.

The positions of all proton-bearing carbons except the methyls at C-8 and C-15 and the acetyl groups were analysed by APT and HECTOR spectra. Seven signals due to the oxygen-carrying carbons: δ73.0, 84.1, 73.7, 76.6, 73.0, 69.7 and 76.5 were assingned to C-2, C-5, C-7, C-9, C-10, C-13 and C-20, respectively, through correlation with their corresponding proton signals at: δ5.46 (H-2β), 4.94 (H-5α), 4.38 (H-7α), 4.33 (H-9β), 6.13 (H-10α) 6.11 (H-13β) and 4.21/4.48 (H-20α and H-20β). The up-field signal at δ14.7 showed a cross-peak with the three-proton doublet at δ1.88 and this was assigned to C-18 methyl, whereas the signals at δ12.3, 21.4 and 28.2, which coupled with three-proton singlets at δ1.74, 1.60 and 1.22, were assigned to carbons C-19, C-16 and C-17, respectively.

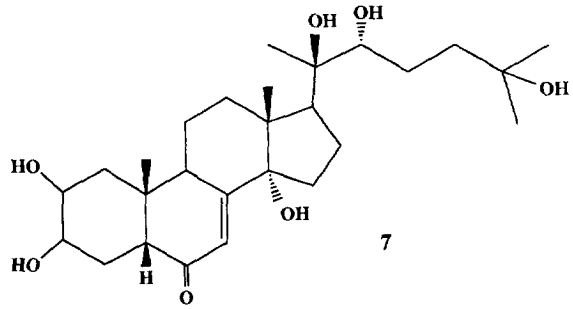
Acetylation of **3** gave a diacetate, the spectral data for which corresponded to those described for baccatin IV (**4**) [9, 10]. Compound **4** was also isolated as one of the components of the bark extract as described here. As for the location of one of the hydroxyls in **3** (whether present at C-9 or C-10), since the signal due to H-10, which is allylic, will appear more down-field than H-9, and since no signal was found in **4**, which was more down-field than δ6.13, it clearly indicated that the newly introduced acetate was at the C-9 hydroxyl. Thus, the structure of **3** was shown to be 7,9-deacetyl baccatin IV, which is being reported here for the first time.



2



	R_1	R_2	R_3	R_4
3	COMe	H	H	COMe
4	COMe	COMe	COMe	COMe
6	COMe	H	H	COC_6H_5



7

Compound **5** was characterized as the known 1β,7β-dihydroxy-4β,20-expoxy-2α,5α,9α,10β,13α-pentaacetoxy-tax-11-ene, isolated earlier from the needles of *T. brevifolia* [8]. We are reporting its ^{13}C NMR data for the first time. The assignments are based on the APT and HECTOR spectra and comparison with data for similar compounds [11].

Compound **6** was identified as 9-dihydro-13-acetyl baccatin III by comparison with the spectral data reported in the literature [12, 13]. This very important compound, (precursor for 9-dihydro-paclitaxel) was isolated earlier from the needles of *T. canadensis*, and the current isolation is the first from the bark of *T. brevifolia*.

Compound **7** was identified as ponasterone A, originally isolated from *Podocarpus* sp., *T. cuspidata* and others [14, 15]. This report describes its first isolation from the bark of *Taxus brevifolia*.

EXPERIMENTAL

^1H and ^{13}C NMR, COSY and the HECTOR spectra: Varian VXR-300 and Varian Gemini-300 spectrometers. HMBC spectrum: Varian 600 spectrometer. Chemical shifts are reported in δ(ppm) using TMS as int. standard. FAB mass spectra: Finnigan Mat 950 Q spectrometer. IR spectra: Perkin-Elmer 1420 ratio recording spectrophotometeer.

Mps (uncorr.): Fisher–Johns apparatus. TLC: silica gel 60 HF_{254} (E. Merck and Aldrich), with MeOH–Me_2CO–CH_2Cl_2 (1:4:15) or MeOH–CH_2Cl_2 (1:10) as solvents, visualiztion by UV (254 nm) and charring with dilute H_2SO_4 spray.

10β-*Benzoyloxy*, 2α,4α-*diacetoxy*-5β,20-*epoxy*-1β,7β,9α,13α-*tetrahydroxy*-11(15 → 1)-*abeotaxane* (**2**). Crystallized from Me_2CO–ligroin; yield 0.004%, mp 242–243° C; IR (KBr), 3450, 2430, 1730, 1450, 1368, 1250, 1175, 1110, 1070, 1025, 985, 938; HRMS(FAB): $[M + H]^+$, 589.2636; Calc. 589.2666; $[M + H - H_2O]$, 571; $[M + H - PhCO_2H]$, 467, $[M + H - PhCO_2H - AcOH - H_2O]$, 389; $[M + H - PhCO_2H - 2 \times AcOH - H_2O]$, 329; ^1H NMR[$CDCl_3$ /DMSO-d_6 (4:1)]: δ1.14 (6H, *s*, H-16, H-17), 1.66 (1H, *dd*, *J* = 14.4, 7.2 Hz, H-14α), 1.71 (1.H, *s*, H-19), 1.78 (1H, *m*, H-6β), 1.82 (3H, *s*, H-18), 1.99 (3H, *s*, 4-$OCOCH_3$), 2.12 (1H, *m*, H-14β), 2.14 (3H, *s*, 2-$OCOCH_3$), 2.48 (1H, *m*, ΣJ = 31.2 Hz, H-6α), 2.82 (1H, *d*, *J* = 7.5 Hz, H-3α), 4.27 (1H, *t*, *J* = 8.3 Hz, H-7α), 4.36 (2H, *bs*, 20 α/β), 4.45 (1H, *t*, *J* = 8 Hz, H-13β), 4.51 (1H, *dd*, *J* = 8, 10 Hz, H-9β), 4.93 (1H, *d*, *J* = 8.4 Hz, H-5α), 5.92 (1H, *d*, *J* = 7.5 Hz, H-2β), 6.10 (1H, *d*, *J* = 7.5 Hz, 9-αOH), 6.27 (1H, *d*, *J* = 11.2 Hz, H-10α), 7.44 (2H, *t*, *J* = 7.5 Hz, *m*-ArH), 7.56 (1H, *t*, *J* = 7.2 Hz, *p*-ArH), 7.95, (2H, *d*, *J* = 7.5 Hz, *o*-ArH); ^{13}C NMR [($CDCl_3$/DMSO-d_6(4:1)]: δ10.5 (C-19), 10.7 (C-18), 20.5 (OAc), 24.4 (C-16), 26.7 (C-17), 36.3 (C-6), 38.1 (C-14), 40.7 (C-8), 43.1 (C-3), 65.7 (C-1), 67.1 (C-2), 70.0 (C-10), 70.9 (C-7), 73.0 (C-20), 74.2 (C-13), 74.3 (C-9), 76.3 (C-15), 77.9 (C-4), 83.5 (C-5), 127.1 (ArC-*m*), 128.2 (ArC-*o*), 129.2 (ArC-CO), 131.6 (C-11), 133.3 (ArC-*p*), 148.8 (C-12), 163.7 (O-CO-Ph), 168.5 (C2-$OCOCH_3$), 169.5 (C4-$OCOCH_3$). Anal. Calc. for $C_{31}H_{40}O_{11}$, H_2O: C, 61.32; H, 6.97. Found C, 61.68; H, 6.86.

7,9-*Deacetyl baccatin IV* (**3**). Crystalline solid from Me_2CO–ligroin), yield, 0.005%, mp 210–214°, IR (KBr, Cm^{-1}), 3480, 1742, 1738, 1430, 1368, 1230, 1165, 1050,

1015, 980; ^{1}H NMR ($CDCl_3$ δ), 1.22 (3H, *s*, H-17), 1.60 (3H, *s*, H-16), 1.74 (3H, *s*, H-19), 1.88 (3H, *d*, J = 1.2 Hz, H-18), 1.96 (1H, *m*, H-6β), 2.18 (2H, *m*, H-14α, H-14β), 2.09 (3H, *s*, OAc), 2.12 (3H, *s*, OAc), 2.16 (6H, *s*, 2 × OAc), 2.56 (1H, *m*, H-6α), 2.91 (1H, *d*, J = 5.7 Hz, H-3α), 4.21 (1H, *d*, J = 8 Hz, H-20α), 4.33, (1H, *d*, J = 9.3 Hz, H-9β), 4.38, (1H, *m*, ΣJ = 17 Hz, H-7α), 4.48 (1H, *d*, J = 8 Hz, H-20β), 4.94 (1H, *d*, J = 8.1 Hz, H.5α), 5.46 (1H, *d*, J = 5.7 Hz, H-2β), 6.11 (1H, *m*, H-13β), 6.13 (1H, J = 10.5 Hz, H-10α); ^{13}C NMR ($CDCl_3$, δ) 12.3 (C-19), 14.7 (C-18), 21.1 (OAc), 21.3 (2 × OAc), 21.4 (C-16), 22.7 (OAc), 28.2 (C-17), 35.0 (C-14), 37.7 (C-6), 43.0 (C-8 or C-15), 44.6 (C-8 or C-15), 47.0 (C-3), 69.7 (C-13), 73.0 (C-2 and C-10), 73.7 (C-7), 76.5 (C-20), 76.6 (C-9), 78.3 (C-1), 81.7 (C-4), 84.1 (C-5), 134.8 (C-11), 139.3 (C-12), 169.6 ($OCOCH_3$), 170.5 ($OCOCH_3$), 170.7 ($OCOCH_3$), 171.9 ($OCOCH_3$); HRMS, [M + H], 569.2671; Calc. for $C_{28}H_{40}O_{12}$: 569.2615.

Baccatin IV (**4**). A mixt. of **3** (50 mg), Ac_2O (1 ml) and pyridine (0.5 ml) was heated at 70° for 30 min, cooled, diluted with H_2O and the solid filtered and crystallized from Et_2O, mp 263–265° (lit. 265–266° [9, 10]). The compound was also isolated in a yield of 0.004% as one of components from chromatography of the bark extract in the present work. TLC and spectral data for the two were identical.

9-*Dihydro*-13-*acetyl baccatin III* (**5**). Crystallized from Me_2CO–ligroin, yield, 0.003%, mp 243–244°, MS(FAB): $[M + H]^+$, 631. ^{1}H and ^{13}C NMR spectra matched those reported in lit. [12, 13].

1β,7β-*Dihydroxy*-4β,20-*epoxy*-2α,5α,9α,10β,13α-*penta-acetoxy-tax*-11-*ene* (**6**). Crystalline solid (from Et_2O), mp 234–236°, yield 0.005%, MS (FAB): $[M + H]^+$, 611; ^{13}C NMR ($CDCl_3$): 12.6 (C-19), 15.5 (C-18), 20.5, 20.8, 21.3, 21.6, 21.8 (Ac × 5), 21.8, (C-17), 28.5 (C-16), 32.3 (C-6), 38.4 (C-14), 40.4 (C-3), 43.3 (C-15), 47.1 (C-8), 49.9 (C-20), 59.2 (C-4), 69.1 (C-7), 70.4 (C-10), 71.8 (C-13), 72.5 (C-2), 76.1 (C-1), 78.1 (C-5), 135.7 (C-11), 140.7 (C-12), 168.3 ($OCOCH_3$), 169.1 ($OCOCH_3$), 169.5 ($OCOCH_3$), 169.6 ($OCOCH_3$), 170.1 ($OCOCH_3$).

Ponasterone A (**7**). Purified by crystallization from MeOH, mp 257–260°, yield 0.006%, MS (FAB): $[M + H]^+$, 465. Spectral data identical with those described in lit. [14, 15].

Acknowledgements—The authors express their grateful appreciation to Drs David H. Powell and Roy King of the Chemistry Department, University of Florida, for assistance with the NMR and mass spectral data, and to Mr Daniel Plant of the Center for Structural Biology, University of Florida, for assistance with the HMBC spectral determination.

REFERENCES

1. Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. and McPhail, A. T. (1971) *J. Am. Chem. Soc.* **93**, 2325.
2. Blechert, S. and Guenard, D. (1990) in *The Alkaloids* (Brossi, A. ed.), Vol. 39, p. 195. Academic Press, Orlando, FL.
3. McGuire, W. P., Rowinsky, E. K., Rosenshein, N. B., Grumbine, F. C., Ettinger, D. S., Armstrong, D. K. and Donehover, R. C. (1989) *Ann. Int. Med.* **111**, 272.
4. Rowinsky, E. K., Cazennove, L. A. and Donehover, R. C. (1990) *J. Natl Cancer Inst.* **82**, 1247.
5. Rao, K. V. (1993) *Pharm. Res.* **10**, 521.
6. Rao, K. V., Hanuman, J. B., Alvarez, C., Stoy, M., Juchum, J., Davies, R. M. and Baxley, R. (1995) *Pharm. Res.* **12**, 1003.
7. Chu, A., Furlan, M., Davin, L. B., Zajicek, J., Towers, G. H. N., Soucy-Breau, C. M., Rettig, S. J., Croteau, R. B. and Lewis, N. G. (1994) *Phytochemistry* **36**, 975.
8. Chu, A., Davin, L. B., Zajicek, J., Lewis, N. G. and Croteau, R. B. (1993) *Phytochemistry* **34**, 473.
9. Della Casa de Marcano, D.P. and Halsall, T. G. (1975) *J. Chem. Soc., Chem. Commun.* 365.
10. Rojas, A. C. De Marcano, D. Mendez, B. and De Mendez, J. (1983) *Org. Magn. Reson.* **21**, 257.
11. Barboni, L., Garibaldi, P., Torregiani, E., Appendino, G., Garbetta, B., Zini, G. and Bombardelli, E. (1993) *Phytochemistry* **33**, 145.
12. Zamivr, L. O., Nedea, M. E., Belair, S., Sauriol, F., Mamer, O., Jacqmain, E., Jean, F. I. and Garneau, F. X. (1992) *Tetrahedon Letters* **33**, 5173.
13. Gunawardana, G. P., Premachandran, U., Burres, N. S., Whitten, D. N., Henry, R., Spanton, S. and McAlpine, J. B. (1992) *J. Nat. Prod.* **55**, 1686.
14. Yakemoto, T., Ogawa, S., Nishimoto, N. and Hoffmeister, H. (1967) *Z. Naturforsch.* **22b**, 681.
15. Imai, S., Nakanishi, K., Koreeda, M. and T. Kurokawa, T. (1967) *Steroids* **10**, 557.