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*J. Nat. Prod.*, **1993**, 56 (4), 514-520• DOI: 10.1021/np50094a010 • Publication Date (Web): 01 July 2004

*Journal of Natural Products*
*Vol. 56, No. 4, pp. 514-520, April 1993*

# TAXANES FROM THE SEEDS OF *TAXUS BACCATA*[1]

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ABSTRACT.—The seeds of the European yew, *Taxus baccata*, gave two alkaloidal taxanes **2** and **4**, related to taxine B [**1**], and an oxygenated derivative **5** of brevifoliol. Conformational flexibility of the tricyclic taxane skeleton was detected in **5**.

Current interest in the European yew (*Taxus baccata* L., Taxaceae) focuses on obtaining the non-alkaloidal diterpenoid 10-deacetylbaccatin III from the needles of the plant. This compound is in fact the starting material for the synthesis of the very promising anticancer compounds taxol and taxotere (1). However, *T. baccata* is also a poisonous plant, containing a complex mixture of toxic alkaloids collectively referred to as taxine (1,2). A great deal of work has been done on taxine; however, owing to the instability of the alkaloids and the difficulty of their separation, only two constituents (taxines A and B) have been structurally characterized so far (3–5).

The most common form of yew poisoning is the accidental swallowing of seeds by children, who are attracted by the colorful and sweet-tasting aril surrounding the seeds (6). Unless seeds are broken or chewed, only very mild effects or no toxic signs at all are observed; serious poisoning is thus relatively rare, but nevertheless potentially lethal, since no clinical treatment for its management is known (6). A better knowledge of the chemistry and the toxicology of the seed constituents is therefore of considerable interest.

## RESULTS AND DISCUSSION

A defatted $CHCl_3$ extract from the seeds was subjected to an acid-base extraction scheme. A crude alkaloidal fraction, mainly consisting of taxine B [**1**] (5), was obtained. The extract remaining after the acidic washings still contained alkaloids that did not partition as $H_2O$-soluble salts on account of a higher lipophilic character. The main constituent was **2**, isolated in ca. 0.10% yield from dried plant material and representing by far the major taxane diterpenoid from the seeds. Compound **2** has already been isolated from the seeds of the Japanese yew (*Taxus cuspidata* Sieb. et Zucc.) (7). Most assignments of the $^1$H- and $^{13}$C-nmr resonances of **2** (Tables 1 and 2) were done by comparison with its corresponding cinnamate **3**, whose spectra have been fully assigned (8), or by selective decoupling experiments for the methyls, C-2′ and C-3′. More polar

[1]Part IV in the series "The Chemistry and Occurrence of Taxane Derivatives." For Part III, see G. Appendino, P. Gariboldi, B. Gabetta, R. Pace, E. Bombardelli, and D. Viterbo, *J. Chem. Soc., Perkin Trans. 1*, 2925 (1992).

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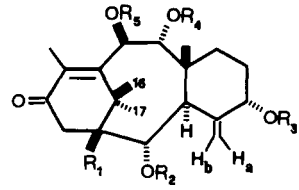
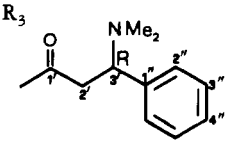
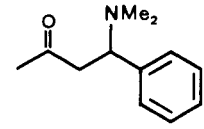
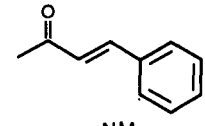
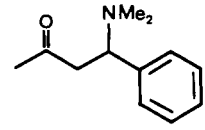


| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | OH | H |  | H | Ac |
| **2** | H | Ac |  | Ac | Ac |
| **3** | H | Ac |  | Ac | Ac |
| **4** | OH | Ac |  | Ac | Ac |

fractions gave the diacetate **4** of taxine B, a compound already prepared from taxine B (5) but not yet isolated from yew. Structures **2** and **4** correspond to the alkaloids referred to in the literature as taxine II and taxine I, respectively (2). Taxines I and II were characterized after conversion of a mixture of alkaloids from *T. baccata* to their crystalline desdimethylamino peracetyl derivatives (2); however, the number of the acetyl groups introduced could not be precisely determined. In 1968 Lythgoe (2) pointed out that, paradoxically, the constituents of taxine "which have been isolated (taxine A, B, C) have not received structure elucidation and those whose structure has been determined (taxine I and II) have not been isolated." With the present isolation of **2** and **4** from yew and the structure elucidation of taxines A and B (3–5), this does not hold any more. It is worth noting, however, that **2** and **4** do not partition as simple $H_2O$-soluble salts. Thus, little if any of these compounds might actually have been present in the HCl-soluble mixture of the mono- or bisdesacetylderivatives (8) from which taxines I and II were characterized.

The major non-alkaloidal taxane was the crystalline triol **5**. At room temperature, the $^1$H-nmr spectrum of **5** showed two sets of broad signals in a ca. 5:1 ratio. The spectrum showed marked changes with temperature, suggesting the presence of a slow (on the nmr time scale) conformational equilibrium. At elevated temperatures (60–100°) most pairs of signals coalesced to broad humps, and spectral analysis was not possible. Further increase of temperature was prevented by the thermal instability of **5**, but at low temperature (0°) most signals sharpened, allowing the identification of the spin systems. The same temperature-dependent phenomenon was observed in the $^{13}$C-nmr spectrum of **5**. Further evidence for the presence of a conformational equilibrium was obtained by the analysis of the ROESY spectrum taken at 25°. On account of chemical exchange (9), several cross-peaks of the same sign of the diagonal were in fact observed between corresponding protons of the two conformers. The $^1$H-nmr spectrum of the

TABLE 1. $^1$H-nmr Data (300 MHz, TMS as Reference, $CDCl_3$, $J$ in Hz) for Compounds **2**, **5**, and **6**.

| Proton | Compound | | |
|---|---|---|---|
| | **2** | **5**[a] | **6** |
| H-2 | 5.50 dd (6.6, 2.2) | 6.02 d (9.0) | 6.00 d (8.0) |
| H-3 | 3.17 d (6.6) | 3.30 d (9.0) | 3.00 d (8.0) |
| H-5 | 5.10 br s | 4.30 br | — |
| H-6α | [b] | 1.70 m | 2.68 dd (15.0, 6.7) |
| H-6β | [b] | 1.90 m | 2.56 dd (15.0, 11.2) |
| H-7α | [b] | 5.48 dd (11.0, 5.2) | 5.13 dd (11.2, 6.7) |
| H-7β | [b] | — | — |
| H-9 | 5.85 d (10.2) | 5.98 d (10.5) | 5.26 br d (4.0) |
| H-10 | 6.00 d (10.2) | 6.54 d (10.5) | 6.34 d (4.0) |
| H-13 | — | 4.42 br t (7.1) | — |
| H-14α | 2.80 dd (19.6, 7.0) | 1.96 m | 2.57 s |
| H-14β | 2.30 d (19.6) | 2.29 dd (14.2, 7.1) | |
| H-16 | 1.01 s | 1.08 s | 0.94 s |
| H-17 | 1.72 s | 1.17 s | 1.18 s |
| H-18 | 2.20 s | 2.04 s | 1.96 s |
| H-19 | 0.84 s | 1.00 s | 1.81 s |
| $H_a$-20 | 5.25 br s | 5.16 br s | 5.94 br s |
| $H_b$-20 | 4.81 br s | 4.56 br s | 5.06 br s |
| Ac | 2.03 s | 2.03 s | 2.02 s |
| | 2.04 s | 1.97 s | 2.00 s |
| | 2.04 s | 1.75 s | 1.94 s |
| Arom | 7.35–7.20 m | 7.81 d (8.0) | 8.00 d (8.0) |
| | | 7.39 t (8.0) | 7.48 t (8.0) |
| | | 7.53 t (8.0) | 7.60 t (8.0) |
| $H_a$-2′ | 2.93 dd (14.8, 7.1) | | |
| $H_b$-2′ | 2.55 dd (14.8, 7.4) | | |
| H-3′ | 3.79 dd (7.4, 7.1) | | |
| $N(Me)_2$ | 2.24 s | | |

[a]Major conformer (temperature 0°); selected resonances for the minor conformer (temperature 0°): δ 6.24 (d, $J$ = 4.0 Hz, H-10), 5.85 (d, $J$ = 9.8 Hz, H-2), 4.90 (d, $J$ = 4.0 Hz, H-9), 3.04 (d, $J$ = 9.8 Hz, H-3), 1.70 (s, H-19).

[b]Could not be assigned because of overlapping signals.

major conformer of **5** was similar to that of brevifoliol, a recently described constituent of *Taxus brevifolia* Nutt. (10), and the differences could be rationalized in terms of introduction of an α-acetyloxy group at C-2. The most striking difference between the conformers of **5** was the coupling constant between H-9 and H-10 (10.5 Hz in the major conformer, 4.0 Hz in the minor conformer). Oxidation with activated $MnO_2$ gave the diketone **6**, whose nmr spectra showed only one set of signals, unaffected by temperature changes. This showed that **6** is only one conformer in solution. Some $^1$H-nmr coupling constants ($J_{9,10}$ = 4.0 Hz) and chemical shift values [δ (H-19) = 1.81; δ (H-9) = 5.26] were quite unusual for taxane derivatives and closely resembled those of the minor conformer of **5**. A thorough spectroscopic analysis enabled assignment of all $^1$H and $^{13}$C resonances (Tables 1 and 2). A comparative analysis of the coupling constant pattern and the ROESY spectrum (Table 3) showed that the eight-membered ring adopts a chair-chair (=crown) conformation (11,12). Especially diagnostic were the cross-peaks H-9/H-10, H-14α/$H_b$-20, H-3/$H_b$-20, and H-19/H-17. The two conformers of **5** thus differ in the conformation of the eight-membered ring. The usual (1) chair-boat conformation with H-9 and H-10 pseudo-axial ($J_{9,10}$ = 10.5 Hz) is present

TABLE 2. $^{13}$C-nmr Data (75.4 MHz, TMS as reference, $CDCl_3$) for Compounds **2**, **5**, and **6**.

| Carbon | Compound | | |
|---|---|---|---|
| | **2** | **5**[a,b] | **6**[b] |
| C-1 | 48.47 d | 75.58 s | 75.46 s |
| C-2 | 69.47 d | 67.50 d | 68.60 d |
| C-3 | 42.99 d | 42.11 d | 42.69 d |
| C-4 | 141.61 s | 143.76 s | 143.44 s |
| C-5 | 77.77 d | 74.41 d | 197.91 s |
| C-6 | 28.25 t | 37.20 t | 38.57 t |
| C-7 | 27.42 t | 69.31 d | 69.10 d |
| C-8 | 44.43 s | 68.52 s | 61.58 s |
| C-9 | 75.77 d | 76.10 d | 73.95 d |
| C-10 | 73.30 d | 69.64 d | 70.39 d |
| C-11 | 151.80 s | 132.02 s | 161.38 s |
| C-12 | 137.83 s | 152.93 s | 147.69 s |
| C-13 | 199.00 s | 76.95 d | 206.50 s |
| C-14 | 35.94 t | 40.43 t | 44.21 t |
| C-15 | 37.60 s | 45.22 s | 43.16 s |
| C-16 | 37.31 q | 27.67 q | 28.16 q |
| C-17 | 25.12 q | 25.61 q | 29.32 q |
| C-18 | 14.05 q | 12.19 q | 9.48 s |
| C-19 | 17.41 q | 13.37 q | 13.10 q |
| C-20 | 117.55 t | 112.92 t | 127.62 t |
| Ac | 169.37 s | 171.34 s | 170.10 s |
| | 169.69 s | 170.05 s | 169.92 s |
| | 169.81 s | 169.66 s | 169.33 s |
| | 20.66, 20.85, 21.37 q | 21.74, 21.35, 20.63 q | 21.86, 20.91, 20.68 q |
| C-1′ | 170.85 s | — | — |
| C-2′ | 38.33 t | — | — |
| C-3′ | 66.31 d | — | — |
| Arom | — | 164.50 s | 165.48 s |
| | 128.34 s | 129.10 s | 129.48 s |
| | 128.56 d | 129.45 d | 129.70 d |
| | 128.02 d | 128.74 d | 128.78 d |
| | 127.40 d | 133.34 d | 133.60 d |
| $N(Me)_2$ | 42.22 q | — | — |

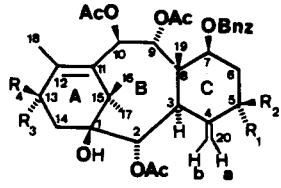
[a]Major conformer.

[b]$^1$H and $^{13}$C long-range correlations of quaternary carbons in the FLOCK experiment [W. Reynolds, S. McLean, M. Perpic-Dumont and R.G. Enriques, *Magn. Reson. Chem.*, **27**, 162 (1989)]:

For **5**:
δ 75.58 (C-1): H-16, H-17
δ 143.76 (C-4): H-3
δ 68.52 (C-8): H-3, H-10, H-19
δ 133.02 (C-11): H-10, H-18
δ 152.93 (C-12): H-18
δ 45.22 (C-15): H-10, H-16, H-17

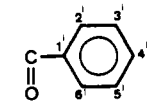
For **6**:
δ 75.46 (C-1): H-10, H-17
δ 143.44 (C-4): H-3, H-20a
δ 197.91 (C-5): H-20b
δ 61.58 (C-8): H-3, H-10, H-19
δ 161.38 (C-11): H-9, H-10, H-18
δ 147.69 (C-12): H-18
δ 206.50 (C-13): H-14, H-18
δ 43.16 (H-15): H-10, H-16, H-17

in the major rotamer, whereas the crown conformation with these protons pseudo-equatorial ($J_{9,10} = 4.0$ Hz) is assumed by the minor conformer. [Small values of $J_{9,10}$ (2–3 Hz) have been observed in taxagifine-type taxanes, where the presence of an additional ether bridge between C-12 and C-16 and of a β-hydroxyl at C-11 produce considerable distortion in the C-8–C-10 fragment (G. Chauvière, D. Guénard, C. Pascard, F. Picot, P. Potier, and T. Prangé, *J. Chem. Soc., Chem. Commun.*, 495 (1982).] These conformations of the eight-membered ring correspond to topologically quite different




**5** $R_1=R_3=OH$, $R_2=R_4=H$

**6** $R_1,R_2=O$; $R_3,R_4=O$

Bnz= 

geometries of the tricyclo [9.3.1.0.$^{3,8}$] pentadecene skeleton (Figure 1). The presence of the ROE cross peaks $H_b$-20/H-14α and H-3/$H_b$-20 (Table 3) shows that ring C adopts a boat conformation in the diketone **6**.

Little attention has been paid so far to the conformational flexibility of the highly folded taxane skeleton. Indeed, only one conformation of the eight-membered ring, the chair-boat, was detected in all the nmr and X-ray investigations done to date (1), and the strained tricyclic taxane skeleton has been considered conformationally locked, a view also supported by molecular mechanics calculations (13). The fluxional behavior of **5** is therefore surprising and unprecedented but not unique, inasmuch as we observed a conformational equilibrium also in the nmr spectra of other brevifoliol derivatives (G. Appendino and P. Gariboldi, manuscript in preparation), and conformational interconversion has also been reported for some synthetic tricyclo [9.3.1.0$^{3,8}$] pentadecenes related to taxanes (14). In brevifoliol derivatives, the conformer populations and their energy difference depend upon a complex and delicate balance of torsional and steric influences that have yet to be rationalized. When information on the conformation

TABLE 3. Cross peaks Detected in the ROESY Spectrum of **5** (major conformer) and **6** [only peaks of opposite sign to the diagonal (ROE's) have been reported].

| Proton | **5**[a,b] Cross-peaks with | Proton | **6**[b] Cross-peaks with |
|---|---|---|---|
| H-2 | H-17 m, H-19 m | H-2 | H-17 m, H-19 s, H-6β w |
| H-3 | H-7 s, H-14α s | H-3 | H-14 s, $H_b$-20 s |
| H-5 | $H_a$-20 s, H-6α m, H-6β m | — | |
| H-6α | H-6β s, H-5 m, H-7 m | $H_a$-6 | H-6β s, H-7 w |
| H-6β | $H_a$-6 s, H-19 m, H-5 m | $H_b$-6 | H-6α s, H-19 m, H-2 w |
| H-7 | H-6α m, H-3 s, H-10 s | H-7 | H-6α w |
| H-9 | H-19 w | H-9 | H-10 s, H-19 m |
| H-10 | H-7 s, H-18 s | H-10 | H-9 s, H-18 m |
| H-13 | H-14β w, H-18 m, H-16 w | — | |
| H-14α | H-14β s, H-3 s | H-14α + β | H-3 s, $H_b$-20 m, H-16 m |
| H-14β | H-16 s, H-14α s, H-13 w | | |
| H-16 | H-14β s, H-13 w | H-16 | H-14(β) m, H-17 m |
| H-17 | H-2 m | H-17 | H-16 m, H-19 w, H-2 m |
| H-18 | H-13 m, H-10 s | H-18 | H-10 m |
| H-19 | H-2 m, H-9 w, H-6β m | H-19 | H-2 s, H-6β m, H-17 w |
| $H_a$-20 | H-5 s, H-20b s | | |
| $H_b$-20 | $H_a$-20 s, 2-OAc m | $H_b$-20 | H-3 s, H-14(α) m |
| 2-Ac | $H_b$-20 | | |

[a]Major conformer.

[b]s = strong, m = medium, w = weak.

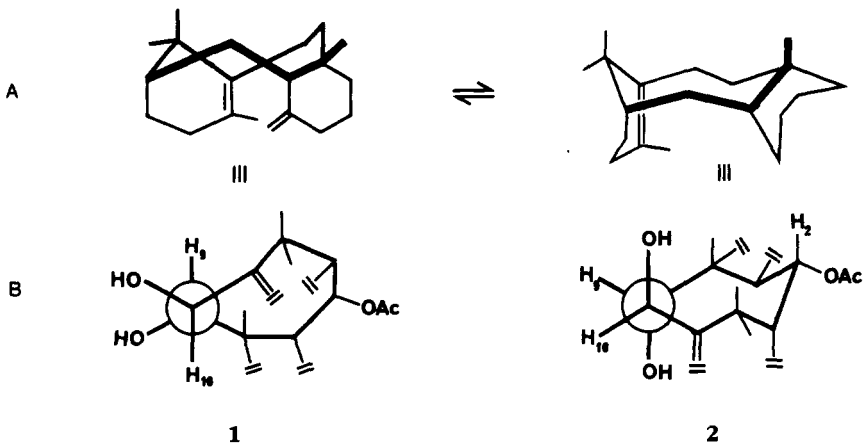



FIGURE 1. Conformation of the taxane skeleton (A) and Newman projection along C-10/C-9 (B) in brevifoliol-type compounds: (1) conformation of the major conformer of **5**; (2) conformation of **6** and the minor conformer of **5**.

is lacking, simple inspection of the coupling constants is insufficient to define the stereochemistry of these derivatives. If the usual chair-boat conformation of the B ring had been assumed for **6**, where this ring adopts instead the chair-chair conformation, an "unnatural" 9β,10α- or a cis- configuration would have been deduced for the vicinal hydroxyls at C-9 and C-10, since H-9 and H-10 are synclinal ($J_{9,10} = 4.0$ Hz) (Figure 1).

A detailed procedure for the purification of taxine B from the leaves of yew has been reported (5), and a rapid method for the isolation of **2** from the undefatted seeds is presented in the Experimental part. The availability in pure form of the major alkaloids from the leaves and the seeds of yew should now prompt studies on their biological activity. Indeed, in spite of studies spanning more than a hundred years, the high toxicity of these heart poisons still lacks a sound mechanistic and molecular pharmacological basis. Preliminary investigations on crude taxine pointed to the cardiac calcium channels as the molecular target of this poison (15,16). In view of the current interest in the development of drugs active on heart calcium channels (17), taxine might represent a new potential tool in pharmacological research.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Buchi SMP 20 apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter. Uv and ir spectra were taken on Beckman DB-GT and Perkin-Elmer model 237 spectrophotometer, respectively. $^1$H-nmr (300 MHz) and $^{13}$C-nmr (75.4 MHz) spectra were recorded on a Varian VXR 300 spectrometer at 25° unless otherwise specified. Ci mass spectra were taken on a VG EQ 70/70 apparatus. Cc was carried out on Si gel (Merck, 70–230 mesh). A Waters microPorasil column (0.8 × 30 cm) was used for preparative hplc, with detection by a Waters differential refractometer 3401.

PLANT MATERIAL.—Commercially available seeds of *T. baccata* were used. A voucher specimen is kept at the laboratory of the University of Torino.

ISOLATION.—Crushed seeds (500 g) were defatted with petroleum ether (bp 40–60°) (4 × 3 liters), and the remaining plant material was extracted with $CHCl_3$ (3 × 3 liters). The residue remaining after removal of the solvent was dissolved in 250 ml $CHCl_3$ and extracted with 2% HCl (4 × 100 ml). The acidic phase was washed with $CHCl_3$, filtered over Celite, and then made basic with 6 N $NH_3$ and extracted with $CHCl_3$. The crude alkaloid phase was obtained as a yellowish gum (350 mg, yield 0.070%); the latter was chromatographed on Si gel [10 g, eluent $CHCl_3$-EtOH (9:1)] to give 72 mg taxine B [**1**] as an amorphous powder (5). The neutral phase was washed with brine and evaporated. The residue, a brownish gum (4.7

g), was separated by cc (50 g Si gel, $CHCl_3$ containing increasing amounts of EtOH as eluent). Fractions eluted with $CHCl_3$-EtOH (95:5) gave **5** (59 mg; yield 0.0012%), **2** (480 mg; yield 0.096%), and **4** (14.4 mg; yield 0.0029%).

In a short method for the isolation of **2**, crushed seeds (500 g) were extracted with $CHCl_3$ at room temperature (3 × 3 liters). The crude extract was evaporated, the residue was dissolved in 1 liter EtOH and 1 liter of 5% aqueous $Pb(OAc)_2$ was added. After standing overnight, Celite was added (300 g) and the mush was filtered through a bed of Celite. The clear yellowish filtrate was diluted with $H_2O$ (2 liters) and extracted with $CHCl_3$. The organic phase was evaporated and the residue (7 g) separated by cc [50 g Si gel, $CHCl_3$-EtOH (95:5) as eluent] to yield **2** (519 mg).

*1-Deoxydiacetyltaxine B* [**2**].—Gum: $[\alpha]^{25}D$ +27 ($CH_2Cl_2$, $c$ = 2.8); uv (EtOH) λ max nm 207, 275; ir (liquid film) $\nu$ max $cm^{-1}$ 3450, 1735, 1670, 1370, 1240, 1030, 730; cims ($NH_3$) $[M+NH_4]^+$ 669 ($C_{37}H_{49}NO_9+NH_4$) (80).

*2α-Acetoxybrevifoliol* [**5**].—Colorless needles ($Me_2CO/Et_2O$): mp 198°; $[\alpha]^{25}D$ −24° ($CH_2Cl_2$, $c$ = 0.83); uv (EtOH) λ max nm 223, 279; ir $\nu$ max (KBr) $cm^{-1}$ 3400, 1730, 1365, 1240, 1170, 1060, 1020, 700; cims ($NH_3$) $[M+NH_4]^+$ 632 ($C_{33}H_{42}O_{11}+NH_4$) (70).

OXIDATION OF **5**.—Compound **5** (449 mg) was dissolved in EtOAc (14 ml), and 4.6 g activated $MnO_2$ (Merck) was added. After stirring at room temperature for 72 h, the reaction mixture was filtered over Celite and evaporated. The residue was purified by hplc [hexane-EtOAc (3:7) as eluent] to give **6** (198 mg; yield 44%) as a white powder: mp 195° (dec); $[\alpha]^{25}D$ +125 ($CH_2Cl_2$, $c$ = 0.83); uv (EtOH) λ max nm 225, 280; ir (KBr) $\nu$ max $cm^{-1}$ 1750, 1715, 1610, 1370, 1240, 1110, 1050, 1030; cims ($NH_3$) $[M+NH_4]^+$ 628 ($C_{33}H_{38}O_{11}+NH_4$) (100).

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*Received 3 August 1992*