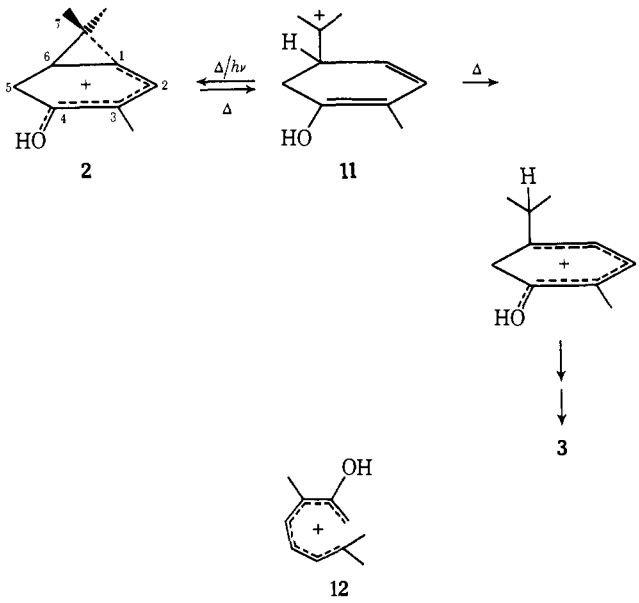

The formation of **2** photochemically from **1** could be thought of in terms of a concerted $[_{\sigma}2_a + {}_{\pi}2_a]$ cycloaddition reaction;[11] however, such a mechanism does not account for the formation of **3**.[15] An alternative pathway, which can account for the production of both **2** and **3**, is an electrocyclic ring-opening reaction that breaks the C_6C_7 bond of **1** to give the open-chain cation **12**. Such a process is directly comparable to that observed upon the photoisomerization of the isoelectronic cyclohexa-1,3-dienes to the bicyclo[3.1.0]-hexenes.[11,16] While the direct isomerization of **12** to give **2** is possible, in view of the constant ratio of **2** to **3** observed in these reactions, it is attractive to consider that a cation resembling **11** might be formed either photochemically, or thermally, from **12** and that this gives the observed products, **2** and **3**.

(15) A trans ring juncture would result from the alternative $[_{\sigma}2_s + {}_{\pi}2_a]$ cycloaddition reaction.

(16) J. Meinwald and P. H. Mazzocchi, *J. Amer. Chem. Soc.*, **88**, 2850 (1966).

(17) Holder of National Research Council of Canada Scholarship.

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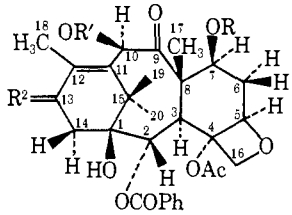
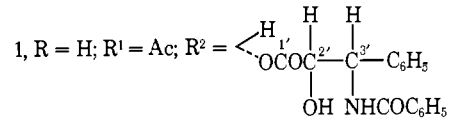
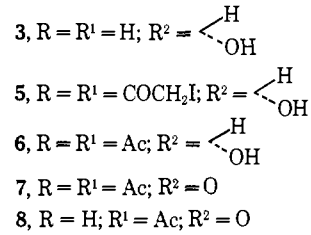
Plant Antitumor Agents. VI. The Isolation and Structure of Taxol, a Novel Antileukemic and Antitumor Agent from *Taxus brevifolia*[1,2]

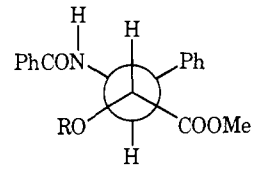
Sir:

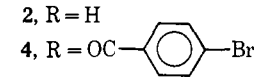
We wish to report on the structure of a novel compound named taxol (**1**), isolated from the stem bark of the western yew, *Taxus brevifolia*.[3,4] *Taxol has potent antileukemic and tumor inhibitory properties*[5] *and is the first compound possessing the taxane*[6] *ring which has been demonstrated to have such activity.*











The alcohol extract of the stem bark was concentrated and partitioned between water and chloroform. Guided by assay in 9KB and various leukemia systems, three successive chromatographies of the residue from the chloroform extract on Florisil, Sephadex LH-20, and silica gel followed by crystallization from aqueous methanol gave taxol (**1**) as needles:[7] yield 0.02%; M^+ at *m/e* 853, calcd for $C_{47}H_{51}NO_{14}$, 853; mp 213–216° dec; $[\alpha]^{20}D$ −49° (MeOH); λ_{max} (MeOH) 227 nm (ε 29,800), 273 (1700); ν_{max}^{Nujol} 3300–3500 (OH, NH), 1730 (ester), 1710 (ketone), 1650 (amide) cm^{-1}. The characteristic chemical shifts[8] of **1** are shown in Table I.

Because of the extremely limited quantity of taxol and its evident complexity, attempts were made to prepare derivatives suitable for X-ray analysis. Al-

(1) Previous paper in this series: M. C. Wani, J. A. Kepler, J. B. Thompson, M. E. Wall, and S. G. Levine, *Chem. Commun.*, 404 (1970).

(2) This investigation was conducted under Contract No. SA-43-ph-4322, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health. X-Ray investigations were carried out at Duke University and were supported by a Duke Endowment Grant.

(3) A preliminary report dealing only with the isolation of **1** was presented by M. E. Wall and M. C. Wani at the 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., 1967; Paper No. M-006.

(4) Taxol has been isolated from several other species of the Taxus genus, including *T. Cuspidata* and *T. baccata*. We thank Dr. Robert E. Perdue, New Crops Research Branch, Plant Inudstry Station, Beltsville, Md., for obtaining the plant material.

(5) Taxol shows confirmed activity [for description of bioassay procedures and leukemia and tumor systems, *cf. Cancer Chem. Rept.*, **25**, 1 (1962)] in L-1210, P-388, and P-1534 leukemias, being highly active in the latter two systems, is also highly active as an inhibitor of WM-256 carcinosarcoma, and shows considerable cytotoxicity in 9KB assay, $ED_{50} = 5.5 \times 10^{-5}$. Less pure concentrates containing taxol were also active in Sarcoma 180 and Lewis lung tumors.

(6) B. Lythgoe, K. Nakanishi, and S. Uyeo, *Proc. Chem. Soc.*, 301 (1964).

(7) All compounds reported in this communication have been characterized spectrally (ir, uv, nmr) and analytically (elemental and mass spectrum).

(8) Spectral assignments are based on the nmr spectra of taxane derivatives reported in the literature.[9-11]

(9) M. C. Woods, K. Nakanishi, and N. S. Bhacca, *Tetrahedron*, **22**, 243 (1966).

(10) I. W. Harrison, R. M. Scrowston, and B. Lythgoe, *J. Chem. Soc. C*, 1933 (1966).

(11) D. P. Della Casa de Marcano and T. G. Halsall, *Chem. Commun.*, 1382 (1970).

Table I. Nmr Data (δ Units, J Values in Hertz)[a]

Position	Compound 1	Compound Baccatin-V[b]	Compound 8
C-17 CH_3	1.14 (s)	1.04 (s)	1.20 (s)
C-19 CH_3	1.22 (s)	1.10 (s)	1.26 (s)
C-20 CH_3	1.67 (s)	1.62 (s)	1.66 (s)
C-18 CH_3	2.20 (s)	1.99 (s)	2.18 (s)
C-4 OAc	1.80 (s)		2.08 (s)
C-10 OAc	2.36 (s)		2.28 (s)
C-3 H	3.80 (d, J = 6)	4.02 (d, J = 6)	3.96 (d, J = 6)
C-16 2 H	4.24 (s)	4.38 (s)	4.16 (d, J = 6), 4.36 (d, J = 6)
C-5 H	4.92 (d, J = 10)	4.99 (m)	4.96 (broad d, J = 10)
C-2 H	5.68 (d, J = 6)	5.74 (d, J = 6)	5.71 (d, J = 6)
C-13 H	6.20 (broad t, J = 8)	6.18 (broad t, J = 8)[c]	
C-10 H	6.28 (s)	6.83 (s)	6.46 (s)

[a] s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet. [b] Reference 11. [c] In baccatin-V 13α-acetate.

though a number of crystalline halogenated compounds were obtained, none had properties suitable for X-ray analysis. Taxol was therefore subjected to a mild base-catalyzed methanolysis[12] at 0° yielding a nitrogen containing α-hydroxy methyl ester (**2**, $C_{17}H_{17}NO_4$), a tetraol (**3**, $C_{29}H_{36}O_{10}$), and methyl acetate (not isolated, eq 1).

$$\underset{\mathbf{1}}{C_{47}H_{51}NO_{14}} + 2CH_3OH \longrightarrow \underset{\mathbf{2}}{C_{17}H_{17}NO_4} + \underset{\mathbf{3}}{C_{29}H_{36}O_{10}} + CH_3CO_2CH_3 \quad (1)$$

The methyl ester (**2**) crystallized from chloroform as needles: $C_{17}H_{17}NO_4$ (combustion method) (mass spectrum m/e 281.1061, $C_{17}H_{15}NO_3$ (M − 18) = 281.1051); mp 183–185°; $[\alpha]^{23}D$ −49.6° (MeOH); λ_{max} (MeOH) 217 nm (ϵ 17,700); $\nu_{max}^{CHCl_3}$ 3515, 3455 (OH, NH), 1730 (ester CO), 1660 (amide CO), 1600 (aromatic C=C) cm^{-1}; nmr (100 MHz, $CDCl_3$, TMS) δ 3.26 (s, 1, OH), 3.74 (s, 3, ester Me), 4.56 (d, J = 2 Hz, 1, C-2′), 5.68 (q, J = 2, 10 Hz, 1, C-3′), 6.92 (d, J = 10 Hz, 1, NH), 7.22–7.76 (m, 10 aromatic). X-Ray analysis was performed on the *p*-bromobenzoate (**4**),[13] $C_{24}H_{20}BrNO_5$, mp 161–163°, which crystallized in the monoclinic system, space group C_2, with cell dimensions a = 22.91, b = 5.18, c = 19.66 Å, β = 99.80°, and Z = 4. The structure was solved by the heavy-atom method and the molecular parameters (anisotropic Br, isotropic C, N, and O) were refined by full-matrix least-squares calculations to the present R of 0.125 over 1772 observed reflections.

The tetraol (**3**) crystallized from methanol as needles: $C_{29}H_{36}O_{10}$ (combustion method) (mass spectrum m/e 526.2188, $C_{29}H_{34}O_9$ (M − 18) = 526.2203); mp 245–247°; $[\alpha]^{23}D$ −42.05° (MeOH); λ_{max} (MeOH) 227 nm (ϵ 14,700); ν_{max}^{KBr} 1680–1720 (ester and ketone C=O) cm^{-1}; nmr could not be obtained for lack of solubility in $CDCl_3$ or DMSO-d_6. X-Ray analysis was performed on the bisiodoacetate (**5**):[13] $C_{33}H_{38}O_{12}I_2$, mp 202–203° dec; nmr (100 Mz, $CDCl_3$, TMS) δ 3.72 (s, 2, C-7 iodoacetate CH_2), 3.82 (s, 2, C-10 iodoacetate CH_2), 6.20 (s, 1 H, C-10). The bisiodoacetate (**5**) crystallized in the orthorhombic system, space group $P2_12_12_1$, with cell dimensions a = 9.67, b = 15.34, c = 23.69 Å, and Z = 4. The structure solution was by the heavy-atom method, all nonhydrogen atoms being located in two successive Fourier syntheses. Positional and thermal parameters (anisotropic I, isotropic C and O) were refined by full-matrix least-squares calculations, and the present R is 0.142 for 2305 observed reflections.

It is interesting to note that Halsall and coworkers[11] have also recently reported the isolation of the diterpenoid baccatin-V, a naturally occurring oxetan similar to the tetraol (**3**) differing only in the configuration of the hydroxyl group at C-7. The reported chemical shifts of baccatin-V are reproduced in Table I for comparison with those of taxol.

Assuming that no rearrangement of **1** occurs under the mild methanolysis conditions,[14] the final structure of taxol requires the placement of the two hydrolyzed ester functions of **1** on the tetraol (**3**). Taxol could not be oxidized by carefully washed (neutral to litmus) and activated manganese dioxide[15,16] indicating that the two esters were located at the allylic positions 10 and 13. The chemical shifts of protons at C-10 and C-13 (*cf.* Table I) were also in accord with this observation. Oxidation of **1** with activated manganese dioxide[15] under mild basic conditions (pH of the aqueous suspension was 8) in acetone yielded compound **8** (mp 210–212°). The molecular composition (mass spectrum m/e 584.2266, $C_{31}H_{36}O_{11}$ = 584.2257) of **8** suggested that it was formed by the loss of the nitrogen containing α-hydroxy ester function and oxidation of the liberated allylic hydroxyl group. Several independent lines indicate that the hydrolyzed ester function was at C-13. The ultraviolet (λ_{max} (MeOH) 272 nm (ϵ 4800)) and infrared spectra (ν_{max} ($CHCl_3$) 1680 cm^{-1}) of **8** are in complete accord with this structure and rule out the alternative Δ^{11}-9,10-dioxo formulation.[10] Secondly, the nmr spectrum of **8** (*cf.* Table I) clearly shows the presence of a singlet due to the C-10 proton at δ 6.46 as required by formulation **8**.

Bioassay data[17] indicate that the methyl ester (**2**) is inactive and the tetraol (**3**) only 0.001th as active as taxol. It is interesting to speculate that the activity of taxol may to some extent be due to the easily cleaved allylic α-hydroxy ester function at C-13 which may be capable of acting as a leaving group under physiological conditions.

Acknowledgments. We wish to thank Drs. S. G. Levine, North Carolina State University, and J. A. Kepler for helpful discussions and Mr. J. B. Thompson and Mrs. L. W. Ambrosio for expert technical assistance. We wish also to thank Dr. David Rosenthal and Mr. Fred Williams of the Research Triangle Center for Mass Spectrometry for mass spectral data. Their work was supported by the Biotechnology Resources

(12) G. Zemplen, A. Gerecs, and I. Hadacsy, *Ber.*, **69**, 1827 (1936).

(13) The absolute configurations of **4** and **5** were established using the anomalous scattering of the halogen atoms and they are as represented. Full details of the X-ray analysis will be reported elsewhere.

(14) This is a reasonable assumption in view of the fact that the methanolysis of taxinine gives dideacetyltaxinine which gives taxinine upon reacetylation (*cf.* M. Kurono, Y. Nakadaira, S. Onuma, K. Sasaki, and K. Nakanishi, *Tetrahedron Lett.*, 2153 (1963)).

(15) I. M. Goldman, *J. Org. Chem.*, **34**, 1979 (1969).

(16) Under the same conditions the 7,10-diacetate (**6**, $C_{33}H_{40}O_{12}$, mp 225–227°, obtained from **3**) was smoothly oxidized to the corresponding conjugated ketone (**7**, $C_{33}H_{38}O_{12}$, mp 236–238°).

(17) The cytotoxicities of **1**, **2**, and **3** have been determined and have ED_{50} values 5.5×10^{-5}, 1.0×10^{2}, and 2.2×10^{-2}, respectively.

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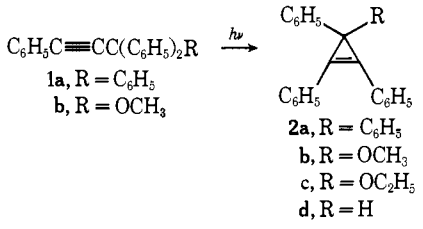
Photocyclization of Aryl-Substituted Acetylenes; Application of Di-π-methane-like Rearrangements to Arylcyclopropene Syntheses[1]

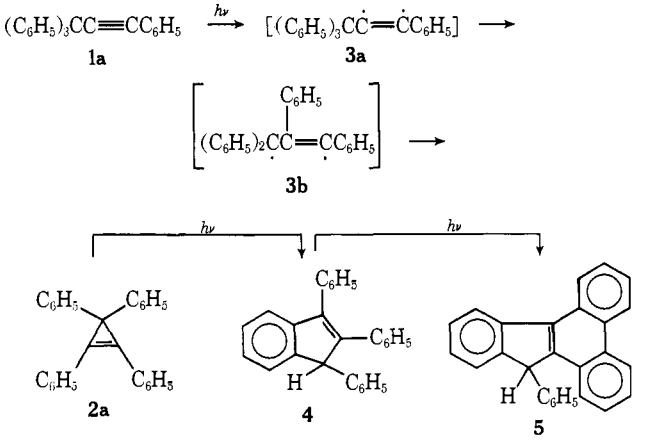
Sir:

In a continuation of previous studies on the photocyclization of aryl-substituted propenes[2] and related functionalized propenyl systems, such as 3-alkoxy-substituted propenes,[3] we have investigated the photochemistry of several aryl-substituted acetylenes with the intent of developing a convenient route to cyclopropenes including functionalized systems which in turn might serve as precursors for cyclopropenium derivatives.[4] Our continuing interest in the photochemistry of cyclopropenes[5a-d] provided an additional incentive for this study.



Tetraphenylpropyne (**1a**) was synthesized by a procedure[6a] which is a modification of the Wieland and Closs method.[6b] Irradiation of **1a** (500 mg) in 500 ml of cyclohexane (0.003 *M*) at 253.7 nm for relatively short periods (3.4 hr) in a preparative photochemical reactor[7a] while simultaneously sparging with a slow stream of argon provided 230 mg (46%) of **2a**, mp 177.5–9° (lit.[5b] mp 176–178°) which may be isolated by elution chromatography on alumina.

For convenience the mechanism for the rearrangement of **1a** is formulated as a diradical process as outlined below.[4]



The formation of **2a** indicates that the intermediate, perhaps **3b**, formed by rearrangement of a diradical-like intermediate such as **3a** cyclizes at least in part in a process analogous to the photochemical rearrangement of propenes to cyclopropanes.[2,3] Alternatively, and perhaps more likely, the reaction proceeds in a concerted manner.[8]

Additional photoproducts begin to form when the irradiation time is extended as evidenced by the appearance of nmr signals at τ 4.94 and 4.67 ($CDCl_3$), characteristic of the benzyl protons of **4** and **5**, respectively.[5a,b] An nmr study[9a] of the variation in product composition as a function of time under standard irradiation conditions in benzene[7b] proved instructive. The quantitative data summarized in Table I were

Table I

Time, hr	% 2a	4	5
24	25	42	19
4	46	35	9
1	70	Trace	Trace

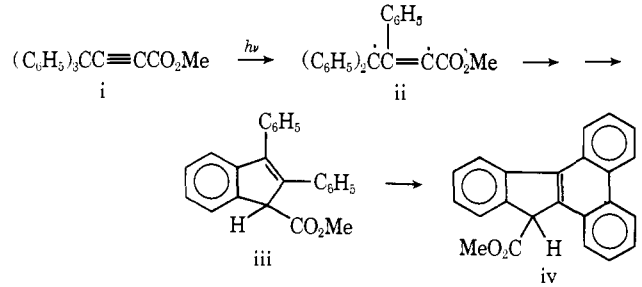
determined utilizing the four-proton aromatic multiplet for **2a** centered at τ 2.30 ($CDCl_3$) in addition to the singlets for the benzyl protons of **4** and **5**. Although these data confirm that the photolysis of **1a** in cyclohexane[7b] proceeds in high conversion (~80%) upon

(1) A preliminary report of this work was presented at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970.

(2) G. W. Griffin, A. F. Marcantonio, H. Kristinsson, R. C. Petterson, and C. S. Irving, *Tetrahedron Lett.*, 2951 (1965).

(3) J. J. Brophy and G. W. Griffin, *ibid.*, 493 (1970).

(4) Recently, J. W. Wilson and K. L. Huhtanen (*Chem. Commun.*, 454 (1968)) described the photochemical rearrangement of methyl 3,3,3-triphenylpropyne carboxylate (i) to the indenophenanthrene (iv). The diradical species ii was proposed as a possible intermediate leading to the indene iii which in turn undergoes subsequent dehydrocyclization to iv. A similar diradical species has been postulated by us as an intermediate in the thermal[5a] and photochemical[5b] rearrangement of tetraphenylcyclopropene (**2a**) to triphenylindene **4**.



(5) (a M. A. Battiste, B. Halton, and R. H. Grubbs, *Chem. Commun.*, 907 (1967); (b) R. H. Grubbs, M.S. Thesis, University of Florida, 1965; (c) H. Kristinsson and G. W. Griffin, *J. Amer. Chem. Soc.*, **88**, 1579 (1966); (d) H. Kristinsson, *Tetrahedron Lett.*, 2343 (1966); (e) A. S. Monahan, J. D. Freilich, and J. J. Fong, *ibid.*, 1865 (1970); (f) H. Dürr, *Justus Liebigs Ann. Chem.*, **723**, 102 (1969).

(6) (a) A. W. Herriot, Ph.D. Thesis, University of Florida, 1967; (b) H. Wieland and H. Closs, *Justus Liebigs Ann. Chem.*, **470**, 201 (1929).

(7) (a) The preparative irradiations were conducted in a Rayonet RPR 208 Reactor (The Southern New England Ultraviolet Co., Middletown, Conn.) with 8 RUL 253.7-nm lamps; (b) an RPR-100 unit equipped with 16 8-W 253.7-nm lamps; (c) an RPR-100 unit fitted with 16 8-W 310.0- or 350.0-nm lamps.

(8) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 781 (1969).

(9) (a) All nmr spectra were recorded on a Varian A-60 instrument in the solvent indicated with tetramethylsilane as an internal standard; (b) all new compounds gave satisfactory combustion and/or mass spectral analyses; (c) mass spectral data were obtained on a Perkin-Elmer Hitachi RMU-6 spectrometer.