Pergamon

PII: S0031-9422(96)00281-6

Phytochemistry, Vol. 43, No. 2, pp. 439–442, 1996

0031-9422/96 $15.00 + 0.00

TAXANES OF THE NEEDLES OF *TAXUS* × *MEDIA**

KOPPAKA V. RAO,† G. CHANDRASEKHARA REDDY and JOHN JUCHUM

Department of Medicinal Chemistry, College of Pharmacy, Box J-100485, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610, U.S.A.

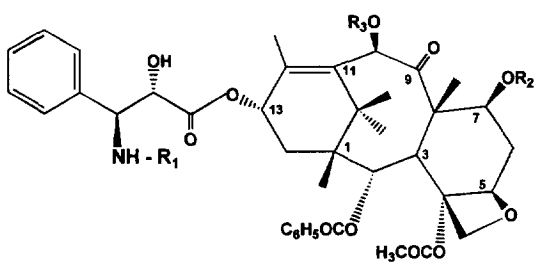
(*Received in revised form* 14 *March* 1996)

Key Word Index—*Taxus* × *media*; Taxaceae; ornamental yew; needles; brevifoliol; paclitaxel; deaminoacyl taxine A; decinnamoyl taxicin I; taxinine M, 10-deacetyl paclitaxel; 10-deacetyl paclitaxel-7-xyloside.

Abstract—The chloroform-soluble portion of the methanolic extract of the needles of *Taxus* × *media* was chromatographed on a C-18 reverse-phase column and the major components were separated by direct crystallization. Further fractionation by chromatography on normal phase silica gel, of the filtrates from the region surrounding brevifoliol, yielded two taxanes belonging to the 2(3 → 20)abeo-taxane group, one of the taxicin-type, in addition to taxinine M, 10-deacetyl paclitaxel, 10-deacetyl paclitaxel-7-xyloside, 10-deacetyl paclitaxel-C-7-xyloside, apigenin and *p*-hydroxybenzaldehyde.

INTRODUCTION

Paclitaxel (**1**) [1], an antitumour drug, isolated from the bark of the Pacific yew (*Taxus brevifolia*) has demonstrated clinical effectiveness in ovarian and breast carcinomas [2, 3]. Although compound **1** is still isolated from this bark, alternative sources, such as semisynthesis from the 10-deacetyl baccatin III [4] and also isolation from the needles of ornamental yew (*Taxus* × *media* Hicksii) [5], have been receiving increasing attention. A new process, suitable for large-scale application [6], that yields paclitaxel and other constituent taxanes from the bark of *T. brevifolia* has been developed, based on the use of a single reversed phase (C-18 bonded silica) chromatographic column and direct crystallization. Recently, this large-scale process was also applied to the extract of the needle biomass of *T.* × *media* Hicksii, from which compound **1** and five other taxanes were isolated [7].



1: R_1 = CO-C_6H_5, R_2 = H, R_3 = CH_3CO

9: R_1 = CO-C_6H_5, R_2 = H, R_3 = H

10: R_1 = CO-C_6H_5, R_2 = Xylosyl, R_3 = H

11: R_1 = CO-C_5H_{11}, R_2 = Xylosyl, R_3 = H

RESULTS AND DISCUSSION

Currently isolated compounds and their source

Elution of the reverse-phase column on the extract of *T.* × *media* Hicksii with a step gradient of 25–60% acetonitrile in water gave successive taxanes of decreasing polarity. Those fractions covering the brevifoliol (**2**) region were processed further by chromatography on a normal-phase silica gel column, which gave a number of crystalline compounds whose characterization is described here. First, two nontaxane compounds were identified as *p*-hydroxybenzaldehyde and 5,7,4′-trihydroxyflavone (apigenin). Of the rest, a new crystalline taxane **3** was isolated. Also isolated for the first time from *T.* × *media* Hicksii, were compound **5**, a taxane closely related to compound **3**, compound **6**, a member of the taxicin group, taxinine M **8**, 10-deacetyl paclitaxel (**9**) 10-deacetyl paclitaxel-7-xyloside (**10**) and 10-deacetyl paclitaxel-C-7-xyloside (**11**).

Compound **3**

The mass spectrum gave the molecular formula of $C_{26}H_{36}O_9$ with three acetate groups. The ^{1}H NMR spectrum showed four CH_3 (δ 1.18, 1.20, 1.31 and 1.94) and three CH_3COO (δ 2.02 × 2 and 2.19) groups

*For Part 4 of the series see ref. [7].

†Author to whom correspondence should be addressed.

and seven one-proton signals between δ 4.20 and 5.75, one of which was D_2O-exchangeable. Acetylation of compound **3** gave the acetate **4**, with five acetate signals, thus showing that compound **3** has two hydroxyls and three acetoxyls. In compound **4**, two downfield shifts (δ 4.49–5.50 and δ 5.46–6.30) were also seen. The ^{13}C spectrum of compound **3** showed four peaks in the alkene carbon region, (δ 124.8, 133.9, 135.3 and 138.6), of which the one at 124.98 carried a H, while the others were quaternary. This was confirmed by the HETCOR spectrum, in which the H-20 coupled with C-20 at δ 124.8. Thus, the presence of only one vinylic proton suggested that compound **3** has an altered taxane skeleton: a 2(3 → 20)abeotaxane, as in taxine A [8, 9] but without the *N,N*-dimethyl phenylisoserine side chain at 5. The complete assignment of the structure of compound **3** was made on the basis of the COSY and HETCOR spectral analysis.

In the COSY spectrum of compound **3**, the H-2 proton (δ 5.71 *dd*) coupled with H-20 at δ 5.65, and the H-5 (δ 4.49 *br s*), likewise, with H-6 protons which appeared at δ 2.08. Similarly, the H-7, (δ 5.06 *dd*) coupled with the H-6 protons at δ 2.08. H-13 (δ 5.35) coupled with H-14 protons (δ 1.96 and δ 2.70), which in turn, were strongly coupled. The H-3 protons (δ 1.65 and δ 2.70) were strongly coupled.

The location of one of the acetates at C-7 instead of C-5 was based on the spectral shifts in compound **4**, as a result of acetylation. The signal at δ 5.5 was assigned to H-5 (an allylic H), which is relatively downfield from that of H-7 (δ 5.22). Based on the coupling constants, the protons at 2, 5, 7, 10 and 13 were assigned β, β, α, α and β, respectively, and surmized as having the same configuration as found in taxine A, thus leading to the assigned structure **3**, as a new member of the deaminoacyl taxine subgroup.

Compound **5**

Compound **5**, also a crystalline taxane, showed close relationship to compound **3**. Its ^1H and ^{13}C NMR spectra were in good agreement with those for the deaminoacyl taxine A [9], except for two signals: δ 38.9 (C-6) and 47.0 (C-1), as opposed to δ 30.9 and 44.8 given in ref. [9]. A new sample of compound **5** was isolated and it also showed the same signals: δ 38.9 and 47.0. Furthermore, the signal at δ 47.0 (C-1) is in line with that seen in the closely related **3** and **4**, and with those in the literature [8–10] for the taxine A type compounds. For the signal at δ 38.9 (C-6), no example with hydroxyls at both 5 and 7, other than the deaminoacyl taxine A [9] is known, and those in which one or both are acetylated appear near δ 35. The presently observed 38.9 (versus 30.8) appears justifiable on the basis of deshielding, usually resulting from the presence of hydroxyl groups. Despite these differences, compound **5** is considered to be the same as the deaminoacyl taxine A [9].

On acetylation, compound **5** gave a triacetate which was identical to compound **6**.

Compound **6**

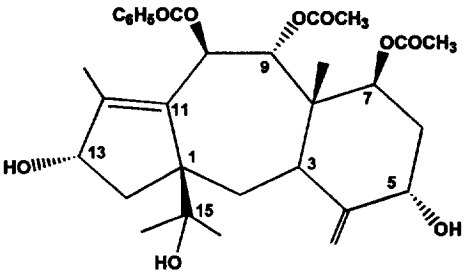
The ^1H and the ^{13}C NMR spectra of compound **6** showed that it was identical to the triacetyl-5-decinnamoyl taxicin I obtained from the needles of *T. baccata* [10]. Acetylation gave the monoacetate **7**.

The next taxane fraction, with an R_f of 0.7 (brevifoliol, R_f 0.6), was isolated and identified as taxinine M (**8**), reported from the bark of *T. brevifolia* [11], by comparison of the spectral data.

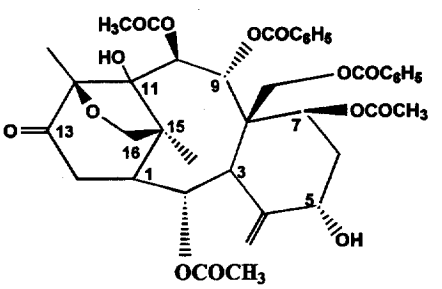
Another taxane fraction, with the same R_f as that of brevifoliol, but differing in its colour tests (with sulphuric acid spray), was isolated and found to be identical with 10-deacetyl paclitaxel **9** by HPLC and spectral comparison.

The final taxane fraction with an R_f of 0.1 (**9**, R_f 0.6), was separated on a reverse-phase column into two components. These were crystallized and found to be identical to 10-deacetyl paclitaxel-7-xyloside (**10**) and 10-deacetyl paclitaxel-C-7-xyloside (**11**) [6].

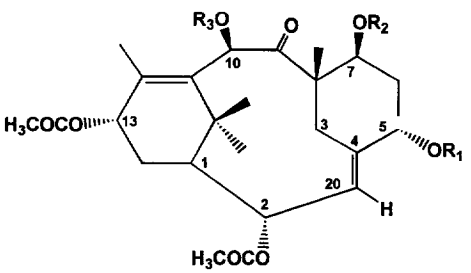
The presence of 10-deacetyl paclitaxel (**9**) and the two xylosides **10** and **11** in the needle biomass of *T.* × *media* Hicksii is of considerable practical significance and has not been reported. Because both compounds **9** and **10** can serve as precursors for the semisynthesis of paclitaxel [12, 13], their isolation and subsequent conversion to compound **1** can, therefore, increase the total yield of paclitaxel, obtainable from



2



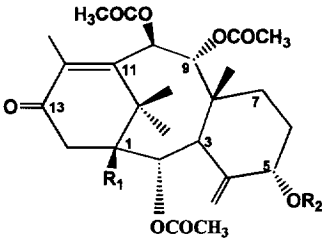
8



3: $R_1 = R_3 = H$, $R_2 = COCH_3$

4: $R_1 = R_2 = R_3 = COCH_3$

5: $R_1 = R_2 = R_3 = H$



6: $R_1 = OH$, $R_2 = H$

7: $R_1 = OH$, $R_2 = COCH_3$

this source. Their isolation and also the separation of compound **10** from **11** is best carried out by the use of a reverse-phase (C-18 silica) column; the recovered yields may be improved by further optimization.

It appears that the most abundant taxane components of the needles of *T* × *media* Hicksii are those having the 11,4/20-taxadiene type structure, typified by the acetates of taxicins I and II [7]. Related to these are the structural variants represented by brevifoliol (**2**) taxinine M (**8**) and the two 2(3 → 20)abeo-taxanes: compound **3** and the known compound **5**. The third type is represented by the oxetane ring-containing compounds: paclitaxel (**1**) and its close analogues, compounds **9**, **10** and **11**.

EXPERIMENTAL

General. ^1H and ^{13}C NMR, COSY and the HETCOR spectra: Varian VXR-300 and Varian Gemini-300 spectrometers. Chemical shifts are reported in δ (ppm) using TMS as int. standard. FAB-MS: Finnigan Mat 950 Q spectrometer. IR spectra: Perkin–Elmer 1420 ratio recording infrared spectrophotometer.

Mps (uncorr.): Fisher–Johns apparatus. Analytical HPLC: Waters 501 pump, with a U6K injector, a 486 tunable absorbance detector and a Goetz Servogor 120 recorder. Columns: standard (4.6 × 250 mm) analytical columns packed with C-8 bonded silica gel (5 μm, Fisher Scientific Company). Solvent system: 1:1 CH_3CN–H_2O or a 5:4:1 mixture of CH_3CN–H_2O–MeOH at 0.5 ml min^{-1}. TLC: silica gel 60 HF_{254} (E. Merck and Aldrich) with solvent systems: MeOH–Me_2CO–CH_2Cl_2 (5:20:75) or MeOH–CH_2Cl (1:10) and visualization by UV (254 nm) and charring with a 1 N H_2SO_4 spray.

A C-18 bonded silica column (12.5 kg, 6″ × 6 ft) was charged with 2.5 kg of an extract of *T. media* × Hicksii needles, obtained from a 50 kg batch of the dried needles. The column was eluted with CH_3CN–H_2O (1:3 to 3:1) and 21 frs collected and tested by UV-absorbance (275 nm), TLC and analytical HPLC, as previously described [7]. Frs that contained brevifoliol as the major component were combined and concd until solids began to appear. After 2–3 days, the solid was filtered and the filtrate, together with filtrates from neighbouring frs was concd to a syrup (320 g).

A portion of this syrup (15 g) was chromatographed (silica gel 150 g) in CH_2Cl_2 and ligroin (1:1), with the solvent sequence of CH_2Cl_2, 2–5% Me_2CO in CH_2Cl_2, 2–5% MeOH in CH_2Cl_2 and 10% MeOH in CH_2Cl_2. The Me_2CO–CH_2Cl_2 eluate (4 g) gave, on CC on silica gel, two crystalline compounds, identified as *p*-hydroxybenzaldehyde (0.4 g) and apigenin (0.1 g). The 2–5% MeOH–CH_2Cl_2 eluate (3 g) gave brevifoliol as the major product. The mother liquors from the *p*-hydroxybenzaldehyde on further chromatography on Florisil with the same solvent sequence, followed by preparative TLC (CH_2Cl_2–Me_2CO–MeOH, 26:3:1) gave compounds **3**, **5**, **6** and **8** (0.12 g, 0.08 g, 0.12 g and 0.06 g, respectively). The frs collected with 5% MeOH–CH_2Cl_2 (3 g) on further fractionation gave 10-deacetyl paclitaxel (**9**, 0.2 g), and finally a mixture (0.3 g) of 10-deacetyl paclitaxel-7-xyloside (**10**) and 10-deacetyl paclitaxel-C-7-xyloside (**11**).

2α,7β,13α-Triacetoxy-5α,10β-dihydroxy-9-keto-2(3 → 20)abeo-taxane (**3**). Crystalline solid (Me_2CO–hexane), yield, 0.12 g (0.005% of the dried needles), mp. 172–174° C; $[\alpha]_D$ −147°; ^1H NMR ($CDCl_3$ δ: 1.18 (3H, *s*), 1.20 (3H, *s*), 1.31 (3H, *s*), 1.65 (1H, *d*, *J* = 8 Hz, H-3), 1.94 (3H, *s*), 1.96 (1H, *m*, H-14), 2.02 (6H, *s*), 2.08 (2H, *m*, H-6-*a* and *b*), 2.19 (3H, *s*), 2.70 (2H, *m*, H-3 and H-14), 4.21 (1H, *s*, OH), 4.49 (1H, *br s*, H-5), 5.06 (1H, *dd*, *J* = 11.5 and 4.5 Hz, H-7), 5.35 (1H, *d*, *J* = 9.5 Hz, H-13), 5.46 (1H, *s*, H-10), 5.65 (1H, *d*, *J* = 9.75 Hz, H-20) and 5.71 (1H, *dd*, *J* = 9.75 and 1.5 Hz, H-2); ^{13}C NMR: 46.8 (C-1), 70.3 (C-2), 34.8 (C-3), 138.3 (C-4), 68.2 (C-5), 35.4 (C-6), 70.5 (C-7), 52.5 (C-8), 213.2 (C-9), 76.7 (C-10), 134.0 and 135.3 (C-11 and 12), 69.7 (C-13), 26.3 (C-14), 37.1 (C-15), 35.1 (C-16), 23.8 (C-17), 18.2 (C-18), 20.7 (C-19), 124.8 (C-20), 20.8, 20.9, 21.2 (CH_3CO), 170.0, 170.1, 170.1 (CH_3CO). FAB-MS: *m/z* 515 $[M + Na]^+$, 475 $[M + 1 - 18]^+$, 433 $[M + 1 - 60]^+$, 415 (475 − 60), 373 (433 − 60), 313 (373 − 60), 295

(373 − 18 − 60), 267 (295 − 28), Anal. Calc. for $C_{26}H_{36}O_9$: C, 63.40; H, 7.37. Found. C, 63.11; H, 7.52.

Acetylation of **3** (50 mg, Ac_2O, 2 ml, pyridine, 0.5 ml at 80°, 3 hr) gave compound **4**, crystallized from Me_2CO–ligroin, 35 mg, mp 240–241°; ^1H NMR: 1.11 (3H, *s*), 1.26 (3H, *s*), 1.29 (3H, *s*), 1.70–2.20 (5H), 1.95 (3H, *s*), 2.00 (3H, *s*), 2.06 (3H, *s*), 2.14 (3H, S), 2.17 (3H, *s*), 2.24 (3H, S), 2.72 (2H, *m*), 5.22 (1H, *dd*, $J = 12$ and 3 Hz), 5.4–5.51 (3H, *m*), 5.72 (1H, *dd*, $J = 10$ and 2 Hz) and 6.30 (1H, *s*); ^{13}C NMR: 46.8 (C-1), 70.4 (C, 2), 32.3 (C-3), 138.8 (C-4), 69.3 (C-5), 35.4 (C-6), 70.8 (C-7), 53.1 (C-8), 205.6 (C-9), 77.8 (C-10), 133.1 (C-11), 128.6 (C-12), 70.0 (C-13), 27.1 (C-14), 37.8 (C-15), 31.6 (C-16), 25.0 (C-17), 16.8 (C-18), 20.3 (C-19), 133.7 (C-20), 20.7, 20.7, 21.3, 21.4, 21.4 (CH_3CO), 169.4, 169.7, 170.1, 170.3, 170.3 (CH_3CO). FAB-MS: 599 $[M + Na]^+$, 577 $[M + H]^+$, 457, 415, 397, 373, 355, 313, 295 and 253. Anal. Calc. for $C_{30}H_{40}O_{11}$: C, 62.49; H, 6.99. Found: C, 62.78; H, 7.12.

2α,13α-Diacetoxy-5α,7β,10β-trihydroxy-9-keto-2(3 → 20)abeo-taxane (**5**). Obtained as a microcrystalline powder from CH_2Cl_2–ligroin in a yield of 0.08 g (0.003% of the needles). NMR spectral comparison showed that it was identical to the deaminoacyl taxine described in ref. [9].

Acetylation of compound **5** was carried out as for compound **3** and the acetate crystallized from Me_2CO–ligroin, mp 240–241°. It was identical to compound **4**.

Triacetyl-5-decinnamoyl taxicin I **6**. Purified by preparative TLC and obtained as a powder, yield, 0.12 g (0.005% of the needles). NMR spectral data indicated that it was identical to the triacetyl-5-decinnamoyl taxicin I described in ref. [10].

Acetylation of compound **6** gave the monoacetate **7**, which has not previously been described. ^1H NMR: 0.93 (3H, *s*), 1.22 (3H, *s*), 1.70 (3H, *s*), ^1H NMR: δ 0.93 (3H, *s*), 1.21 (3*H*, *s*), 1.69 (3H, *s*), 1.72–1.83 (2H, *m*, H-6*a* and *b*), 1.98 (3H, *s*), 2.07 (3H, *s*), 2.08 (1H, *m*, H-7), 2.09, (3H, *s*), 2.16 (3H, *s*), 2.15 to 2.18 (1H, *m*, H-7), 2.25, 3H, *s*), 2.62 (1H, *d*, $J = 20$ Hz, H-14), 2.78 (1H, *d*, $J = 20$ Hz, H-14), 3.38 (1H, *d*, $J = 7$ Hz, H-3), 4.70 (1H, *s*, H-20), 5.24 (1H, *br s*, H-5; in COSY spectrum, this was coupled to H-6 (2) protons at δ 1.72 and 1.83), 5.34 (1H, *s*, H-20), 5.59 (1H, *d*, $J = 10$ Hz, H-2), 5.92 (1H, *d*, $J = 10$ Hz, H-9) and 6.10 (1H, *d*, $J = 10$ Hz, H-10); ^{13}C NMR: 13.7, 17.4, 19.8, 20.7, 20.9, 21.1, 21.3, 27.5, 28.5, 34.3, 42.7, 43.6, 44.7, 45.8, 71.9, 72.9, 75.2, 77.7, 117.3, 113.8, 141.9, 151.9, 169.6, 169.9, 170.1, 171.8 and 198.8; HRFAB-MS: $[M + 1]^+$, 535.2966, Calc. for $C_{28}H_{39}O_{10}$: 535.3002.

Compound **8**. Frs obtained from the 2–5% Me_2CO–CH_2Cl_2 containing this component (R_f 0.8 versus brevifoliol, 0.6) were subjected to prep. TLC (10% Me_2CO in CH_2Cl_2) to obtain an essentially homogeneous powder (yield 0.06 g, 0.003% of the needles). A comparison of the ^1H and ^{13}C NMR spectra with the published data showed that it was identical to taxinine M [11].

10-Deacetyl paclitaxel (**9**). Obtained from the 2–5% MeOH–CH_2Cl_2 eluate, purified further by prep. TLC (7% MeOH in CH_2Cl_2) and crystallized from MeCN to give needles: Yield, 0.2 g, 0.008% of the needles), mp. 194–196°. Its chromatographic and spectral properties were identical to those of an authentic sample [6].

10-Deacetylpaclitaxel-7-xyloside (**10**) *and 10-deacetyl paclitaxel-C-7-xyloside* (**11**). The 5–10% MeOH–CH_2Cl_2 eluates on concentration deposited a crystalline solid, which was found to be a mixt. of compounds **10** and **11**. The mixt. (0.3 g) was taken up in 25% MeCN in H_2O and applied to a column of C-18-bonded reverse-phase silica gel (25 g, 15–35 μm) and the column developed with 35% MeCN in H_2O. Based on the results of analytical HPLC, frs containing the two components were combined separately and concd to dryness. The major component, **11**, was crystallized from Me_2CO to give needles, mp 247–249°, yield 120 mg (0.004%). The slower, minor component, **11**, was likewise crystallized to give 70 mg (0.002%), mp 218–220°. A comparison of the chromatographic (HPLC) and spectral data showed that compounds **10** and **11** were identical to 10-deacetyl paclitaxel-7-xyloside and 10-deacetyl paclitaxel-C-7-xyloside, respectively [6].

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. 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.
3. Rowinsky, E. K., Cazennove, L. A. and Donehover, R. C. (1990) *J. Natl Cancer Inst.* **82**, 1247.
4. McNeil, C. (1995) *J. Natl Cancer Inst.* **87**, 1106.
5. Witherup, K. M., Look, S. A., Stasko, M. W., Muschil, G. M. and Cragg, G. M. (1990) *J. Nat. Prod.* **53**, 1249.
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. Rao, K. V., Bhakuni, R. S., Juchum, J. and Davies, R. M. (1995). *J. Liq. Chrom.* **19**, 427.
8. Graf, E., Kirfel, A., Wolff, G. J. and Breitmaier, E. (1982) *Liebigs Ann. Chem.* 376.
9. Appendino, G., Cravotto, G., Enriu, R., Jakupovich, J., Garibaldi, P., Gabetta, B. and Bombardelli, E. (1994). *Phytochemistry* **36** 407.
10. Barboni, L., Garibaldi, P., Appendino, G., Enriu, R., Gabetta, B. and Bombardelli, E. (1995). *Liebigs Ann. Chem.* 345.
11. Beutler, J. A., Chmurny, G. M., Look, S. A. and Witherup, K. M. (1991). *J. Nat. Prod.* **54**, 893.
12. Rao, K. V., Bhakuni, R. S., Johnson, J. and Oruganti, R. (1995). *J. Med. Chem.* **38**, 3411.
13. Rao, K. V. (1994). *Abstracts of International Congress on Natural Products, Halifax, Nova Scotia, July/Aug. 1994.* O:43.