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ISOLATION OF 9-DIHYDRO-13-ACETYLBACCATIN III FROM *TAXUS CANADENSIS*

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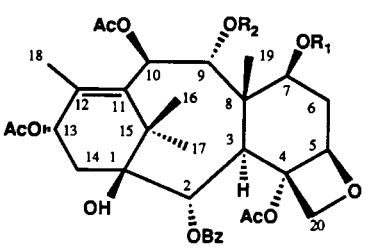
ABSTRACT—The investigation of *Taxus canadensis* (Canada yew) needles as a renewable source of taxol and its congeners led to the isolation of a novel taxane derivative, 9-dihydro-13-acetylbaccatin III [**1**], which was characterized by spectral analyses and confirmed by single crystal X-ray diffraction studies.

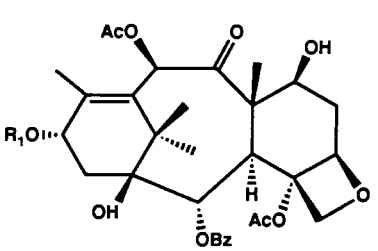
Since the discovery of the promising anticancer activity of taxol [**2**], attention has focused on the acute need for alternate sources of taxol and efficient isolation methods (1–3). The members of the family Taxaceae, the genus *Taxus* in particular, have been investigated extensively; however, only limited chemical work has been reported on *Taxus canadenis* Marsh. (4), a species relatively abundant in North America.

Canada yew is an evergreen shrub growing in the mixed conifer-hardwood forests of northeastern United States and southeastern Canada (5). Analysis of an EtOH extract of the needles and twigs of this plant by hplc according to literature methods (6) showed the taxol content to be 0.1%. An enriched fraction containing 1.27% of taxol, obtained by solvent extraction, was separated by high speed planetary coil countercurrent chromatography (pccc). The fractions were analyzed by a combination of tlc, ms, and P-388 cytotoxicity. The biological activity identified the taxol-containing fractions which were further purified by pccc to furnish about 60% of the taxol present in the extract. A similar pccc method for the separation of a taxol-containing column chromatographic fraction was reported recently (7).

The mass spectral analysis of the fractions indicated the presence of $[M]^+$ ions corresponding to a number of previously reported taxane diterpenoids (8), and their isolation will be described separately. A fraction producing a hitherto undescribed $[M]^+$ ion, at *m/z* 630, was recrystallized from MeOH to give compound **1**. The formula of the novel compound, $C_{33}H_{42}O_{12}$, determined by hrfabms, was found to differ from that of 13-acetylbaccatin III [**3**] (9) by two hydrogen atoms. The comparison of the ^{1}H-nmr spectra of **1** and baccatin III [**4**], recorded in $CDCl_3$, indicated that the two compounds are closely related but differ at C-9 and C-13. Further, the spectra of **1** displayed signals for an additional acetate function. The ^{1}H signal due to H-10 in **4** appeared as a singlet at 6.32 ppm,



1 $R_1=R_2=H$
5 $R_1=R_2=Ac$



2 R_1=COCH(OH)CH(Ph)NHCOPh
3 R_1=Ac
4 R_1=H

while that due to H-10 in **1** appeared as a doublet at 6.2 ppm coupled to a doublet at 4.45 ppm (J=10.9 Hz), suggesting that **1** has a hydroxyl function at C-9 instead of the carbonyl function present in **4**. The signal due to H-13 in **4** appeared at 4.48 ppm while that in **1** appeared at 6.17 ppm, indicating that the additional acetate function is at C-13 in the latter compound. The only other significant difference is in the chemical shift of the signal due to H-3. In compound **4** this appeared at 3.9 ppm while in compound **1** this signal has moved up-field to 3.05 ppm. The analysis of one- and three-bond C-H correlations by HMQC (10) and HMBC (11) experiments and nOe information allowed the unambiguous assignment of all ^{1}H- and ^{13}C-nmr signals (Table 1) and the assignment of the structure 9-dihydro-13-acetylbaccatin III for compound **1**. Finally, the structure was confirmed by single crystal X-ray crystallography (Figure 1).

The antiproliferative activity of compounds and fractions was assessed by

TABLE 1. Nmr Data of 9-Dihydro-13-acetylbaccatin III [**1**].[a]

Position	δ ^{13}C ($CDCl_3$)	δ ^{1}H ($CDCl_3$), J in Hz	δ ^{1}H (CD_3OD), J in Hz
1	79.51		
2	74.3	5.76, d, J=5.95	5.76, d, J=6.34
3	49.83	3.05, d, J=5.95	3.64, d, J=6.34
4	82.85		
5	84.83	4.96, dd, J=8.72, 1.3	4.96, dd, J=8.3, 1.5
6	38.66	α 2.53, ddd, J=14.8, 9.1, 7.5	2.46, ddd, J=15.1, 8.3, 7.5
		β 1.96, ddd, J=14.8, 9.8, 1.3	1.83, ddd, J=15.1, 9.8, 1.5
7	74.65	4.45, m	4.38, dd, J=9.8, 7.5
8	45.50		
9	77.51	4.45, d, J=10.9	4.48, d, J=11.2
10	73.96	6.2, d, J=10.9	6.19, d, J=11.2
11	135.74		
12	140.26		
13	70.68	6.17, m	6.14, ddd, J=15.14, 9.28, 7.8, 2.2
14	36.10	α, β 2.2, m	2.21, dd, J=15.1, 7.8
			2.29, dd, J=15.1, 9.28
15	43.80		
16	23.34	1.25, s	1.21, s
17	29.04	1.67, s	1.65, s
18	15.59	1.92, d, J=1.4	1.91, d, J=2.2
19	13.25	1.89, s	1.77, s
20	77.32	α 4.32, d, J=8.3	4.21, d, J=7.8
		β 4.17, d, J=8.3	4.16, d, J=7.8
2-*CO*	167.75		
q-Ph	130.00		
o-Ph	130.79	8.09, dd, J=6.7, 1.4	8.1, dd, J=7.3, 1.4
m-Ph	129.37	7.46, m	7.5, m
p-Ph	134.42	7.62, m	7.62, m
4-*COCH*$_3$	170.13		
4-COCH$_3$	23.57	2.3, s	2.29, s
10-*COCH*$_3$	171.37		
10-COCH$_3$	21.96	2.1, s	2.1, s
13-*COCH*$_3$	171.23		
13-COCH$_3$	22.07	2.2, s	2.17, s

[a]The ^{1}H- and inverse detected nmr spectra were recorded at 499.96 MHz while the ^{13}C spectra were recorded at 125.64 MHz using corresponding solvent signals as internal reference. Chemical shifts are in δ ppm.

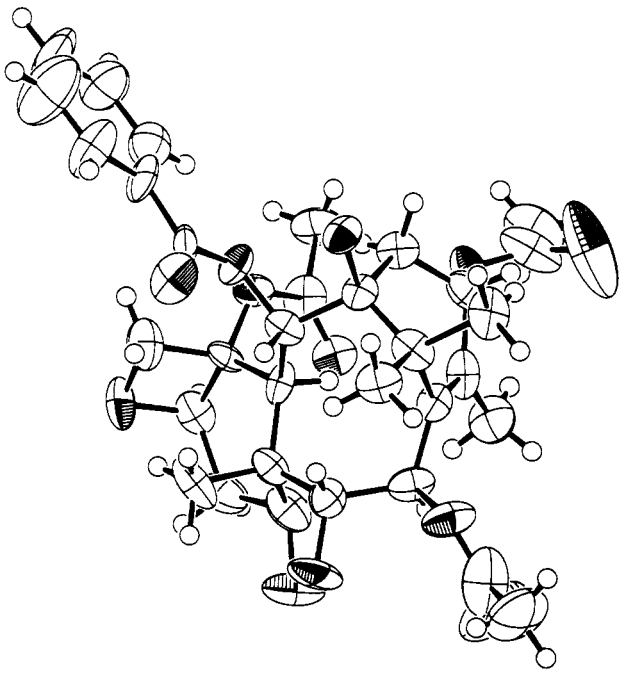


FIGURE 1. The ORTEP representation of the X-ray crystallographic structure of 9-dihydro-13-acetylbaccatin III [**1**] shown with 30% probability ellpsoids. The two molecules of H_2O have been omitted for clarity.

MTT reduction as previously described (12). In parallel determinations (means±SD, n=6), taxol [**2**] (IC_{50}=0.05±0.01 μg/ml) was a 400-fold more potent inhibitor of P-388 leukemia cell proliferation than 9-dihydro-13-acetylbaccatin III [**1**] (IC_{50}=20±1 μg/ml).

To the best of our knowledge this is the first report of this compound; however, the isolation of the corresponding diacetate **5** from *Taxus baccata* has been reported (13). We believe that this compound, one of the major constituents of the *T. canadensis* extract, could be used as a synthetic precursor of 9-dihydrotaxol analogues that cannot be obtained from taxol itself due to the resistance of the C-9 carbonyl function to chemical reduction (14). The superimposition of both solid state and solution structures of taxol [**2**] and **1** showed that the presence of the 9-hydroxy function has a minimal effect on the conformation of the taxol skeleton.

EXPERIMENTAL

ISOLATION.—An EtOH extract of the needles and twigs of *T. canadenis* (30 g) obtained from Biolyse Corporation (137, de la Pointe, Port Daniel, Quebec, Canada G0C 2N0) was partitioned between aqueous MeOH and hexane, and the MeOH phase was extracted with CH_2Cl_2 to furnish an extract (3.2 g) containing taxol (1.27% by hplc). A portion of this extract (1.0 g) was separated by pccc using the lower phase of the CCl_4-CH_2Cl_2-MeOH-H_2O (5:5:6:4) system as the mobile phase.

Taxol [**2**].—Fractions containing taxol were combined (60 mg) and further separated by pccc using the upper layer of the isooctane-EtOAc-MeOH-H_2O (7:3:6:4) system as the mobile phase to give taxol (8 mg, 90% pure by nmr, the impurities were identified as cephalomannine and 7-*epi*-taxol by hplc), mp 198° [lit. (1) mp 213–216°]. The identity was confirmed by hplc and spectral comparison with a reference sample.

9-Dihydro-13-acetylbaccatin III [**1**].—White needles from MeOH (35 mg): mp 221°, fab hrms *m/z* $[M+H]^+$ 631.2771, calc for $C_{33}H_{43}O_{12}$, 631.2754 (Δ 1.7 mmu); cims (NH_3) *m/z* $[M+H]^+$ 631 (28%), $[M-OH]^+$ 613 (23), $[M-OAc]^+$ 571

(100), 553 (28), 511 (32), 431 (35), 329 (65); ir ν max (KBr) 3450 (br), 1735 (sh), 1720, 1370, 1240, 1065, 1050, 1010, 980, 714 cm^{-1}; ^1H and ^{13}C nmr see Table 1.

X-RAY CRYSTALLOGRAPHY.[1]—Compound **1** was crystallized from MeOH as clear rods. The crystals displayed monoclinic symmetry, and the unit cell constants of $a=8.513$ (3), $b=16.164$ (2), $c=12.772$ (2) Å, and $\beta=100.16$ (2)° were determined from 25 diffractometer-measured 2θ values. Systematic extinction and density considerations were uniquely consistent with space group $P2_1$ with one molecule of composition $C_{33}H_{40}O_{12}\cdot 2H_2O$ in the asymmetric unit. A total of 1605 reflections were collected using CuKα radiation, and 1055 reflections were taken as observed [$I>3.00\sigma(I)$]. The structure was solved by direct methods (15) using TEXSAN crystallographic software package (16). The neutral atom scattering factors were taken from Cromer and Weber (17). In the final model the non-hydrogen atoms are anisotropic; the final discrepancy indices were $R=0.057$ and $R_w=0.065$.

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[1]The atomic coordinates for the structure have been deposited with the Cambridge Crystallographic Data Center, and are available on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, UK.