Taxodione and Taxodone, Two Novel Diterpenoid Quinone Methide Tumor Inhibitors from *Taxodium distichum*[1,2]

Sir:

In the course of a continuing search for tumor inhibitors of plant origin, extracts of *Taxodium distichum* Rich (*Taxodiaceae*)[3] showed significant activity *in vivo* against the Walker intramuscular carcinosarcoma 256 in rats and *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[4] We report herein the isolation and structural elucidation of two novel diterpenoid quinone methides, taxodione (**1**) and taxodone (**5**); each showed significant inhibitory activity against the Walker carcinosarcoma 256 in rats, at 40 and 25 mg/kg,[4] respectively.

Fractionation of the chloroform extract, guided by assay against KB, revealed that the cytotoxic activity was concentrated in the methanol layer of a 10% aqueous methanol–petroleum ether partition. Further fractionation involving silica gel chromatography yielded six crystalline compounds listed in order of decreasing R_f in Table I.

Table I

Compound	Formula	M$^+$ at *m/e*[a]	Mp, °C	$[\alpha]^{28}$D, deg	Cytotoxicity ED_{50}, μg/ml
Royleanone (**10**)	$C_{20}H_{28}O_3$	316	179–181	+134	80
Taxodione (**1**)	$C_{20}H_{26}O_3$	314	115–116	+56	3
Taxoquinone (**11**)	$C_{20}H_{28}O_4$	332	212–214	+340	73
Taxodone (**5**)	$C_{20}H_{28}O_3$	316	164–165	+50	0.6
Sugiol (**8**)	$C_{20}H_{28}O_2$	300	292–294	+26	>100
Δ^5-Dehydrosugiol (**13**)	$C_{20}H_{26}O_2$	298	284–286	+13	>100

[a] We thank Dr. R. D. Brown and Dr. F. W. McLafferty of Purdue Mass Spectrometry Center, supported under U. S. Public Health Service Grant FR-00354, for the mass spectral data.

Taxodione (**1**, golden plates) showed λ_{max}^{MeOH} 320, 332, 400 mμ (ε 25,000, 26,000, 2000); $\lambda_{max}^{CCl_4}$ 2.99, 5.95, 6.07, 6.13–6.17 (d), 6.23, 10.99 μ; τ (benzene-d_6) 2.30 (1 H, s, 11-O*H*), 3.58 (1 H, s, 14-*H*), 4.13 (1 H, s, 7-*H*), 7.05 (2 H, 1 H, septet, J = 7 cps, 15-*H* + 1 H, m), 7.68 (1 H, s, 5-*H*), 8.62, 8.75, 8.82 (9 H, s, three CH_3), 8.96, 9.03 (6 H, d, J = 7 cps, 16- and 17-CH_3).

Taxodone (**5**, yellow plates) showed λ_{max}^{MeOH} 316 mμ (ε 20,000); $\lambda_{max}^{CCl_4}$ 2.77, 2.86, 3.00, 6.14–6.18 (d), 6.34, 10.96 μ; τ ($CDCl_3$) 2.51 (1 H, s, 11-O*H*), 3.19 (1 H, s, 14-*H*), 3.45 (1 H, d, J = 2.5 cps, 7-*H*; collapsible to a singlet upon irradiation at τ 5.31), 5.30 (1 H, m, half-width = 23 cps, 6-β-axial H), 7.00 (2 H, 1 H, septet, J = 7 cps, 15-*H* + 1 H, m), 8.78, 8.83, 8.90 (15 H, five CH_3).

Catalytic hydrogenation of taxodione (**1**) (Scheme I) gave colorless dihydrotaxodione (**2**, M$^+$ *m/e* 316): mp 176–177°; λ_{max}^{MeOH} 283 mμ (ε 1000); $\lambda_{max}^{CHCl_3}$ 5.82 μ; τ ($CDCl_3$) 3.60 (1 H, s, 14-*H*), 3.97, 4.55 (2 H, s, 11- and 12-O*H*), 7.35 (1 H, s, 5-*H*). Taxodone (**5**) upon treatment with methanolic HCl also gave **2**, which was readily converted to taxodione (**1**) by aerial oxidation.

Methylation of **2** yielded **3** ($C_{22}H_{32}O_3$, M$^+$ *m/e* 344): mp 117–118°; $[\alpha]^{28}$D +100° ($CHCl_3$); $\lambda_{max}^{CCl_4}$ 5.80 μ; τ ($CDCl_3$) 6.15, 6.21 (6 H, s, 11- and 12-OCH_3), 8.64 (3 H, s, 10-CH_3). Reduction of **3** with $LiAlH_4$ furnished alcohol **4** [$C_{22}H_{34}O_3$, M$^+$ *m/e* 346: mp 107–108°; $[\alpha]^{28}$D +33° ($CHCl_3$); τ ($CDCl_3$) 5.37 (1 H, m, half-width 8 cps, 6-α-equatorial *H*), 8.31 (3 H, s, 10-CH_3)] which was dehydrated with $POCl_3$ to **6** [$C_{22}H_{32}O_2$, M$^+$ *m/e* 328; mp 88–89°; τ ($CDCl_3$) 4.13 (1 H, t, J = 4 cps, 6-*H*), 8.53 (3 H, s, 10-CH_3)]. Catalytic hydrogenation of **6** gave 11-methoxyferruginol methyl ether (**7**), characterized by direct comparison with a sample prepared from sugiol (**8**) by the procedure of Wenkert, *et al.*[5,6]

Royleanone (**10**) was characterized by direct comparison with an authentic sample.[7] Sugiol (**8**) was identified by comparison of its physical and spectral properties with those recorded earlier.[9] Taxoquinone (**11**) [λ_{max}^{MeOH} 276, 408 mμ (ε 12,000, 800); $\lambda_{max}^{CHCl_3}$ 2.80, 2.95, 5.97, 6.05, 6.15, 6.25 μ; τ ($CDCl_3$) 5.20 (1 H, t, 7-*H*), 6.90 (1 H, septet, J = 7 cps, 15-*H*), 8.65, 8.73, 8.85 (9 H, s, three CH_3), 9.05 (6 H, s, two CH_3)] was converted to royleanone (**10**), upon hydrogenation followed by aerial

(1) Tumor Inhibitors. XXXIV. Part XXXIII: S. M. Kupchan, C. W. Sigel, R. J. Hemingway, J. R. Knox, and M. S. Udayamurthy, *Tetrahedron*, in press.

(2) Supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275), and a contract with the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, National Institutes of Health (PH 43-64-551).

(3) Seeds collected in Maryland, Oct 1966. The authors acknowledge with thanks the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the U. S. Department of Agriculture by CCNSC.

(4) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the CCNSC by procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(5) C. H. Brieskorn, A. Fuchs, J. B. Bredenburg, J. D. McChesney, and E. Wenkert, *J. Org. Chem.*, **29**, 2293 (1964).

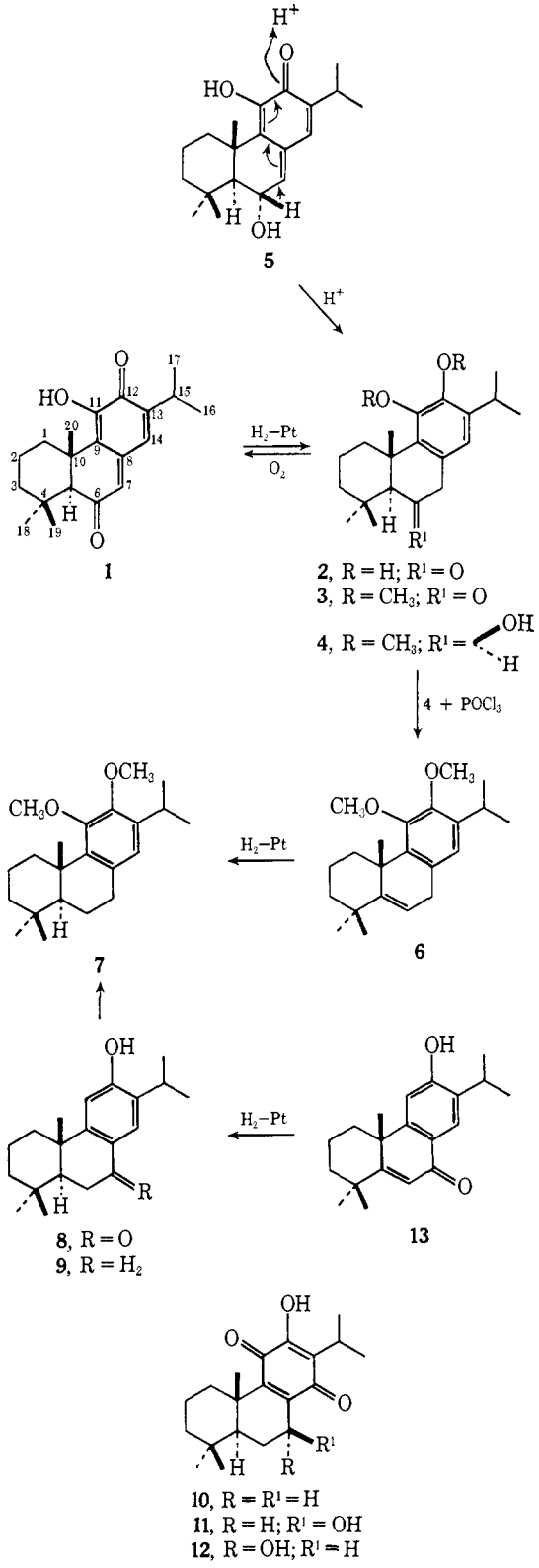
(6) Assignment of 6β-axial configuration to the hydroxyl group in **4** is supported by the downfield shift of the C-10 methyl group signal (Δτ 0.33) relative to the corresponding signal for **3** [*cf.* R. L. Clarke, S. J. Daum, P. E. Shaw, and R. K. Kullnig, *J. Am. Chem. Soc.*, **88**, 5865 (1966)]. Hydride reduction of a hindered 6-ketone to the β-axial alcohol has been reported earlier by J. S. E. Holker (in "Terpenoids in Plants," J. B. Pridham, Ed., Academic Press, London, 1967, pp 34–35). Assignment of α-axial configuration to the C-5 proton in taxodione, taxodone, and the related compounds is supported by the facile dehydration of **4** to **6** and the failure of ketone **3** to epimerize under acid equilibrating conditions known to effect isomerization of 3β-acetoxy-4,4-dimethyl-5β-androstan-6-one [T. G. Halsall, E. R. H. Jones, E. L. Tan, and G. R. Chaudhry, *J. Chem. Soc.*, 1374 (1966)].

(7) O. E. Edwards, G. Feniak, and M. Los, *Can. J. Chem.*, **40**, 1540 (1962). We thank Dr. O. E. Edwards cordially for authentic samples of royleanone and hydroxyroyleanone ("horminone"[8]).

(8) M. Janot and P. Potier, *Ann. Pharm. Franc.*, **22**, 387 (1964).

(9) T. Sengupta, S. N. Choudhuri, and H. N. Khastgir, *Tetrahedron*, **10**, 45 (1960).

Scheme I



oxidation. Taxoquinone thus differs from horminone[8] (**12**) only in the configuration of the hydroxy group. In the nmr spectrum of taxoquinone (**11**) the 7-proton signal is a triplet centered at τ 5.20 with a splitting of 7 cps, indicative of an α-axial proton, while the corresponding broad signal for horminone (τ 5.23, half-width 8 cps) indicates a β-equatorial 7-proton.[10] The structure of Δ^5-dehydrosugiol (**13**) [λ_{max}^{MeOH} 246, 313 mμ (ϵ 22,000, 13,200); λ_{max}^{KBr} 6.10, 6.19, 6.31, 6.39, 6.65 μ; τ (pyridine-d_5) 1.55 (1 H, s, 7-*H*), 2.70 (1 H, s, 11-*H*), 3.32 (1 H, s, 6-*H*), 3.50 (1 H, br s, 12-O*H*), 6.33 (1 H, septet 15-*H*), 8.52, 8.65, 8.78, 8.82 (15 H, five C*H*$_3$)] was established by conversion to ferruginol (**9**) upon catalytic hydrogenation.

The observed growth-inhibitory activity of taxodione (**1**) and taxodone (**5**) confirms and extends an earlier report of antitumor activity of quinone methides.[11]

Investigations are in progress to determine the significance of the reactive quinone methide and of other structural features in relation to the tumor-inhibitory activity of taxodione and taxodone.

(10) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, pp 77–83.

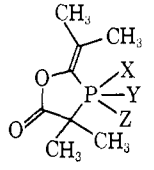
(11) E. Schwenk, *Arzneimittel-Forsch.*, **12**, 1143 (1962).

S. Morris Kupchan, Aziz Karim, Carol Marcks
Department of Pharmaceutical Chemistry
University of Wisconsin, Madison, Wisconsin 53706
Received August 12, 1968

A New Phosphorane Obtained from Dimethylketene

Sir:

The synthesis and intermediacy of pentacovalent compounds of phosphorus (phosphoranes) have received considerable attention in the past few years.[1,2] We report here a new series of cyclic phosphoranes, I,



Ia, X, Y, Z = OCH_3
Ib, X, Y = OCH_3; Z = $N(CH_3)_2$
Ic, X = OCH_3; Y, Z = $-OCH_2-CH_2O-$
Id, X = OCH_3; Y, Z = $-OCH_2-CH_2N(CH_3)-$
Ie, X = $N(CH_3)_2$; Y, Z = $-OCH_2CH_2O-$
If, X = $N(CH_3)_2$; Y, Z = $-OCH_2CH_2N(CH_3)-$
Ig, X = OCH_3; Y, Z = $-(CH_3)-NCH_2CH_2N(CH_3)-$

from the smooth reaction of dimethylketene with trivalent phosphorus derivatives.[3] These 2:1 adducts contain the vinyl ester functionality as part of the ring system, a structural feature not accessible by other methods. In addition, these phosphoranes undergo a variety of clean and characteristic reactions. Preparation of the phosphorane Ia is achieved by addition of a solution of dimethylketene to trimethyl phosphite cooled to Dry Ice temperature followed by removal of volatile materials under vacuum yielding quantitative

(1) For reviews, see (a) F. Ramirez, *Accounts Chem. Res.*, **1**, 168 (1968); (b) F. H. Westheimer, *ibid.*, **1**, 70 (1968).

(2) For recent work, see: (a) D. B. Denney, D. Z. Denney, and L. A. Wilson, *Tetrahedron Letters*, 85 (1968); (b) W. Hawes and S. Trippett, *Chem. Commun.*, 295 (1968); (c) A. N. Hughes and S. Uaboonkul, *Tetrahedron*, **24**, 3437 (1968); (d) B. Akermark, *Acta Chem. Scand.*, **21**, 584 (1967); (e) J. Wulff and R. Huisgen, *Angew. Chem. Intern. Ed. Engl.*, **6**, 457 (1967); (f) B. A. Arbuzov, N. A. Polezhaeva, and V. S. Vinogradova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 2281 (1967); (g) F. Ramirez, M. Nagabhushanam, and C. P. Smith, *Tetrahedron*, **24**, 1785 (1968).

(3) The reaction of diphenylketene with triethyl phosphite was reported[4] to yield a 2:1 adduct for which the structure was not determined. It is possible that the structure of this adduct is analogous to that proposed for I.

(4) T. Mukaiyama, H. Nambu, and M. Okamoto, *J. Org. Chem.*, **27**, 3651 (1962).