A BRIEF SURVEY OF *TAXUS* ALKALOIDS AND OTHER TAXANE DERIVATIVES

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ABSTRACT.—Alkaloids of the Taxaceae are briefly surveyed. Compounds containing the taxane nucleus are reviewed, including discussions of their isolation, nomenclature, and ^{1}H nmr, ms and X-ray analyses.

The Taxaceae are generally considered to include the five following genera: *Amentotaxus* Pilger, *Austrotaxus* Compton, *Pseudotaxus* Cheng, *Taxus* Linnaeus and *Torreya* Arn (1); apparently, alkaloids have been found only in species of *Taxus*.

The constituents of the needles and other parts of the English yew (*Taxus baccata* L.) have been studied for over a hundred years, and there has been a confusing proliferation of very similar names for the isolated components and their derivatives, e.g., taxicatin; taxicin I and II; taxine; taxine A, B, C, I and II; taxinine; taxinine A, B, E, H, J, K and L; taxininol and anhydrotaxininol; taxinol; taxiphyllin; taxiresinol and *iso*-taxiresinol; taxol; and taxusin. Many of these names do not refer to alkaloids, and a number of them are not derivatives of taxane. The most recent review of the different constituents of *T. baccata* was published in 1976 (2).

In addition to *T. baccata*, the following species also contain alkaloids: *T. baccata* var. *barroni* Barron (3), *T. baccata* var. *fructu luteo* Pilg. (3), *T. brevifolia* Nutt. (4), *T. canadensis* Willd. (5), *T. chinensis* (Pilg.) Rehd. (6), *T. cuspidata* Sieb. & Zucc. (7), *T. cuspidata* var. *nana* Rehd. (7), *T. fastigiata* Lindl. & Gord. (8), *T. floridana* Nutt (7) and *T. speciosa* Florin (9). Not all of these species have had their alkaloids identified.

After examining the taxane nucleus and surveying briefly the literature of taxine, this review will discuss the available formulas, ^{1}H nmr, ms and X-ray data of various taxane derivatives.

NOMENCLATURE AND STRUCTURE OF TAXANE

Prior to 1964, every worker in the field used his own system of numbering the taxane derivatives, but in that year three groups recommended numbering the molecule in the same manner (10, see **1**). For several years this recommendation was followed; but in 1969, without explanation, another system numbered the methyl groups differently (11), and both systems have been used since. In 1978 the IUPAC published "Section F: Natural Products and Related Compounds," recommending the latter system (12, see **2**), which was based on an unpublished report also used by *Chemical Abstracts* (13).

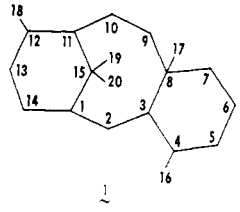
It is difficult to illustrate the caged structure of taxane and its derivatives in a two-dimensional drawing. One possibility is shown in **3**; rings A and C are folded back so that they are almost perpendicular to the "plane" of ring B, which is in a boat-chair conformation. This structure (**3**), numbered according to the IUPAC

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

system, will be used as the basis for the taxane compounds discussed in the following sections.

A BRIEF CHRONOLOGY

In 1856, Lucas isolated from *T. baccata* an alkaloid called taxine (14) for which the early research was reviewed by Henry in 1949 (15). Associated with crude taxine were color reactions which, as Lefebvre discovered, were due to a glucoside that he isolated and called taxicatin (16).



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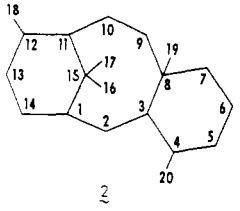

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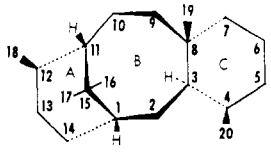

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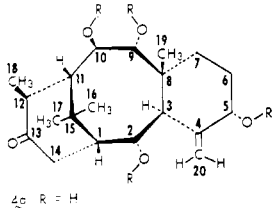
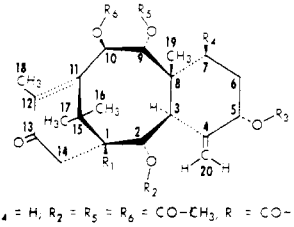


4a R = H
4b R = CO-CH$_3$



5a $R_1 = R_4 = H$; $R_2 = R_5 = R_6 = CO-CH_3$; $R = CO-CH=CH-C_6H_5$
5b $R_1 = OH$; $R_2 = R_3 = R_4 = R_5 = R_6 = H$
5c $R_1 = R_3 = R_4 = H$; $R_2 = R_5 = R_6 = CO-CH_3$
5d $R_1 = R_4 = H$; $R_2 = R_3 = R_5 = R_6 = CO-CH_3$
5e $R_1 = H$; $R_2 = R_5 = R_6 = CO-CH_3$; $R_3 = CO-CH=CH-C_6H_5$; $R_4 = O-CO-CH_3$
5f $R_1 = OH$; $R_2 = R_5 = R_6 = CO-CH_3$; $R_3 = CO-CH=CH-C_6H_5$; $R_4 = H$

In 1921, Winterstein and Iatrides assigned to taxine the empirical formula $C_{37}H_{51}NO_{10}$ (17); treatment with acid produced a crystalline nitrogenous compound, $C_{11}H_{15}NO_2$, which appeared to be 3-dimethylamino-3-phenyl propionic acid (18). In 1922, taxine was found in the Japanese yew (*T. cuspidata*) (19); in 1925, formulas were given for taxinolamine ($C_{21}H_{31}NO_5$), taxic acid ($C_{16}H_{20}O_5$) and taxinol ($C_{19}H_{24}O_5$) produced from taxine (20). Besides taxine, Kondo and Takahashi found a nitrogen-free compound, taxinine, in the alcohol extract of the needles of the Japanese yew (20). Later work revealed that taxinine was also a decomposition product of taxine (21). Reduction of taxinine by $LiAlH_4$ produced, among several compounds, an ether-insoluble product called taxininol (22). After many years of work, the structures for taxinol and taxinine were finally

established as $C_{20}H_{32}O_5$ (**4a**) and $C_{35}H_{42}O_9$ (**5a**), respectively (23). Bourbeau assigned the name taxinine to a $C_{37}H_{57}N_3O_{10}$ compound isolated from the Canadian yew (*T. canadensis*) (5); no further research on this compound seems to have been reported.

In 1934, Jesser erroneously stated that Amato and Capparelli found, in addition to taxine, an alkaloid called milossine in the seeds and needles of the yew (24); the colorless, crystalline substance Amato and Capparelli called milossine was nitrogen-free (25).

Graf (26) appears to be the first to recognize that taxine is a mixture of alkaloids, three of which he calls taxine A ($C_{35}H_{49}NO_{10}$), taxine B [$C_{35}H_{51}NO_9$, which he later revised to $C_{35}H_{45}NO_8$ (27)] and taxine C, for which he gave no formula.

Lythgoe and coworkers found that the major alkaloid (40%) of the English yew was taxine-I, occurring as an ester derivative of a tricyclic diterpenoid, taxicin-I ($C_{20}H_{30}O_6$, **5b**) (28). In a ten-year period, they published ten papers describing their work on taxine-I, taxine-II, and other taxane derivatives. In 1968 Lythgoe contributed a chapter on *Taxus* alkaloids (29) which covered the literature into 1966 and his own 1967 publications.

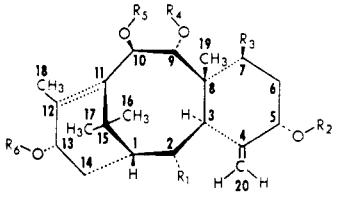
In 1963 Taylor extracted from the heartwood of *T. baccata* a substance he called baccatin ($C_{20}H_{30}O_8$ or $C_{32}H_{48}O_{12}$) (30). A different compound, also called baccatin and probably a methoxy triterpene ($C_{21}H_{48}O_4$), was isolated from yew roots by Preuss and Orth (31). In order to distinguish the two baccatins, Halsall[2] and coworkers called Taylor's compound baccatin-I ($C_{32}H_{44}O_{12}$). At the same time, they discovered baccatin-II ($C_{32}H_{44}O_{14}$), baccatin-III (possibly $C_{31}H_{38}O_{11}$), baccatin-IV (mw 592) and a taxane tetraol ($C_{20}H_{32}O_4$, **6a**) (32).

In 1967, Nakanishi and coworkers isolated four new taxinine congeners from the Japanese yew: taxinine A ($C_{26}H_{36}O_8$, **5c**), taxinine H ($C_{25}H_{38}O_9$, **5d**), taxinine K ($C_{26}H_{36}O_8$, **7a**) and taxinine L ($C_{28}H_{38}O_9$, **7b**) (34). In the following year, they reported the discovery of three more congeners: taxinine B ($C_{37}H_{44}O_{11}$, **5e**), taxinine E ($C_{37}H_{46}O_{10}$, **6b**) and taxinine J ($C_{39}H_{48}O_{12}$, **6c**) (35). That same year, other Japanese workers reported isolation of taxusin ($C_{28}H_{40}O_8$, **6d**) from the heartwood of *T. cuspidata* (36). Taxusin has also been found in *T. baccata*, *T. brevifolia*, *T. floridana* (37) and *T. mairei* (Lemée & Léveillé) S. Y. Hu (38). The hydrolysis product of taxusin was identified as the same tetraol ($C_{20}H_{32}O_4$, **6a**) (36) found naturally occurring by Chan *et al.* (32).

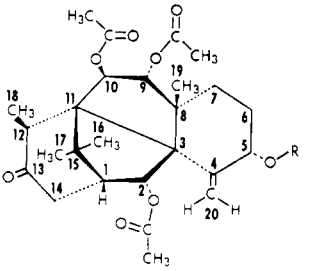
In 1969, Della Casa de Marcano and Halsall reported the isolation of seven new taxane derivatives as well as taxusin from the heartwood of *T. baccata*: taxa-4(20),11-diene-5α,9α,10β,13α-tetraol 9,10-diacetate ($C_{24}H_{36}O_6$, **6e**), taxa-4(20),11-diene-5α,7β,9α,10β,13α-pentaol pentaacetate ($C_{30}H_{42}O_{10}$, **6f**), taxa-4(20),11-diene-2α,5α,9α,10β,13α-pentaol pentaacetate ($C_{30}H_{42}O_{10}$, **6g**), taxa-4(20),11-diene-2α,5α,7β,9α,10β,13α-hexaol hexaacetate ($C_{32}H_{44}O_{12}$, **6h**), taxa-4(20),11-diene-2α,5α,7β,10β-tetraol 5,7,10-triacetate 2-α-methylbutyrate ($C_{31}H_{46}O_8$, **8a**), taxa-4(20),11-diene-2α,5α,7β,9α,10β-pentaol 7,9,10-triacetate 2-α-methylbutyrate ($C_{31}H_{46}O_9$, **8b**) and taxa-4(20),11-diene-2α,5α,7β,9α,10β-pentaol 5,7,9,10-tetraacetate 2-α-methylbutyrate ($C_{33}H_{48}O_{10}$, **8c**) (11).

Della Casa de Marcano *et al.* in 1970 revised the proposed formula $C_{33}H_{42}O_{13}$ for baccatin-III (39). They also established the structure of baccatin-I (now $C_{32}H_{44}O_{13}$, **9a**) and isolated the 5-deacetyl derivative of baccatin-I ($C_{30}H_{42}O_{12}$, **9b**),

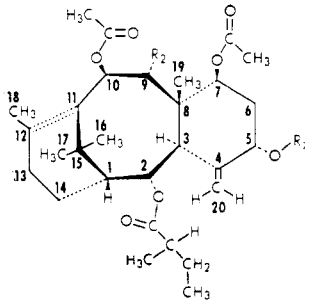
[2]In his publications (32, 33), Halsall repeatedly gives $C_{32}H_{44}O_{12}$ as the composition for Taylor's baccatin and $C_{21}H_{44}O_4$ for that of Preuss and Orth; both formulas are inexplicably at variance with statements of the authors quoted.



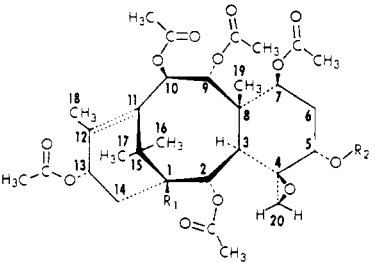
6a $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$
6b $R_1 = O-CO-CH_3$; $R_2 = CO-CH=CH-C_6H_5$; $R_3 = H$; $R_4 = R_5 = R_6 = CO-CH_3$
6c $R_1 = R_3 = O-CO-CH_3$; $R_2 = CO-CH=CH-C_6H_5$; $R_4 = R_5 = R_6 = CO-CH_3$
6d $R_1 = R_3 = H$; $R_2 = R_4 = R_5 = R_6 = CO-CH_3$
6e $R_1 = R_2 = R_3 = R_6 = H$; $R_4 = R_5 = CO-CH_3$
6f $R_1 = H$; $R_2 = R_4 = R_5 = R_6 = CO-CH_3$; $R_3 = O-CO-CH_3$
6g $R_1 = O-CO-CH_3$; $R_2 = R_4 = R_5 = R_6 = CO-CH_3$; $R_3 = H$
6h $R_1 = R_3 = O-CO-CH_3$; $R_2 = R_4 = R_5 = R_6 = CO-CH_3$



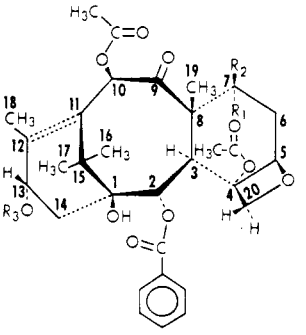
7a $R = H$
7b $R = CO-CH_3$



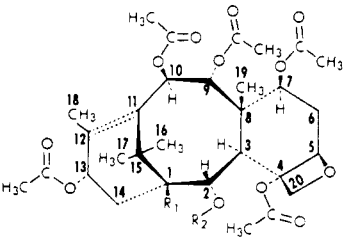
8a $R_1 = CO-CH_3$; $R_2 = H$
8b $R_1 = H$; $R_2 = O-CO-CH_3$
8c $R_1 = CO-CH_3$; $R_2 = O-CO-CH_3$



9a $R_1 = H$; $R_2 = CO-CH_3$
9b $R_1 = R_2 = H$
9c $R_1 = OH$; $R_2 = CO-CH_3$



10a $R_1 = OH$; $R_2 = R_3 = H$
10b $R_1 = H$; $R_2 = OH$; $R_3 = CO-CH(OH)-CH(C_6H_5)-NH-CO-C_6H_5$
10c $R_1 = R_3 = H$; $R_2 = OH$



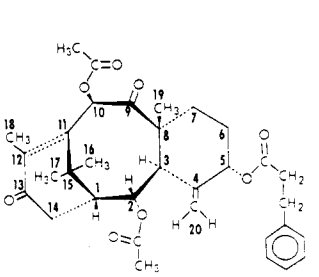
11a $R_1 = OH$; $R_2 = CO-CH_3$
11b $R_1 = H$; $R_2 = CO-CH_3$
11c $R_1 = OH$; $R_2 = CO-C_6H_5$
11d $R_1 = OH$; $R_2 = CO-(CH_2)_4-CH_3$

as well as 1β-hydroxybaccatin-I ($C_{32}H_{44}O_{14}$, **9c**) (33) and baccatin-V ($C_{31}H_{38}O_{11}$, **10a**) (40).

Whereas Tyler had found taxine in the needles of the Pacific yew (*T. brevifolia*) in 1960 (4), Wani *et al.* isolated taxol ($C_{47}H_{51}NO_{14}$, **10b**) from its stem bark in 1971

(41). They also isolated taxol from several other *Taxus* species, including *T. baccata* and *T. cuspidata*. This compound showed considerable antileukemic and tumor inhibitory properties.

In 1975, Della Casa de Marcano and Halsall determined the structures of baccatin-III (now revised back to the 1966 formula, $C_{31}H_{38}O_{11}$, **10c**), baccatin-IV ($C_{32}H_{44}O_{14}$, **11a**), 1-dehydroxybaccatin-IV ($C_{32}H_{44}O_{13}$, **11b**), baccatin-VI ($C_{37}H_{46}O_{14}$, **11c**) and baccatin-VII ($C_{36}H_{52}O_{14}$, **11d**) (42).



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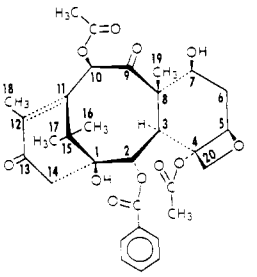

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From 1966 to 1975, nineteen different taxane derivatives were reported by Halsall and coworkers in seven brief communications; a full paper covering this work has yet to appear. Their earliest report (32) listed the empirical formula of baccatin-II as $C_{32}H_{44}O_{14}$; since that time, they have published the structures of two isomeric compounds, 1β-hydroxybaccatin-I (33) and baccatin-IV (42). The compound they originally called baccatin-II may have been identified later as 1β-hydroxybaccatin-I. The original baccatin-IV had a molecular weight of 592 (32), but the compound called baccatin-IV in 1975 (42) had a molecular weight of 652, whereas 5α-deacetylbaccatin-I (33) has a molecular weight of 594. Perhaps the latter was the compound originally called baccatin-IV.

EXTRACTION PROCEDURES

Extraction methods, when described in the literature, often have employed sulfuric acid—a rather harsh treatment for labile components. For example, Callow *et al.* extracted yew needles by soaking them in 1% H_2SO_4 (43), which has a pH of 0.9; Nicholson used 1% H_2SO_4 for 4 days (3); Bourbeau suggested that the alkaloid was most suitably extracted with 1% H_2SO_4 (5); and Lythgoe soaked yew needles seven days in 0.65% H_2SO_4 (44), which has a pH of 1.1. Kondo and Taga, however, extracted the needles with methanol, evaporated the solvent from the residue, but then extracted the residue with 2% H_2SO_4 (45), which has a pH of 0.65. In contrast, petroleum ether extraction of the heartwood of *T. baccata* was used to obtain the original baccatins (31, 32), and Wani *et al.* used alcohol to extract the stem bark of *T. brevifolia* (41).

Since baccatin-III is converted to baccatin-V under rather mild conditions (46), taxine might be an artifact produced by sulfuric acid extraction. Perhaps that is why taxine-I and taxine-II have never been isolated themselves and our knowledge of their structures comes only from their derivatives (29). Milder extraction procedures, such as those used by King *et al.* (47), Wani *et al.* (41) and Chan *et al.* (32), might be necessary to obtain the alkaloids that are actually present in yew.

TABLE 1. ^{1}H nmr data for various taxane derivatives. Chemical shifts in δ and couplings in Hz.

Compound (reference)	Protons of carbon number								
	1	2	3	5	6α	6β	7	9	10
4b (51)		5.77 br d $J=\sim1, \sim3$	3.06 br s	5.31				5.92 d $J=10$	5.26 dd $J=\sim2, 10$
5a (51)	2.3 m	5.58 dd $J=2, 6.5$	3.42 d $J=6.5$	5.37				5.90 d $J=10$	6.06 d $J=10$
5e (35)				$\sim$5.38 m $J=\sim4$	$\sim$2.23 m $J=\sim4, 5.5, 14.5$	1.74 ddd $J=\sim4, 11, 14.5$	5.43 dd $J=5.5, 11$		
5f (48)	—	5.6 d $J=6.5$	3.53 br d $J=6.5$	5.33 br s				5.9 d $J=10$	6.1 d $J=10$
6b (35)									
6d (11)			3.0 m	5.36 t $J=3$				5.88 d $J=10.8$	6.05 d $J=10.8$
6e (11)			3.29	4.31 br s				5.84 d $J=10.8$	6.10 d $J=10.8$
6f (11)			2.95 m	5.42 t $J=3$			5.59 q $J=6,10.5$	5.97 d $J=11.5$	6.25 d $J=11.5$
6g (11)		5.48 q $J=2,7$	3.22 d $J=6$	5.32 br s				5.95 d $J=10.5$	6.05 d $J=10.5$
6h (11)		5.55 q $J=3,7$	3.22 d $J=7$	5.48 t			5.33 q $J=3,8$	5.94 d $J=10.5$	6.18 d $J=10.5$
7b (34)	2.14 m $J=5$	6.12 d $J=5$	—	5.56 t $J=8.5$	←——— 1.2–2.3 m ———→			5.71 d $J=10$	5.56 d $J=10$
8a (11)		5.40 q $J=2.5,6$	2.96 d $J=6$	5.32			5.00 q $J=4,9$		6.09 q $J=6,11$
8b (11)		5.43 q $J=3,7$	3.34 d $J=7$	4.23 br s			5.07 q $J=5,9$	5.84 d $J=10$	6.12 d $J=10$
8c (11)		5.46 q $J=2.5,6$	3.01 d $J=6$	5.36			5.00 q $J=5,9$	5.85 d $J=11$	5.98 d $J=11$

TABLE 1. ^{1}H nmr data for various taxane derivatives. Chemical shifts in δ and couplings in Hz.

Protons of carbon number										Acetate signal
11	12	13	14α	14β	16	17	18	19	20	
1.7 m $J=\sim2$, ~4	2.75 m $J=\sim4$, 7	——	2.44 dd $J=2$, 20	2.58 dd $J=7$ 20	1.55 s	0.95 s	1.38 d $J=7$	0.95 s	5.56, 5.39 s s	1.95, 2.06 2.06, 2.10
——	——	——	2.41 dd $J=1$, 20	2.85 dd $J=6.5$, 20	1.77 s	1.17 s	2.28 s	0.94 s	4.88, 5.37 s s	2.06, 207
——	——	——								
——	——	——		2.77 q $J=19$	1.70 s	1.23 s	2.27 s	0.95 s	4.7, 5.33 br s br s	
——	——	5.80 ddq $J=1.5$, 7,10	1.48 br dd $J=\sim1,7$, 15	2.64 ddd $J=9,10$, 15			2.30 d $J=1.5$			
——	——	5.86 q $J=3.8$			1.63[a]	1.12[a]	2.25	0.75	4.79, 5.21	
——	——	4.34 q $J=5.8$			1.52[a]	0.91[a]	2.22	0.71	4.74, 5.08	
——	——	5.99			1.65[a]	1.15[a]	2.14	0.87	4.99, 5.32	
——	——	5.91 q $J=2,8$			1.68[a]	1.14[a]	2.17	0.90	4.80, 5.35	
——	——	5.95			1.78[a]	1.17[a]	2.25	1.03	4.87, 5.40	
——	3.45 q $J=7$	——	2.60 dd $J=2.5$, 20	2.44 dd $J=5,20$	1.68 s	1.21 s	1.24 d $J=7$	1.31 s	5.81, 5.64 s s	
——	——				1.68[a]	1.14[a]	2.17	0.82	4.82, 5.30	
——	——				1.74[a]	1.12[a]	2.17	0.86	4.81, 5.20	
——	——				1.72[a]	1.15[a]	2.19	0.88	4.89, 5.32	

TABLE 1. Continued.

Compound (reference)	Protons of carbon number								
	1	2	3	5	6α	6β	7	9	10
9a (33).....				4.23 $J=3$					
10a (40).....	——	5.74 d	4.02 $J=6$	4.9 m			3.68 sextet	——	6.83 s
10b (41).....	——	5.68 d $J=6$	3.80 d $J=6$	4.92 d $J=10$				——	6.28 s
10c (42).....	——	5.62 $J=7$	3.86 $J=7$	4.99 q $J=4,10$			4.46 q $J=6,10.5$	——	6.30 s
11a (42).....	——	5.62 d $J=6$	3.06 d $J=6$	5.00 d $J=10$			5.52 q $J=7,10$	5.95 d $J=11$	6.22 d $J=11$
11b (42).....		5.60	2.90 d $J=6$	5.00 d $J=10$			5.58 $J=5,9$	5.92 d $J=11$	6.18 d $J=11$
11c (42).....	——	5.90 d $J=6$	3.19 d $J=6$	4.98 d $J=10$			5.57 br t $J=8$	6.05 d $J=10$	6.22 d $J=10$
11d (42).....	——	5.63 d $J=6$	3.05 d $J=6$	4.98 d $J=10$			5.50 br t $J=8$	5.94 d $J=11$	6.19 d $J=11$
12 (49).....		5.24	3.7 br d $J=7$	5.33 br s				——	6.65 s
13[e] (41).....	——	5.71 d $J=6$	3.96 d $J=6$	4.96 br d $J=10$				——	6.46 s
13[f] (42).....	——	5.71 $J=6$	3.92 $J=6$	4.96 q $J=4.9$			4.48	——	6.48 s

[a]Assignment made by present author.
[b]Reassignment made by present author.
[c]Value determined by present author.
[d]These two values may be interchanged (49).
[e]This diketone was prepared from taxol (41).
[f]This diketone was prepared from baccatin-III (42).

^{1}H NUCLEAR MAGNETIC RESONANCE

The most definitive work on the ^{1}H nmr of taxane derivatives has been conducted by Lythgoe and coworkers (48–50) and Nakanishi and coworkers (34, 35, 51). The chemical shifts for the four methyl groups (see e.g., **5f**) have been carefully studied by Lythgoe's group. Since C–18 is attached to a double-bonded carbon, it's protons appear farthest downfield of the four (δ2.0–2.3) (48, 51).

TABLE 1. Continued.

Protons of carbon number										Acetate signal
11	12	13	14α	14β	16	17	18	19	20	
——	——				1.72[a]	1.14[a]	2.25[a]	1.24	3.57, 2.5	
——	——	4.9 m			1.10[a]	1.04[a]	1.99[a]	1.62[a]	4.38 s	
——	——	6.20 br t $J=8$			1.22[b] s	1.14[b] s	1.80[b] br s	1.67[b] s	4.24 s	2.20,[b] 2.36
——	——	4.85 m			1.09	1.09	2.01[c] d $J=1$	1.65	4.17, 4.30 d d $J=8$ $J=8$	2.20,[c] 2.25[b]
——	——	6.20 m			1.70	1.21	2.00	1.55	4.14, 4.55 d d $J=8$ $J=8$	
——	——	5.90 m			1.79	1.12	2.00	1.53	4.22, 4.55 d d $J=8$ $J=8$	
——	——	6.20 m			1.78	1.22	2.12	1.54	4.35, 4.13 d d $J=8$ $J=8$	
——	——	6.15 m			1.71	1.20	2.00	1.55	4.50, 4.17 d d $J=8$ $J=8$	
——	——	——			1.27 s	1.25[d] s	2.05 s	1.18[d] s	5.33, 4.87 br s br s	2.22, 2.22
——	——	——			1.26[b] s	1.20[b] s	2.08[b] s	1.66[b] s	4.16, 4.36 d d $J=8$ $J=8$	2.18,[b] 2.28
——	——	——			1.25	1.20	2.12[c] s	1.67	4.15, 4.35 d d $J=8$ $J=8$	2.22,[c] 2.28[b]

The farthest upfield shift (δ0.7–1.2) has been assigned to the protons of C–19 (48, 50), except when affected by other groups (see below). According to Nakanishi and coworkers, the two geminal methyl groups (C–16 and C–17) are coupled to each other (51);[3] the higher field signals (δ1.18–1.34) of the two have been assigned

[3]However, ^{1}H nmr spectra of these compounds available in the author's laboratory show little, if any, apparent coupling between protons at C–15 and C–16.

to C–17 and the lower field signals (δ1.47–1.69) have been assigned to C–16 (48, 50, 51). See the assignments listed in table 1 for 5-*O*-cinnamoyltaxicin-I triacetate, **5f**. When the methylene group at C–20 is oxidized to give a β-hydroxy group, a transannular interaction produces a downfield shift in the C–19 protons to δ1.30 or 1.38 (50). This reasoning accounts for the assignment of δ1.24 to the C–19 protons of baccatin-I (**9a**, 33). Also, when an oxo group is attached to C–9 (**12**), there are displacements in the shifts for protons on C–2, C–3, C–16 and C–19 (49, 50). (Note assignments for 9-oxo-5-*O*-β-phenylpropionyl-taxicin-II diacetate (**12**) in table 1, particularly the large upfield shift for the protons of C–16.)

The chemical shifts for the four methyl groups of baccatin-V, **10a** (δ1.04, 1.10, 1.62, 1.99) were not assigned by Halsall and coworkers (40), but when more oxetan compounds were found, definitive assignments were made for these methyl groups (42). The β-oxetan ring causes the C–19 protons to move downfield to δ1.54 (42). (See table 1, compounds **11a** and **11b** as examples.) However, when an oxo group at C–9 is also present, as in baccatin-III (**10c**), the value of the C–19 protons is shifted even further downfield to δ1.65 (42). At the same time, the presence of the 9-oxo group causes an upfield shift of about 0.7 ppm in the value of the C–16 protons.

The AB quartet of the C–9 and C–10 methine protons is quite distinctive and is centered at about δ6 (e.g., in **5f**). When an oxo group is present at C–9, as in **12**, the signal of the C–10 proton is a singlet at δ6.65; but in baccatin-III (**10c**) and baccatin-V (**10a**), this singlet is at δ6.30 and 6.83, respectively. The only difference in these two compounds is the configuration of the hydroxyl on C–7. In baccatin-V (**10a**) the OH is α and it can hydrogen bond to the α-acetoxy group at C–4. In baccatin-III (**10c**), the C–7 hydroxyl is β; the α acetate at C–4 is free to rotate and its carbonyl can shield the α proton at C–10 and possibly at C–3 as well.

Reduction of the C–13 oxo group, as in taxusin (**6d**) and taxa-4(20),11-diene-2α,5α,7β,9α,10β,13α-hexaol hexaacetate (**6h**), has little effect on the position of the signal for the C–16 protons but shields the C–17 protons (50). However, the β-H at C–13 is coupled to the protons of C–18, producing a doublet ($J = 1.5$ Hz) (35). If this doublet is not observed, there should be a broad singlet visible; Halsall and coworkers make no comment on the multiplicity observed for the C–18 protons of compounds **6d-6h** (11), **9a-9c** (33), **10a** (40), **10c** (42), **11a-11d** (42). As none of the shifts for acetate groups were given for these compounds, some C–18 values in table 1 may be in error. The chemical shift of the C–13 proton shows the typical upfield shift if the acetoxy group at C–13 is replaced with hydroxyl (40); compare **10b** or **11a** with **10c**.

The chemical shifts, multiplicities and couplings for the protons of carbons 6, 7, 13 and 14 were based on nuclear Overhauser effect experiments for taxinine B (**5e**) and taxinine E (**6b**) (35).

There is disagreement in the literature surrounding some of the chemical shifts for baccatin-V (**10a**) and the oxidation product (**13**) of taxol. The δ-values assigned to the methyl groups of baccatin-V by Wani *et al.* (41) were not so assigned in their literature source (40). The chemical shifts quoted by Della Casa de Marcano and Halsall for the methyl groups of the diketone (**13**) from taxol (42) differed from those given by Wani *et al.* (41) (table 2). Apparently, Wani *et al.* were unaware of Lythgoe's work demonstrating the effect of the C–9 oxo group on the methyl protons at C–16 and C–19 (49, 50) for baccatin-V, taxol, and its corresponding diketone (41). On the other hand, Della Casa de Marcano and Halsall

appear to be unaware of the small coupling between the C-13 βH and the C-18 methyl protons (35) for baccatins-III and -V or they would not have assigned a peak at δ2.25, probably due to an acetate, for the C-18 methyl group in baccatin-III (**10c** in table 1) (42).[4] The protons of the acetate methyl group produce a strong, sharp singlet and should be distinguishable from other methyl signals. Experiments in our laboratory indicate a doublet or broad singlet at δ1.78 as the shift for the C-18 methyl protons of taxol (**10b**) and the two acetate signals are at 2.20 and 2.36. Further, results for the diketone (**13**) indicate that the two downfield signals, δ2.18 and 2.28 in table 2, are due to the two acetate protons and that the C-18 methyl signal is at δ2.08.

TABLE 2. Chemical shifts (δ) for methyl groups of the diketone (**13**) from taxol.[a]

Carbon number	Assignments by:		
	Wani *et al.* (41)	Della Casa de Marcano and Halsall (42)	Present author
4 OAc	2.08	——	2.18 & 2.28
10 OAc	2.28	——	
16	1.66	1.26	1.26
17	1.26	1.20	1.20
18	2.18	2.28	2.08
19	1.20	1.66	1.66

[a]Values determined by Wani *et al.* (41).

X-RAY CRYSTALLOGRAPHIC ANALYSIS

Chan *et al.* determined the structure of taxa-4(20),11-diene-5α,9α,10β,13α-tetraol (**6a**) on the basis of the X-ray analysis of the *p*-bromobenzoate of the dihydro-anhydro-acetonide of the tetraol (32).

The configuration of taxinine (**5a**) has been determined by X-ray analysis of the 14-bromo derivative of taxinol tetraacetate (**4b**) (52, 53). The six-membered ring A (see **3**) is a distorted boat *cis*-fused to the eight-membered ring B, which has a "boat-chair" conformation; the six-membered ring C has a distorted chair conformation and is *trans*-fused to ring B (53). The X-ray analysis of baccatin-V (**10a**) similarly shows a distorted boat conformation for ring A, the "boat-chair" form of ring B, the distorted chair conformation for ring C, and, in addition, an essentially planar oxetan ring (54).

In order to obtain a crystalline derivative suitable for X-ray analysis, Wani *et al.* hydrolyzed taxol to a tetraol and a nitrogen-containing α-hydroxy methyl ester (41). The latter two compounds were derivatized and subjected to X-ray crystallography, but full details of the X-ray analysis apparently were never published.

MASS SPECTROMETRY

Little ms data on taxane derivatives is in the literature. Kurono *et al.* have stated that the low volatility of taxinine derivatives makes them unsuitable for

[4]Unpublished irradiation experiments in our laboratory established δ2.01 (d, $J=1$) as the signal due to the C-18 protons of baccatin-III (**10c**) and δ1.99 (d, $J=1.5$) as the signal due to the C-18 protons of baccatin-V (**10a**). The acetate signals were at δ2.20 and 2.24 for **10c** and at 2.20 and 2.35 for **10a**.

ms, although they did report ms data on taxinol (**4a**) and its derivatives (23). Wani *et al.* mention only four ms peaks: M^+ for taxol (**10b**), M^+ for the diketone (**13**) obtained from taxol, M–18 for the nitrogen-containing α-hydroxy methyl ester from methanolysis of taxol, and M–18 for the tetraol obtained from the same reaction (41). Della Casa de Marcano and Halsall mention ms in two papers without giving any data (11 and 33) and in a third paper give only one value, *m/e* 575, for baccatin-VII (**11d**) (42). They attribute this value to a loss of water and the hexanolyoxy group from the molecular ion but list it as M–115 instead of M–133. No details are given in any of the above references as to how the results were obtained.

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LITERATURE CITED

1. J. C. Willis, "A Dictionary of the Flowering Plants and Ferns," 7th Ed., H. K. A. Shaw, ed., Cambridge University Press, Cambridge, 1966, p. 1102.
2. I. Morelli, *Fitoterapia*, **47**, 31 (1976).
3. J. A. Nicholson, *Univ. Cambridge, Inst. Anim. Pathol., Rep. Dir.*, **3**, 169 (1932/1933); *Chem. Abstr.*, **28**, 4482[6] (1934).
4. V. E. Tyler, Jr., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 683 (1960).
5. G. Bourbeau, *Laval Med.*, **19**, 511, 637 (1954).
6. H. C. Chiang, *Shih Ta Hsueh Pao*, **20**, 147 (1975).
7. T. Kariyone, M. Takahashi, A. Nitta, and Y. Tsunehisa, *Yakugaku Zasshi*, **76**, 611 (1956).
8. R. J. Moss, *Proc. Dublin Soc.*, **12**, 92; *Chem. Abstr.*, **4**, 2864 (1910).
9. I. H. Y. Hsu, *T'ai-wan Yao Hsueh Tsa Chih*, **9**, 5 (1957).
10. B. Lythgoe, K. Nakanishi, and S. Uyeo, *Proc. Chem. Soc., London*, 301 (1964).
11. D. P. Della Casa de Marcano and T. G. Halsall, *Chem. Commun.*, 1282 (1969).
12. IUPAC Commission on Nomenclature of Organic Chemistry, *Eur J. Biochem.*, **86**, 1 (1978).
13. K. L. Loening, personal communication (1979). See also K. Nakanishi, T. Goto, S. Ito, S. Natori, and S. Nozoe (Eds.), "Natural Products Chemistry," Academic Press, Inc., New York, 1974, vol. 1, p. 186, footnote 1.
14. H. Lucas, *Arch. Pharm. (Weinheim, Ger.)*, **95**, 145 (1856).
15. T. H. Henry, "The Plant Alkaloids," 4th Ed., Churchill, London, 1949, p. 769.
16. C. Lefebvre, *J. Pharm. Chim.*, s6, **26**, 241 (1907); *Chem. Abstr.*, **2**, 300 (1908).
17. E. Winterstein and D. Iatrides, *Hoppe-Seyler's Z. Physiol. Chem.*, **117**, 240 (1921).
18. E. Winterstein and A. Guyer, *Hoppe-Seyler's Z. Physiol. Chem.*, **128**, 175 (1923).
19. H. Kondo and U. Amano, *Yakugaku Zasshi*, n490, 1074 (1922).
20. H. Kondo and T. Takahashi, *Yakugaku Zasshi*, n524, 861 (1925).
21. T. Takahashi, *Yakugaku Zasshi*, **51**, 401 (1931).
22. T. Takahashi, K. Ueda, R. Oishi and K. Minamoto, *Chem. Pharm. Bull.*, **6**, 728 (1958).
23. M. Kurono, Y. Nakadaira, S. Onuma, K. Sasaki and K. Nakanishi, *Tetrahedron Lett.*, 2153 (1963).
24. H. Jesser, *Sueddtsch, Apoth.-Ztg.*, **74**, 473 (1934).
25. D. Amato and A. Capparelli, *Gazz. Chim. Ital.*, **10**, 349 (1880).
26. E. Graf, *Angew. Chem.*, **68**, 249 (1956).
27. E. Graf, *Arch. Pharm. (Weinheim, Ger.)*, **291**, 443 (1958).
28. J. N. Baxter, B. Lythgoe, B. Scales, S. Trippett and B. K. Blount, *Proc. Chem. Soc. London*, 9 (1958).
29. B. Lythgoe, in "The Alkaloids," R. H. F. Manske, ed., Academic Press, New York, 1968, vol. 10, chap. 14.
30. D. A. Taylor, *West Afr. J. Biol. Appl. Chem.*, **7**, 1 (1963).
31. F. R. Preuss and H. Orth, *Pharmazie*, **20**, 698 (1965).
32. W. R. Chan, T. G. Halsall, G. M. Hornby, A. W. Oxford, W. Sabel, K. Bjåmer, G. Ferguson and J. M. Robertson, *Chem. Commun.*, 923 (1966).
33. D. P. Della Casa de Marcano and T. G. Halsall, *Chem. Commun.*, 1381 (1970).
34. H. C. Chiang, M. C. Woods, Y. Nakadaira and K. Nakanishi, *Chem. Commun.*, 1201 (1967).
35. M. C. Woods, H. C. Chiang, Y. Nakadaira and K. Nakanishi, *J. Am. Chem. Soc.*, **90**, 522 (1968).
36. M. Miyazaki, K. Shimizu, H. Mishima and M. Kurabayashi, *Chem. Pharm. Bull.*, **16**, 546 (1968).
37. H. Erdtman and K. Tsuno, *Phytochemistry*, **8**, 931 (1969).
38. C. L. Lee, Y. Hirose and T. Nakatsuka, *Mokuzai Gakkaishi*, **21**, 249 (1975).
39. D. P. Della Casa de Marcano, T. G. Halsall and G. M. Hornby, *Chem. Commun.*, 216 (1970).

40. D. P. Della Casa de Marcano, T. G. Halsall, E. Castellano and O. J. R. Hodder, *Chem. Commun.*, 1382 (1970).
41. M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon and A. T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).
42. D. P. Della Casa de Marcano and T. G. Halsall, *Chem. Commun.*, 365 (1975).
43. R. K. Callow, J. M. Gulland and C. J. Virden, *J. Chem. Soc.*, 2138 (1931).
44. J. N. Baxter, B. Lythgoe, B. Scales, R. M. Scrowston and S. Trippett, *J. Chem. Soc.*, 2964 (1962).
45. H. Kondo and J. Taga, *Itsuu Kenyusho Nempo*, **9**, 16 (1958).
46. R. G. Powell, R. W. Miller and C. R. Smith, Jr., *Chem. Commun.*, 102 (1979).
47. F. E. King, L. Jurd and T. J. King, *J. Chem. Soc.*, 17 (1952).
48. J. W. Harrison, R. M. Scrowston and B. Lythgoe, *J. Chem. Soc. C*, 1933 (1966).
49. M. Dukes, D. H. Eyre, J. W. Harrison, R. M. Scrowston and B. Lythgoe, *J. Chem. Soc. C*, 448 (1967).
50. D. H. Eyre, J. W. Harrison and B. Lythgoe, *J. Chem. Soc. C*, 452 (1967).
51. M. C. Woods, K. Nakanishi and N. S. Bhacca, *Tetrahedron*, **22**, 243 (1966).
52. M. Shiro, T. Sato, H. Koyama, Y. Maki, K. Nakanishi and S. Uyeo, *Chem. Commun.*, 97 (1966).
53. M. Shiro and H. Koyama, *J. Chem. Soc. B*, 1342 (1971).
54. E. E. Castellano and O. J. R. Hodder, *Acta Crystallogr., Sec. B*, **29**, 2566 (1973).