THE CHEMISTRY OF TAXOL, A CLINICALLY USEFUL ANTICANCER AGENT[1]

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ABSTRACT.—Earlier studies on the chemistry of taxol from the authors' laboratory are summarized, and recent work is discussed in more detail. The earlier studies include the isolation of new taxanes from *Taxus brevifolia*, the preparation of acyl and ether derivatives of taxol, the deacylation of taxol to baccatin III, and the oxidation of taxol and cephalomannine. Recent work on the reaction of taxol with electrophilic reagents has yielded a taxane **29** with an opened oxetane ring and another taxane **33** with a rearranged A ring. The preparation of a taxol derivative suitable for photoaffinity labeling studies on tubulin is also described.

The search for natural products as potential anticancer agents dates back at least to the Ebers papyrus in 1550 B.C. (1), but the scientific period of this search is much more recent, beginning with the investigations by Hartwell and his co-workers on the application of podophyllotoxin and its derivatives as anticancer agents (2,3). A very large number of plant, marine, and microbial sources have been tested as leads, and many hundreds of active compounds have been isolated. Although relatively few of these lead compounds have survived the rigorous testing required before introduction into normal clinical use, those that have done so have proved enormously beneficial, and cancer treatment would be greatly impoverished without such drugs as vincristine, vinblastine, adriamycin, mitomycin, anthramycin, and other natural products.

Natural products are important in cancer treatment not only in their isolated form, but also as templates for the formation of analogues with improved activity and as probes for studying biochemical processes at the molecular level. It is of some interest in this connection that the podophyllotoxins studied by Hartwell have become the basis of the clinically useful drugs etoposide and teniposide (4); other studies on natural products as models for anticancer drugs are discussed by Cassady and Douros (5).

The novel diterpenoid taxol {**1**} has become one of the most important lead com-
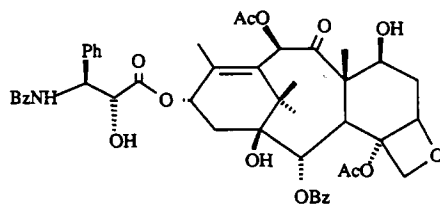
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pounds to emerge from the screening of natural products in recent years. It was first isolated in 1971 by Wall and his collaborators, and it showed significant activity against various leukemias, the Walker 256 carcinosarcoma, Sarcoma 180, and the Lewis lung tumor (6). In spite of this promising spectrum of activity, progress on developing taxol as a drug was slow, largely because of the difficulty of isolating it from the bark of the western yew, *Taxus brevifolia*, and because of its lipophilicity which made formulation

difficult. Interest in taxol was further heightened by the discovery that it has the unique property of acting as a promoter of microtubule assembly (7), and a determined effort on the part of the National Cancer Institute finally resulted in adequate supplies of taxol for formulation studies and eventual clinical trials.

Clinical results on taxol have been extremely encouraging. Although the Phase I trials had some problems, stemming it is thought from the emulsifier used rather than from taxol itself (8), the results available to date from the Phase II trials show that taxol has excellent activity against ovarian cancer (9). The limited supply of taxol has precluded extensive clinical studies, and it is certainly possible that taxol will show significant activity against other cancers. The outlook for taxol as a clinically useful anticancer agent is thus bright.

Our approach to the study of taxol has been driven by the concept that its development as a routinely useful drug will require either an improved source of taxol or a simpler but effective analogue. The total synthesis of taxol is a formidable task, and in spite of some outstanding synthetic work, most notably by Holton and his co-workers (10), it remains an as-yet-unattained goal. The partial synthesis of taxol from the simpler diterpenoid baccatin III [2] has been reported, however, and this approach thus offers in principle a source of taxol from the renewable resource of yew leaves as opposed to yew bark (11).
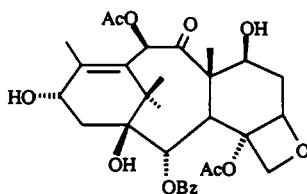

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The approach of modifying taxol to produce taxol analogues had not been investigated when we began our studies. This approach has an advantage over total synthesis in that it permits the relatively rapid preparation of a number of analogues; it is limited, of course, in that the selection of analogues is restricted to those accessible from the natural product. In this review we will briefly summarize our earlier published studies of taxol analogues, and then present some new and as-yet-unpublished work on the preparation of some novel derivatives of taxol and baccatin III.

ISOLATION OF NEW TAXANES.—We initially investigated the constituents of a "post-taxol" fraction that was available as the residue of a large-scale taxol extraction carried out at Polysciences, Inc. Acetylation of the crude fraction followed by isolation yielded 2',7-diacetyltaxol [3] and 2',7-diacetyl-10-deacetyltaxol [4], while a direct isolation of the crude fraction yielded decinnamoyltaxinine J [5] and 10-deacetylbaccatin III [6] (12). A later study on a different crude fraction from a large-scale isolation of taxol yielded 7-*epi*-taxol [7] and the ketotaxol derivative 8. Interestingly enough, treatment of taxol with the free radical initiator AIBN converted it cleanly into 7-*epi*-taxol [7] (13).

We elected to use cytotoxicity as our major criterion of activity for the taxanes, primarily because this assay requires only a small amount of material and because it appears to correlate reasonably well with other measures of activity such as tubulin assembly and tubulin binding (14). The cytotoxicities of the isolated compounds are recorded in Table 1; data are also given for the related compound cephalomannine [9], which in

3   $R_1 = R_3 = Ac$, $R_2 = \beta$-OAc
4   $R_1 = Ac$, $R_2 = \beta$-OAc, $R_3 = H$
7   $R_1 = H$, $R_2 = \alpha$-OH, $R_3 = Ac$

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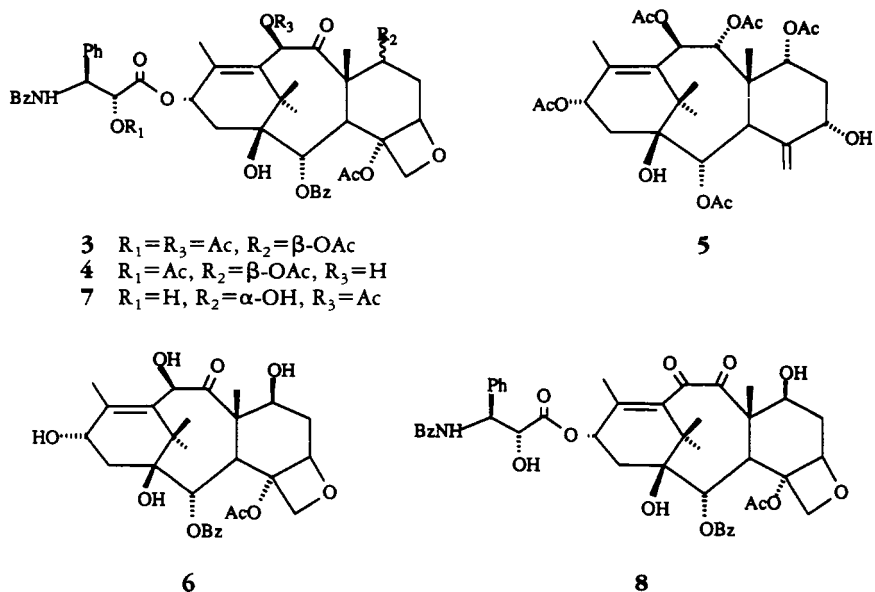
spite of its name was isolated from *Taxus wallichiana* (previously thought to be *Cephalotaxus mannii*) (15).

The conclusions from Table 1 are clear-cut and may be summarized as follows:

1. The C-13 ester side-chain is absolutely required for cytotoxicity. Thus taxol [1] and cephalomannine [9] are both active, while compounds lacking the side-chain, such as baccatin III [2], decinnamoyltaxinine J [5], and 10-deacetylbaccatin III [6], are essentially inactive.

2. The *N*-acyl substituent on the C-13 side-chain appears not to be that important. Thus although the ED_{50} values for taxol vary somewhat when determined in different laboratories, both taxol and cephalomannine show comparable values when determined in the same laboratory (16).

3. The stereochemistry of the hydroxyl group at C-7 does not make a large difference in activity; thus taxol [1] and 7-*epi*-taxol [7] show comparable activities.

The activity of the acyltaxols will be discussed in the next section.

TABLE 1.   Biological Activity of New Taxanes.

| Compound	ED_{50} (μg/mL) in KB cell culture	ED_{50} (M) for J774.2 cells[a]
Taxol [1]	1×10^{-5}, 1.2×10^{-3}[b]	7×10^{-8}
Baccatin III [2]	2.0[b]	$> 1 \times 10^{-5}$
2',7-Diacetyltaxol [3]	3×10^{-2}	8×10^{-7}
2',7-Diacetyl-10-deacetyltaxol [4]	$< 1 \times 10^{-2}$	2×10^{-7}
Decinnamoyltaxinine J [5]	28	
10-Deacetylbaccatin III [6]	1.0	
7-*epi*-Taxol [7]	3×10^{-5}	
10-Oxotaxol [8]	2×10^{-2}	
Cephalomannine [9]	3.8×10^{-3}[b]	1.5×10^{-7}

[a]Data estimated from Parness *et al.* (13).
[b]Data from McLaughlin *et al.* (18).
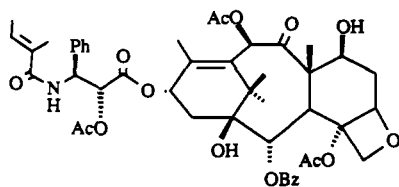
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ACYL AND ETHER DERIVATIVES OF TAXOL.—Any scheme to prepare taxol derivatives for bioassay purposes must logically start with the readily available hydroxyl groups. Although taxol has three hydroxyl groups, at the 1, 7, and 2' position, that at the 1 position is tertiary and is thus not readily acylated. Acetylation of taxol under mild conditions yields 2'-acetyltaxol [10], while acetylation under more vigorous conditions yields 2',7-diacetyltaxol [3] identical with that previously isolated from *T. brevifolia*. The acetyl group at the 7 position is not only more difficult to attach, because of steric crowding at this position, but it is also more difficult to remove. Mild hydrolysis of 2',7-diacetyltaxol [3] thus yields 7-acetyltaxol [11] (17).
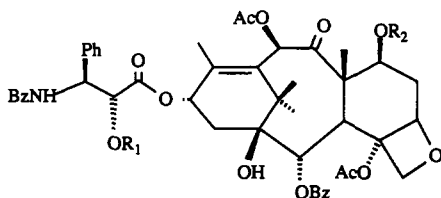
10   R_1=Ac, R_2=H
11   R_1=H, R_2=Ac

Although this chemistry provides a route for the synthesis of 7-acyl derivatives of taxol, the mild hydrolysis of the 2'-acetyl group is tedious to do and is accompanied by some hydrolysis of the C-13 side-chain ester group. The use of the chloroacetyl protecting group provides a convenient way around this problem, because it is removable cleanly under mild conditions by thioethanolamine without accompanying side-chain cleavage.

Prior to our selection of the chloroacetyl group as the reagent of choice for the preparation of C-7 taxol derivatives, we investigated the use of the 2,2,2-trichloroethyloxycarbonyl (troc) protecting group. In an attempt to prepare 2'-epi-taxol we treated 2',7-ditroctaxol [12] with 1,5-diazabicyclo [5.4.0] undec-7-ene (DBU). This reaction caused a cyclization reaction to occur leading to the oxazolone 13, presumably via deprotonation of the amide nitrogen of 12 by DBU followed by intramolecular displacement on the troc carbonyl group. The troc group at C-7 could be removed in the usual way by reaction with Zn dust in methanolic HOAc to yield the unprotected oxazolone 14 (18).
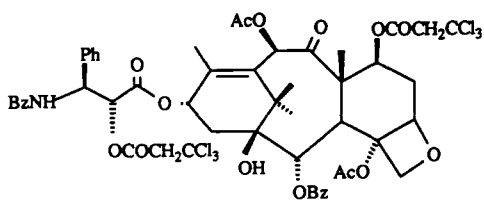
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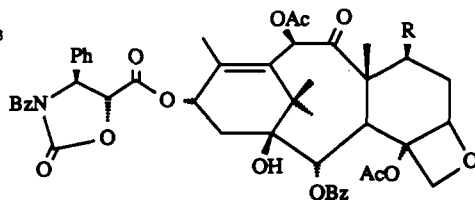
13   R_1=CCl$_3$CH$_2$OCO
14   R_1=OH

As noted earlier, 2'-acyltaxols are readily hydrolyzed to taxol. It thus seemed feasible to prepare suitable 2'-acyltaxols as H_2O-soluble pro-drugs of taxol with the expectation that the derivative would be readily converted to taxol in vivo, and we have prepared a number of such derivatives. As an example, acylation of taxol with N-carbobenzyloxy-β-alanine followed by deprotection of the carbobenzyloxy group gave 2'-(β-alanyl)taxol formate [15], while treatment of taxol with succinic anhydride gave 2'-succinyltaxol [16] (18). Both 15 and 16 showed increased H_2O-solubility as compared with taxol, but 15 proved to be too labile for use as a pro-drug. The succinyltaxol 16 had adequate stability, but its activity in the P-388 in vivo bioassay was not encouraging and we turned to other derivatives. Recently, however, Deutsch *et al.* (17) have published the results of a more extended study of 2'-acyltaxols, in which they show that 2'-succinyl salts and more complex succinyl and glutaryl derivatives have excellent in vivo activity.

15   16
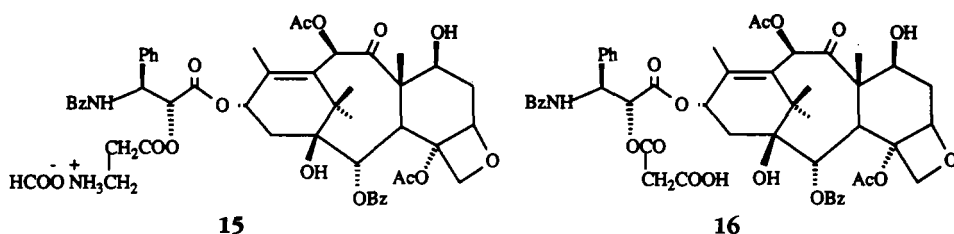
In addition to preparing 2'-acyltaxol derivatives, we also prepared a 2'-ether derivative, 2'-(t-butyldimethylsilyl)taxol [17], and 10-deacetyltaxol [18] was available for testing from an earlier isolation study (16).

Bioassay results for the acyl and ether derivatives discussed in this section are given in Table 2. The following major conclusions emerge from these data:

1. Acylation of taxol at the 2' position does not destroy its cytotoxicity. This result contrasts with the result of a tubulin-assembly study, which showed that 2'-acetyltaxol [10] was not able to promote microtubule assembly (17). The apparent discrepancy is almost certainly due to the fact that the tubulin assembly study is done under conditions where hydrolysis of a 2'-acyl group is slow or nonexistent, while the longer time and the presence of living cells in the cytotoxicity assay presumably promote deacylation at the 2' position. This conclusion is supported by the somewhat variable data for

TABLE 2.   Biological Activity of Acyl and Ether Derivatives of Taxol.

Compound	ED_{50} (µg/mL) in KB cell culture[a]	ED_{50} (M) for J774.2 cells[b]
Taxol [1]	1×10^{-5}	7×10^{-8}
2'-Acetyltaxol [10]	2×10^{-5}	3×10^{-7}
2',7-Diacetyltaxol [3]	2×10^{-1}	8×10^{-7}
7-Acetyltaxol [11]	4×10^{-3}	9×10^{-8}
Oxazolone derivative 14	2×10^{-1}	
2'-(β-alanyl)taxol formate [15]	2×10^{-4}	
2'-succinyltaxol [16]	1×10^{-2}	
2'-(t-Butyldimethylsilyl)taxol [17]	3×10^{-1}	
10-Deacetyltaxol [18]	2.7×10^{-2c}	2×10^{-7d}

[a]Data from Magri and Kingston (16).
[b]Data estimated from Mellado *et al.* (15).
[c]Datum from McLaughlin *et al.* (18).
[d]Datum estimated from Parness *et al.* (13).

17   18
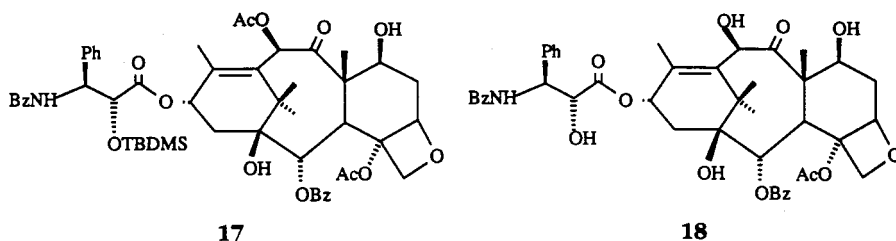
the 2'-acyltaxols: thus 2'-(β-alanyl)taxol formate [15], which is quite labile, is almost as cytotoxic as taxol, while the more stable 2'-succinyltaxol [16] is less active.

2. Taxol derivatives lacking an accessible 2'-hydroxyl group are much less active. Thus the oxazolone 14 and the silyltaxol 17 are both much less active than taxol, suggesting that a free 2'-hydroxyl group is necessary for maximal activity of taxol and its derivatives.

3. Acylation at the 7 position of taxol does not eliminate activity. Thus 7-acetyltaxol [11] is comparably active to taxol, especially in the J774.2 assay, and even 2',7-diacetyltaxol has only a modestly reduced activity in the J774.2 assay. (Compound 3 is much less active in the KB assay; this may be due to solubility problems or to the different bioassay conditions.) Additionally, 7-acetyltaxol is fully competent to assemble microtubules, indicating that 7-acyltaxols can serve as valuable derivatives of taxol for probing the taxol-tubulin interaction (17). One group has taken advantage of this conclusion by the preparation of tritium-labeled 7-acetyltaxol (20).

4. Deacylation at the 10 position, as in 10-deacetyltaxol [18], does not reduce the cytotoxicity of taxol very much, at least to the J774.2 cell line.

DEACYLATION OF TAXOL.—Another approach to the preparation of taxol derivatives is by the deacylation and subsequent reacylation of taxol. Simple base hydrolysis of taxol, similarly to the reported hydrolysis of cephalomannine (15), yields a complex mixture of products including products resulting from epimerization of the C-7 hydroxyl group. However, we found that a reductive cleavage of the C-13 ester side-chain is possible by the use of tetrabutylammonium borohydride in CH_2Cl_2. This reaction proceeds clearly to give baccatin III [2] from either taxol or cephalomannine (21). Although this cleavage is not useful in relation to taxol, it does provide a route for the conversion of the unwanted cephalomannine (which co-occurs with taxol in *T. brevifolia*) into baccatin III and thence into taxol by the synthetic route developed by Denis *et al.* (11).

We have also investigated the selective deacylation of baccatin III, with the idea that reacylation might produce novel derivatives with improved properties. Baccatin III can be hydrogenated (Pt/H_2) and then selectively silylated to yield the reduced derivative 19; the Δ^{11} double bond is not reduced because it is tetrasubstituted and very hindered.

Mild hydrolysis of 19 yielded the fully deacylated derivative 20 as the major product. Although the 10-deacetyl derivative 21 could be isolated as an intermediate in the hydrolysis of 19, the desired 2,10-deacyl derivative was not detected, in spite of the fact that the acetoxy group at the 4 position is quite hindered. The reason for this is presumably that intramolecular transesterification takes place rapidly, leading to acetyl transfer to C-13 or C-2 and subsequent rapid hydrolysis. Further studies of this reaction are in progress.

OXIDATION OF TAXOL AND CEPHALOMANNINE.—Oxidation of taxol provides an opportunity to obtain independent verification of the importance of various structural units to bioactivity. Oxidation of taxol under mild conditions with Jones' reagent

19   R=Ac
21   R=H

20
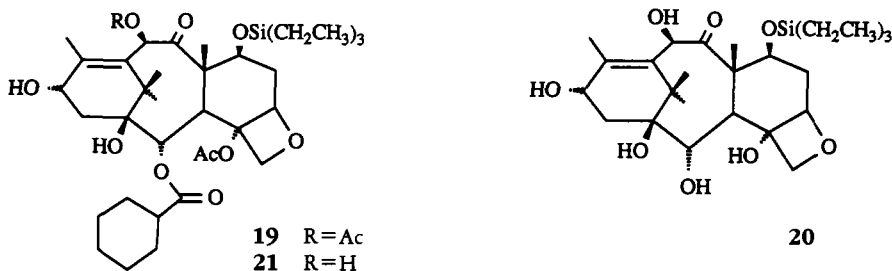
yielded 7-oxotaxol [22], and more vigorous conditions gave 2′,7-dioxotaxol [23]. This latter compound readily formed a hemiketal on standing in MeOH.

7-Oxotaxol [22] could be converted cleanly to the D-secotaxol 24 on treatment with DBU, and this could be reduced (Pt/H$_2$) to the unstable β-diketone 25. Mild heat (40°) on 25 converted it to the lactone 26 by a retro-Claisen reaction followed by lactonization; these conversions are shown in Scheme 1.

Oxidation studies on cephalomannine took a different approach, because cephalomannine has an accessible double bond that is lacking in taxol. The separation of taxol and cephalomannine is a difficult and tedious process, because both compounds have very similar polarity and are thus only separable by careful and repeated chromatography. An improved method to separate these materials would thus be very helpful, and the double bond in cephalomannine provided the vehicle for this process.

Treatment of cephalomannine with osmium tetroxide under catalytic conditions (22) resulted in its clean conversion into a diastereomeric mixture of diols 27. Taxol was unaffected by these conditions, and could be separated readily from the reaction product by simple chromatography. The objective of a simple separation of taxol and cephalomannine was thus achieved.

Bioassay data on the oxidized taxols are shown in Table 3. These confirm that compounds lacking the 2′-hydroxyl group (such as 23) are inactive, and also that major changes in the C and D rings remove the activity (compound 26). The relative lack of activity of 7-oxotaxol [22] cannot be due to a requirement for a hydroxyl group at C-7, since 7-acetyltaxol is much more active. Presumably the ready ring-opening of 22 to 24 occurs under the assay conditions, leading to an inactive D-secotaxol (see below).

REACTION OF TAXOL WITH ELECTROPHILIC REAGENTS.—Although taxol is very labile to base, it is relatively stable to acidic reagents. It does, however, react with Lewis acids under mild to vigorous conditions to yield some important products.

Reaction of taxol with ZnBr$_2$ in MeOH at room temperature is the mildest treatment. Under these conditions taxol undergoes conversion to a mixture of 10-deacetyl-taxol [18] and 10-deacetyl-7-*epi*-taxol [28] in a clean reaction that again serves to demonstrate the greater stability of the epimer over the natural product, presumably due to hydrogen bonding between the 7-hydroxyl group and the 4-acetoxy group.

A more profound and more interesting reaction occurs on treatment of taxol with excess triethyloxonium tetrafluoroborate (Meerwein's reagent). Reaction in CH$_2$Cl$_2$ at 0° for 45 min, followed by an aqueous workup, yielded a mixture of products. The major product, isolated in about 35% yield, had peaks in its fab mass spectrum at *m/z* 911 [MK]$^+$, 894 [MNa]$^+$, 872 [MH]$^+$, and 854 [MH − H$_2$O]$^+$; the peak at *m/z* 872 corresponded to the addition of H$_2$O to taxol. Peaks at *m/z* 609 [MNa − RCOOH]$^+$, 569 [MH − RCOOH − H$_2$O]$^+$, and 527 [MH − RCOOH − HOAC]$^+$, where RCOOH is the protonated C-13 ester side chain, indicated that the additional molecule of H$_2$O is on the taxane skeleton rather than the side chain.
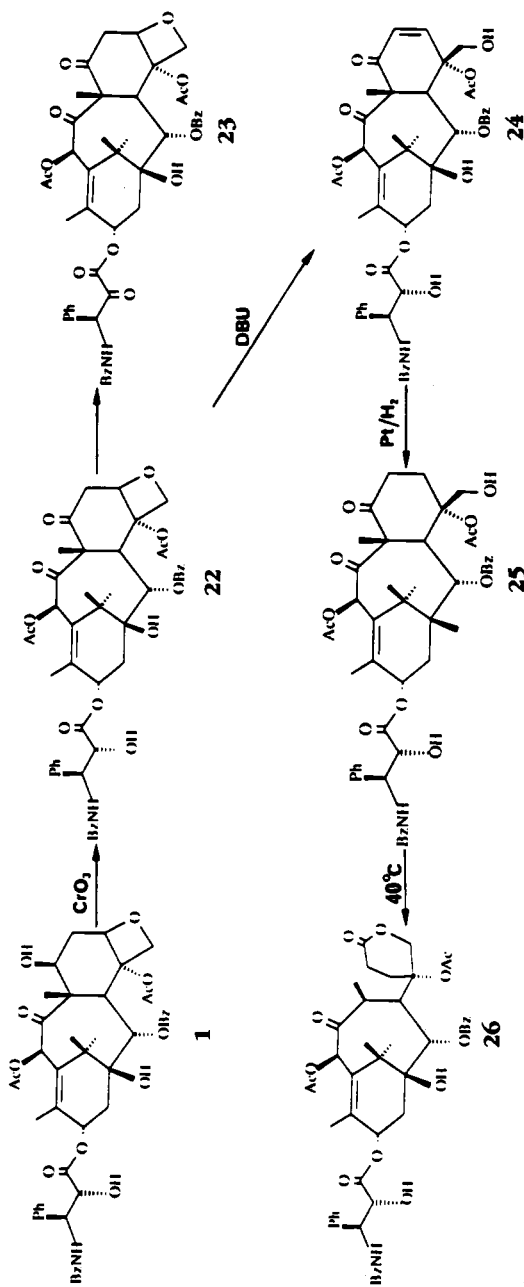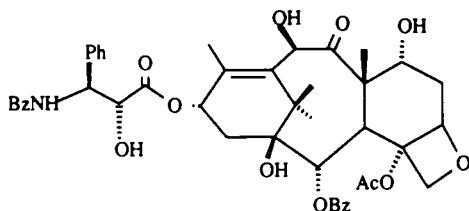
SCHEME 1

TABLE 3.   Biological Activity of Oxidized Taxols.

| Compound	ED_{50} (μg/ml) in KB cell culture
7-Oxotaxol {22}	5×10^{-1}
2',7-Dioxotaxol {23}	12.0
Lactone 26	2.0
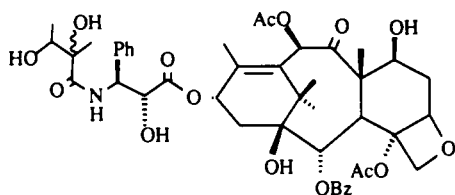
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The ^{1}H-nmr spectrum of the product showed no peaks due to ethyl groups, in spite of the fact that Meerwein's reagent is a powerful ethylating agent. The resonances of the C-5 proton at 3.88 ppm (d, J = 11.5 Hz) and 4.03 ppm (d, J = 11.5 Hz) indicated that the oxetane ring had been opened and that C-5 was substituted with a hydroxyl group, probably in the α orientation. All other resonances were similar to those of taxol, except for changes in the coupling constants of the A-ring protons which were also seen in the D-secotaxol 24. The spectroscopic evidence thus pointed to the diol structure 29 or the isomeric diol 30. Although structure 30 was favored at one time (23), convincing evidence in favor of structure 29 was provided by acetylation experiments. Acetylation of 29 under mild conditions (Ac₂O/DCC/DMAP, 65°) gave a 2',5,7-triacetate derivative. These experiments showed conclusively that the compound lacks an acetylatable primary alcohol function.

The formation of 29 presumably involves an initial ethylation of the oxetane ring by Meerwein's reagent, followed by attack by one of the neighboring ester carbonyl groups, re-ethylation of the resulting ether, and finally hydrolysis of the resulting dication on workup with migration of the acetyl group to the primary position.

In an earlier attempt to acetylate the Meerwein product 29 completely, and thus confirm the presence of a free hydroxyl group at the 5 position, we treated it with excess acetyl chloride and triethylamine at room temperature. Under these conditions the C-5 hydroxyl group was indeed acetylated (the resonance of H-5 shifting from 3.87 ppm to 5.27 ppm), but other and more deep-seated changes also occurred; most striking was the observation of a new pair of vinyl protons in place of one of the methyl groups at C-16 and -17. The same product was also formed directly from taxol on treatment with acetyl chloride under the same conditions.

A consideration of the spectroscopic data led quickly to the postulation of three pos-
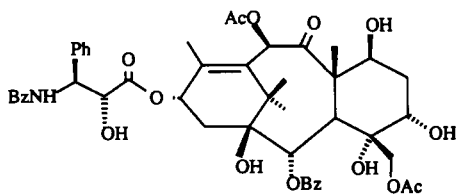

29
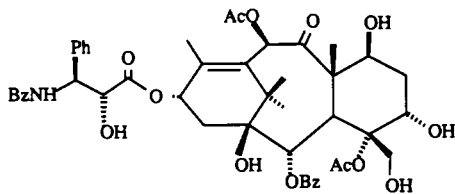
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sible rearranged structures for the new product (Scheme 2). Structures **31** and **32** could be formed from **29** by a 1,2-methyl migration followed by proton loss, while structure **33** could be formed by a 1,2 shift of the C-11, -15 bond followed by proton loss. Structure **32** was excluded because there was no detectable coupling between H-13 and the vinyl protons. Instead, H-13 retained a weak coupling with the vinyl methyl group (C-18), and an additional long range coupling was detected between a second methyl group and the vinyl protons.
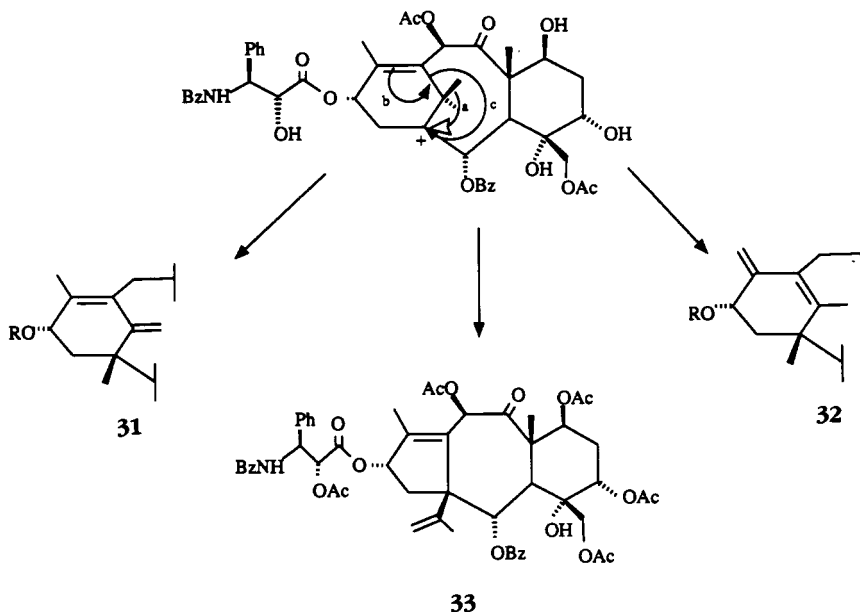
SCHEME 2

The coupling evidence thus favored structure **33**, but positive proof was lacking. We thus hydrogenated the compound (Pt/H$_2$) and looked for the characteristic signals of an isopropyl group. These were detected at 0.70 and 0.71 ppm (3H each) and 1.61 ppm (1H) and were confirmed as such by decoupling experiments. The rearrangement product is thus established as the isopropylidene derivative **33**. A similar rearrangement catalyzed by ZnBr$_2$ in toluene has been reported by Guéritte-Voegelein *et al.* (24) to give a product related to **31**, although the published evidence leaves open the possibility that the structure is in fact related to **33**.

The taxol derivatives **29** and **33** both show much reduced cytotoxicities as compared with taxol. The Meerwein product **29** has an ED$_{50}$ of 0.2 μg/ml in the KB assay, while the rearranged product **33** unsurprisingly has essentially no activity, with an ED$_{50}$ of 2.5 μg/ml. The fact that **29** is so much less active than taxol, even though it is not vastly different in structure, is particularly interesting. Part of the reason may be that the opening of the oxetane ring enables the remaining ring system to flex (as evidenced by the change in coupling constants of the ring A protons) and perhaps makes it unable to fit the binding site on tubulin.

PHOTOAFFINITY-LABELED TAXOL.—As discussed in the introduction, taxol has a unique mechanism of action in that it promotes the assembly of tubulin into microtubules (25). It does this by binding to the microtubule [but not apparently to unassembled tubulin (26)] in an approximately stoichiometric ratio with tubulin, suggest-

ing that there is a taxol binding site on assembled microtubules. The nature of this binding site is clearly of interest, and the technique of photoaffinity labeling offers the most attractive route to obtaining such information.

As a first step towards a study of the taxol-tubulin interaction by photoaffinity labeling, we have prepared a photoaffinity-labeled taxol. The selection of such a derivative requires an appropriate choice of both label and the site of attachment of the label to taxol.

Early studies of photoaffinity labeling were done largely with the α-diazo-β,β,β-trifluoropropionyl group introduced by Chowdhry and Westheimer (27), but this group requires irradiation by light with a wavelength of about 260 nm, which is energetic enough to degrade tubulin rather rapidly (28). The aryl azide photolabels that have been used by some workers are photolabile to light of suitable wavelength, but they generate the less reactive aryl nitrene on photolysis; this species does not undergo C-H insertion very effectively (29). We thus selected the diazirine group as a photolabile carbene-generating group, and made use of the synthesis of the azibenzoic acid [34] developed by Nassal (30).

Before preparing a taxol derivative containing the labile acid 34, we needed to ensure that the labeled taxol was still competent to assemble tubulin. We thus prepared 7-benzoyltaxol [35] by the pathway previously used to prepare other 7-substituted taxol derivatives, and tested its capability to assemble bovine brain tubulin. The tubulin was isolated in our laboratory by published procedures (31), and allowed to assemble and disassemble in the presence of various concentrations of taxol and of 7-benzoyltaxol. The rate of disassembly was determined as a function of drug concentration as described by Potier and co-workers (32) and used to calculate the concentration of drug required to decrease the depolymerization rate by 50% (ID_{50}). Satisfyingly, the ID_{50} of 7-benzoyltaxol (0.17 μM) was similar to that of taxol (0.1 μM), indicating that substitution of a bulky benzoyl group at the 7 position did not destroy the activity of taxol.

The desired photoaffinity-labeled 7-azibenzoyltaxol [36] was then prepared from azibenzoic acid [34] and taxol by the same methodology; its spectroscopic properties (particularly its ^{1}H-nmr spectrum) confirmed the assigned structure. On testing in the tubulin assembly-disassembly assay it gave a reduced but still excellent ID_{50} of 0.34 μM. This taxol derivative is thus appropriate for use as a photoaffinity label, and in future studies we plan to use it to probe the nature of the taxol binding site on microtubules.

35   R=Bz   36   R=
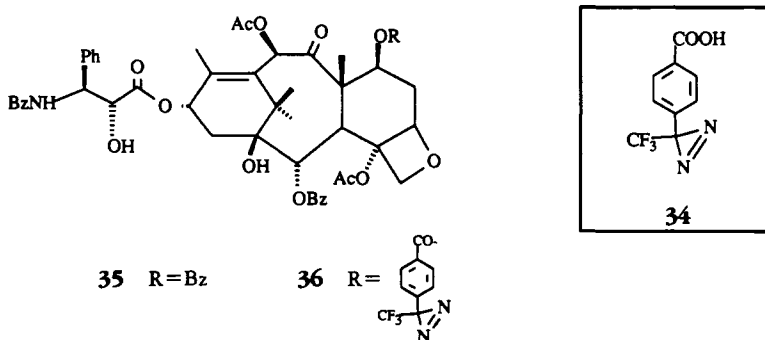
CONCLUSION.—This article has summarized the work that we have done on taxol over the last few years which has led to the development of some useful new derivatives as well as the discovery of some interesting rearrangements. The taxol molecule is however not yet fully investigated, and we expect that our future work will lead to the discovery of even more interesting and useful chemistry of this fascinating molecule.

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