Novel Antitumor Agents CI-920, PD 113270, and PD 113271. 3. Structure Determination[1]

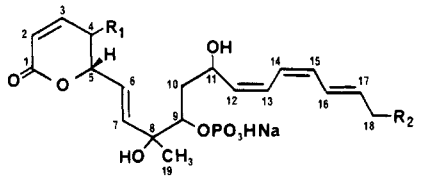
Gerard C. Hokanson* and James C. French

Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, Michigan 48105

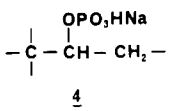
Received July 19, 1984

The chemical structures for the novel phosphate-containing antitumor agents CI-920 (1), PD 113270 (2), and PD 113271 (3) were determined by a combination of spectral and chemical means. Extensive analysis of NMR spectral data allowed tentative structures to be assigned for these compounds and their derivatives. These structures were confirmed by reducing CI-920 to 8-methyl-1-octadecanol and by cleaving dephosphorylated CI-920 (7) with sodium periodate to identified products.

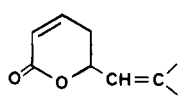
In the course of our screening program for new antitumor agents, a new actinomycete was found that produces a complex of novel compounds possessing good antileukemic activity in mice. A description of the producing organism, identified as a subspecies of *Streptomyces pulveraceous*, and the fermentation conditions used for the production of the complex have been reported.[2] The isolation and chemical properties,[3] as well as the antitumor activity[4] of the major components of the complex have also been described. In the present paper, the structure elucidation of three of these interesting new compounds, CI-920 (1), PD 113270 (2), and PD 113271 (3), will be discussed in detail.



1 $R_1 = H$, $R_2 = OH$
2 $R_1 = R_2 = H$
3 $R_1 = R_2 = OH$



4



5

Preliminary chemical and spectral data for 1–3 revealed that all three compounds were phosphate monoesters containing a conjugated triene moiety.[3] The FAB mass spectrum of CI-920 (1) suggested a molecular weight for the monosodium salt of 452 which, together with elemental analysis and ^1H and ^{13}C NMR spectral information, leads to a molecular formula of $C_{19}H_{26}O_9PNa$. Comparison of the corresponding data for analogues 2 and 3 (Tables I and II) indicates that the three isolated compounds are di-, tri-, and tetrahydroxy derivatives of the same C_{19} parent compound (vide infra). The infrared spectra of these components are nearly identical, displaying a prominent peak at 1710 cm^{-1} which was shown to be due to the presence of an α,β-unsaturated δ-lactone.

Table I. ^{13}C NMR Data for CI-920 (I) and Analogues[a]

C	1	2	3	7
1	170.47, s	170.56, s	169.66, s	170.50, s
2	122.26, d	122.41, d	124.40, d	122.38, d
3	151.89, d	152.01, d	142.27, d	151.89, d
4	31.89, t	31.91, t	64.68, d	31.91, t
5	81.65, d	81.64, d	84.23, d	81.54, d
6	149.46,[b] d	140.42,[b] d	142.27,[b] d	140.44,[b] d
7	137.26,[b] d	136.67,[b] d	137.23,[b] d	137.41,[b] d
8	77.91, d[d]	77.87, d[d]	78.28, d[d]	77.87, s
9	80.55, dd[e]	80.87, dd[e]	79.80, dd[e]	76.41, d
10	41.46, dt[f]	41.85, dt[f]	41.60, dt[f]	41.42, t
11	66.78, d	66.85, d	66.67, d	67.25, d
12	136.87, d	135.94, d	136.87, d	137.31, d
13	127.07, d	127.24, d	126.91, d	126.98, d
14	126.85, d	129.90,[c] d	126.21, d	126.77, d
15	133.34, d	134.66, d	133.36, d	133.56, d
16	128.90, d	129.47,[c] d	127.18, d	128.85, d
17	129.77, d	124.45, d	128.99, d	129.77, d
18	64.93, t	20.70, q	64.90, t	64.89, t
19	24.78, q	25.06, q	24.09, q	24.81, q

[a] Recorded at 90.5 MHz for solutions in D_2O. Chemical shifts are indicated in ppm downfield from Me_4Si. Multiplicities were determined from the proton noise-decoupled spectra and from spectra recorded with the *J*-modulated spin-echo technique. Assignments are based upon chemical shift arguments and selective proton-decoupled spectra. [b,c] Indicated assignments may be reversed. [d] $^3J_{cp} = 2.9$ Hz. [e] $^3J_{cp} = 5.6$ Hz. [f] $^3J_{cp} = 3.0$ Hz.

The ^{13}C NMR spectrum of CI-920 (Table I) exhibits signals for the expected 19 carbon atoms. Notably, close inspection of the fully proton-decoupled ^{13}C spectrum reveals that the signals at 80.55, 77.91, and 41.46 ppm are finely split, showing coupling constants of 5.6, 2.9 and 3.0 Hz, respectively. The magnitude of these couplings is indicative of two- and three-bond couplings of carbon atoms to phosphorus.[5] Consideration of the multiplicities and chemical shifts of these signals defines the structural fragment 4.

Analysis of the 360-MHz ^1H NMR spectrum of CI-920 reveals signals for 22 nonexchangeable protons (Table II). Signals at 6.01 and 7.09 ppm are assigned to the α- and β-protons, respectively, of the α,β-unsaturated lactone. Spin-decoupling experiments show that these olefinic protons are coupled to a pair of nonequivalent allylic methylene protons (signals centered at 2.51 and 2.62 ppm) which in turn are coupled to a methine proton exhibiting a signal at 5.1 ppm. The latter proton is further coupled to an olefinic proton obscured in a three-proton cluster of signals at 5.86–5.98 ppm. The chemical shifts and coupling pattern for the above series of protons are in close agreement with the ^1H NMR properties reported[6-8] for a group

(1) Presented in part at the 186th National Meeting of the American Chemical Society, Washington, D.C., September 3, 1983.
(2) Tunac, J. B.; Graham, B. D.; Dobson, W. E. *J. Antibiot.* **1983**, *36*, 1595.
(3) Stampwala, S. S.; Bunge, R. H.; Hurley, T. R.; Willmer, N. E.; Brankiewicz, A. J.; Steinman, C. E.; Smitka, T. A.; French, J. C. *J. Antibiot.* **1983**, *36*, 1601.
(4) Leopold, W. R.; Shillis, J. L.; Nelson, J. M.; Roberts, B. J. *Cancer Res.* **1984**, *44*, 1928.
(5) Isaac, R. E.; Rose, M. E.; Rees, H. H.; Goodwin, T. W. *J. Chem. Soc., Chem. Commun.* **1982**, 249.
(6) Hlubucek, J. R.; Robertson, A. V. *Aust. J. Chem.* **1967**, *20*, 2199.

0022-3263/85/1950-0462$01.50/0

Table II. 360-MHz ^{1}H NMR Data for CI-920 and Analogues[a]

H	1	2	3
2	6.01, ddd (9, 2.5, 1)	6.01, ddd	6.17, d (10)
3	7.09, ddd (9, 5, 2.5)	7.10, ddd	7.10, dd (10, 6.3)
4A	2.51, dddd (18, 10, 2.5, 2.5)	2.51, dddd	4.34, dd (6.3, 2.7)
4B	2.62, dddd (18, 5, 5, 1)	2.61, dddd	
5	5.11, ddd (10, 5, 5)	5.11, ddd	5.09, dd (6.3, 2.7)
6	5.86–5.98, m	5.83–5.99, m	5.93, dd (16, 6.3)
7	5.86–5.98, m	5.83–5.99, m	6.02, d (16)
9	4.17, m	4.16, ddd[b]	4.17, m
10A	1.58, ddd (14, 11, 2.7)	1.58, ddd	1.59, ddd
10B	1.72, br t[c]	1.74, m	1.71, br t[c]
11	4.92, br t	4.90, br t	4.93, br t
12	5.55, t (10.5)	5.49, t	5.55, t
13	6.57, t (11)	6.55, t	6.56, t
14	6.34, t (11)	6.18, t	6.37, t
15	6.16, t (11)	6.10, t	6.16, t
16	6.86, ddd (15, 11, 1)	6.59, dd	6.77, dd
17	5.86–5.98, m	5.83–5.99, m	5.88–5.99, m
18	4.17, dd (5.5, 1)	1.79, d (7)	4.17, d
19	1.33, s	1.32, s	1.30, s

[a] Recorded for solutions in D_2O. Chemical shifts are listed in ppm downfield from TSP. Coupling constants are indicated in Hz. [b] $J_{9,10A}$ = 10; $J_{9,10B}$ = 2.7; $J_{9,P}$ = 10. [c] Coupling constants obscurred by fine splitting due to long-range coupling to phosphorus.

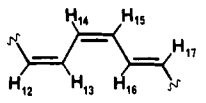
Table III. 360-MHz ^{1}H NMR Data for Certain CI-920 Derivatives[a]

H	7 (D_2O)	8 (D_2O)	10 ($CDCl_3$)
2	6.12, ddd	6.02, ddd (10.3, 2.7, 1.3)	6.11, br d
3	7.15, ddd	7.10, ddd	6.95, m
4A	2.57, dddd	2.48, dddd	2.48, m
4B	2.67, dddd	2.61, dddd	2.61, m
5	5.13, dddd (10.7, 5.5, 5.5, 0.9)	5.10, ddd	5.06, m
6	6.00, dd (15.6, 6.1)	6.05, dd (16, 5.5)	5.88–5.99, m
7	6.11, dd (15.6, 0.9)	5.92, d (16)	5.88–5.99, m
9	3.81, dd (10.3, 2.1)	4.23, t (7)	5.06, m
10A	1.47, ddd (14.3, 10.3, 2.7)	1.95, t (7)	1.89, t
10B	1.87, ddd (14.3, 10, 2.1)		
11	4.95, ddd (10, 10, 2.7)	5.74, ddd (7, 7, 7)	5.75, ddd
12	5.65, t	5.51, t	5.47, t
13	6.61, t	6.64, t	6.57, t
14	6.44, t	6.39, t	6.44, t
15	6.20, t	6.19, t	6.17, t
16	6.89, ddd	6.81, dd	6.77, dd
17	6.00, ddd (15, 5.5, 5.5)	5.91, ddd (15.1, 6.3, 6.3)	5.88–5.99, m
18	4.27, dd (5.5, 1)	4.65, d (6.3)	4.69, d
19	1.40, s	1.40, s	1.34, s
OAc		2.07, s; 2.09, s	2.07, s; 2.13, s; 2.14, s

[a] Chemical shifts are listed in ppm downfield from either TSP or Me_4Si. Coupling constants are indicated in Hz.

of naturally occurring 5,6-dihydropyran-2-one derivatives, substituted at C-5 with an unsaturated side chain, suggesting the second partial structure **5**.

The conjugated triene fragment of **1** is clearly observed in the downfield region of the ^{1}H NMR spectrum. Homonuclear spin-decoupling experiments, beginning with the proton signal at 5.55 ppm and working through the triene to a signal buried in the three-proton multiplet at 5.86–5.98 ppm, allowed the assignment of proton connectivity shown in partial structure **6**. The configuration



6

of the triene system was established as *Z,Z,E* on the basis of first order analysis of coupling constants for the five well-resolved signals. Consistent with model trienes reported in the literature,[9] triene protons (e.g., H-14 at 6.34 ppm) with vicinal single bond and cis couplings (J_{vic} = J_{cis} = 10–11 Hz) appear as apparent triplets, whereas the one observable proton (H-16 at 6.86 ppm) with vicinal and trans couplings (J_{vic} = 11, J_{trans} = 15) is seen as a doublet of doublets.

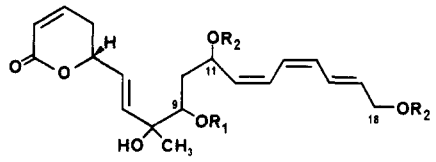
Close inspection of a three-proton multiplet centered at 4.17 ppm in the ^{1}H NMR spectrum of CI-920 suggested the presence of a finely split doublet (J = 5.5, 1 Hz) for an allylic hydroxymethyl group superimposed on a more complex signal due to a methine proton. The chemical shift of the latter signal corresponds to a proton on a carbon bearing a phosphate moiety.[5] These assignments were confirmed by comparison of the NMR data for CI-920 and two key derivatives: the dephosphorylated compound **7** and the diacetyl derivative **8**.

Incubation of CI-920 with alkaline phosphatase afforded **7** in approximately 57% yield after chromatographic purification. Mass spectral, ^{1}H, and ^{13}C NMR data for this derivative were in agreement with the expected molecular formula $C_{19}H_{26}O_6$. The ^{13}C NMR spectrum of **7** (Table I)

(7) Van Puyvelde, L.; Dube, S.; Uwimana, E.; Uwera, C.; Dommisse, R. A.; Esmans, E. L.; Van Schoor, O.; Vlietinck, A. J. *Phytochemistry* **1979**, *18*, 1215.

(8) Van Puyvelde, L.; DeKimpe, N.; Dube, S.; Chagnon-Dube, M.; Boiley, Y.; Borremans, F.; Schamp, N.; Anteunis, M. J. O. *Phytochemistry* **1981**, *20*, 2753.

(9) Baker, S. R.; Jamieson, W. B.; McKay, S. W.; Morgan, S. E.; Rackham, D. M.; Ross, W. J.; Shrubsall, P. R. *Tetrahedron Lett.* **1980**, *21*, 4123 and references therein.

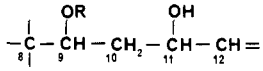


7 $R_1 = R_2 = H$

8 $R_1 = PO_3HNa$, $R_2 = Ac$

10 $R_1 = R_2 = Ac$

was virtually identical with that of CI-920 except for the absence of the carbon–phosphorus couplings described above and the shift of a methine signal from 80.55 to 76.41 ppm. In the ^1H NMR spectrum of 7 (Table III) the signal for the allylic hydroxymethyl group now stands alone at 4.27 ppm. A new one-proton doublet of doublets appears at 3.81 ppm, consistent with its assignment to H-9. An additional change observed in the spectrum of 7 is the sharpening of the signals for the upfield methylene group (1.47 and 1.87 ppm), presumably due to the elimination of long range phosphorus–proton coupling. The above data are consistent with partial structure 4, originally deduced from ^{13}C NMR data. This structural fragment can be expanded to structure 9 by analysis of spin-decoupling

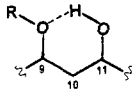


9

experiments which indicate that the nonequivalent protons of the methylene group are coupled to a differing degree with the methine protons observed at 3.81 (assigned above) and 4.95 ppm. The latter chemical shift is consistent with the carbinyl proton (H-11) of a secondary allylic alcohol functionality. Decoupling at 4.95 ppm collapses the signal for the triene proton (H-12) at 5.65 ppm (in 7) to a doublet, establishing connectivity between structural fragments 6 and 9.

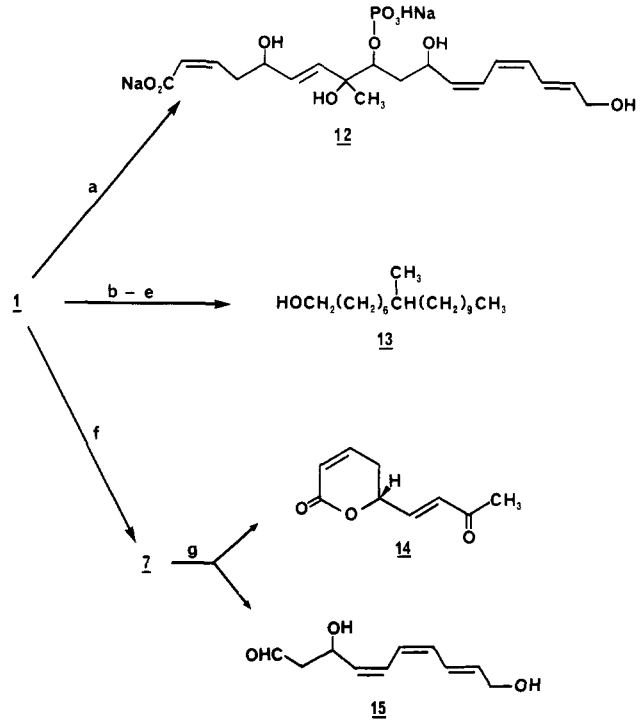
Acetylation of CI-920 with acetic anhydride and pyridine at 5 °C readily afforded the diacetyl derivative 8. A comparison of the ^1H NMR spectrum (Table III) of 8 with that of CI-920 reveals the expected downfield shifts for protons α to acetoxy groups (H-11 and H-18). Similar acetylation-induced shifts were observed in the ^1H NMR spectrum of the triacetyl product 10, obtained from dephosphorylated CI-920 (7). As expected, in addition to the shifts referred to above, the spectrum of 10 (Table III) shows a downfield shift of the H-9 doublet of doublets observed at 3.81 ppm for 7 to 5.06 ppm in the acetylated derivative.

The 360-MHz ^1H NMR spectral data for CI-920 (1), analogues 2 and 3, and the dephosphorylated derivative 7, all exhibit two well resolved one-proton multiplets between 1.45–1.9 ppm, demonstrating the magnetic nonequivalence of the methylene protons on C-10. This nonequivalency can best be explained by introducing rigidity into this portion of the molecule through the formation of a cyclic hydrogen-bonded structure such as 11 (R = PO_3HNa for 1, 2, and 3; R = H for 7). Interestingly, nonequivalence is enhanced in the resonance spectrum of 7 ($\Delta\delta$ = 0.4 ppm as opposed to 0.12–0.16 ppm for 1–3), ruling out the involvement of the phosphate group in this phenomenon. Consistent with this hypothesis, acetylation



11

Scheme I. Degradation of CI-920 (1)[a]



[a] Reagents: (a) NaOH; (b) H_2, Pt; (c) P, HI; (d) $LiAlH_4$; (e) H_2, Pt; (f) alkaline phosphatase; (g) $NaIO_4$.

of 1 or its dephosphorylated derivative (7) results in the elimination of the magnetic nonequivalence of the C-10 protons.

In addition to the above acetylation-induced effects observed in the ^1H NMR spectrum of 8, a partial resolution of the complex four-proton multiplet near 6 ppm is also observed. Extensive spin-decoupling experiments with 8 confirmed the indicated assignment of these protons and completed the assignment of signals for all 22 nonexchangeable protons of CI-920 as listed in Table II. On the basis of these data, structure 1 can be assigned to CI-920. Thus, examination of decoupled ^1H NMR spectra now clearly indicates that the conjugated triene chain terminates with an allylic hydroxymethyl group and that a trans carbon–carbon double bond connects the 5,6-dihydropyran-2-one ring to the quaternary carbon (C-8) of partial structure 9. By necessity, C-8 must also bear the remaining hydroxyl and single methyl group required by the spectral data for 1.

The presence of the dihydropyran-2-one moiety in 1 was readily confirmed by hydrolysis to the carboxylate derivative 12. To confirm the linear carbon skeleton represented in 12, CI-920 was converted to the parent hydrocarbon by using the phosphorus–hydriodic acid method of Cope et al.[10-12] The sequence of reactions described by Cope was modified (Scheme I) to yield a product (13)[13] in which the primary alcohol carbon corresponds to the original lactone carbonyl group. Thus, catalytic hydrogenation of CI-920 with platinum oxide, followed by treatment with phosphorus and hydriodic acid yielded a mixture of iodo compounds. Treatment of this mixture

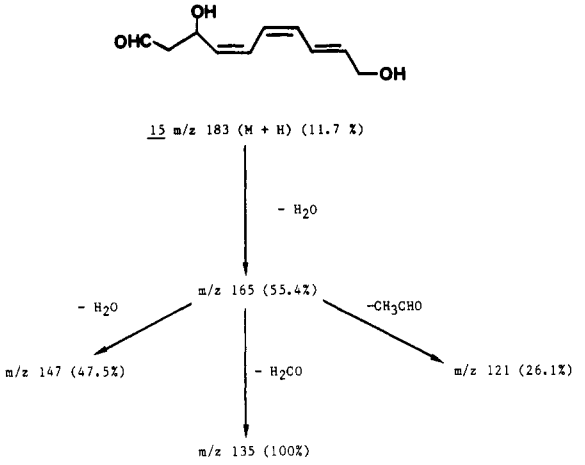
(10) Cope, A. C.; Bly, R. K.; Burrows, E. P.; Ceder, O. J.; Ciganek, E.; Gillis, B. T.; Porter, R. F.; Johnson, H. E. *J. Am. Chem. Soc.* **1962**, *84*, 2170.

(11) Cope, A. C.; Burrows, E. P.; Derieg, M. E.; Moon, S.; Wirth, W. *J. Am. Chem. Soc.* **1965**, *87*, 5452.

(12) Cope, A. C.; Axen, U.; Burrows, E. P.; Weinlich, J. *J. Am. Chem. Soc.* **1966**, *88*, 4228.

(13) Odham, G. *Ark. Kemi* **1965**, *23*, 431.

Scheme II. Mass Spectral Fragmentation of 15



with lithium aluminum hydride, followed by catalytic hydrogenation, yielded 8-methyl-1-octadecanol (**13**) as the principal product, exhibiting spectral properties (IR, NMR, MS) in agreement with the proposed structure. To further confirm the structure of this product, **13** was prepared with standard procedures (see Experimental Section). The synthetic product was chromatographically indistinguishable from the degradation product and the corresponding IR, NMR, and mass spectra were virtually superimposable.

Additional support for the assigned structure of CI-920 was obtained by the rapid periodate cleavage of the C-8/C-9 bond in the dephosphorylated derivative **7** to yield the keto lactone **14** and the unstable triene **15**. Spectral data for **14** readily establish the structure assigned and corroborate the ^{1}H NMR assignments for the lactone portion of CI-920 listed in Table II. Although the aldehyde **15** lacked sufficient stability for complete characterization, its ultraviolet absorption spectrum displayed the expected triene chromophore pattern. In addition, the fragmentation pattern observed in the chemical-ionization mass spectrum of **15** is consistent with the proposed structure (see Scheme II). Thus, the two products of the periodate cleavage of dephosphorylated CI-920 (**7**) account for all 19 carbon atoms of the molecule. This information, combined with the reduction of CI-920 to the alcohol **13** confirms the proposed structure of CI-920.

The (5*R*)-keto lactone **14** was synthesized by an unambiguous and stereoselective method[14] and proved to be identical with the degradation product in all respects (TLC, $[\alpha]_D$, IR, NMR). Thus, the chiral center at C-5 in CI-920 must possess the *R* configuration. The configurations at C-8, 9, and 11 remain to be determined.

Comparison of ^{1}H and ^{13}C NMR spectral data for CI-920 and its congeners, PD 113 270 and PD 113 271, readily allows the assignment of structures **2** and **3**, respectively, to these compounds. Clearly, the hydroxymethyl (C-18) in CI-920 is replaced by a methyl in PD 113 270 (δ 1.79, s, 3 H). For PD 113 271, an additional hydroxyl group must be attached to C-4 based on the observed changes in the chemical shift and multiplicity of neighboring protons. The presence of an additional oxygen atom in **3** was also shown by a 16 mass unit increase in molecular weight observed in the FAB mass spectrum [*m/z* 491 (monosodium salt + Na)$^+$]. The effect of these structural changes on antitumor activity is shown in Table IV. Additionally, these data indicate that acetylation of CI-920 or removal of the phosphate group results in a marked loss of activity.

(14) Details of the synthesis of **14** and other studies related to the synthesis of CI-920 will be published in a separate paper.

Table IV. In Vivo Antitumor Activity for CI-920 and Derivatives vs. L1210 Leukemia in Mice[a]

no.	dose[a]	%T/C[b]
1	12.5	289
2	6.25	271
3	25	180
7	50[c]	109
8	50[c]	101

[a] Dosage (mg/kg/inj) administered with a qd × 5 schedule to achieve the optimal %T/C values as indicated. [b] Ratio of the median survival time for treated animals to that of controls × 100. A value of %T/C ≥ 125 is considered active. [c] Highest dose tested.

Further studies to better define structure activity relationships within this novel class of antitumor agents are in progress.

Experimental Section

General Data. Infrared (IR) spectra were determined by using a Nicolet SX-60 FTIR spectrometer. Ultraviolet (UV) spectra were determined with a Carey 118CX recording spectrometer. Most ^{1}H NMR spectra and all ^{13}C NMR spectra were determined on a Bruker WM-360 spectrometer by Frank Parker at the University of Michigan; other ^{1}H NMR spectra were recorded on Varian XL-200 or XL-300 spectrometers or a Bruker WH-90 spectrometer. Chemical shifts are reported as parts per million (ppm) downfield from internal Me_4Si or sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate (TSP). Mass spectra were recorded on a VG Analytical Model 7070E/HF instrument. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter.

Thin-layer chromatography (TLC) and preparative TLC were carried out on commercial silica gel plates (E. Merck, 0.25 mm and 1.0 mm thickness, respectively). Column chromatography was performed with silica gel 60 (40–43 μm, E. Merck) or reversed-phase silica gels (C-8 or C-18, 40 μm, Analytichem Inc.). Analytical high-pressure liquid chromatography (HPLC) analyses were carried out as previously described.[3]

Samples of CI-920, PD 113 270, and PD 113 271 were isolated and purified as previously reported.[3] The course of chemical reactions and the purity of starting materials and products were determined by HPLC and/or TLC analysis.

Dephosphorylation of CI-920 To Give 7. A solution of alkaline phosphatase (1.0 g, calf intestine, Sigma Chemical Company) and the sodium salt of CI-920 (1.4 g) in water (140 mL) was incubated at 37 °C for 7 h. The reaction mixture was lyophilized and the residue triturated with methanol. The methanol-soluble product was chromatographed over C-8 silica gel (30–50 psi pressure) and, after washing with water, **7** was eluted with water–acetonitrile (85:15). Fractions containing **7** were combined, concentrated, and lyophilized to yield 0.62 g (57%) of dephosphorylated CI-920 as an amorphous white solid: UV (MeOH) λ_{max} (log ϵ) 268 nm (4.53) with inflections at 259 nm and 278 nm; IR ($CHCl_3$) 1720, 1600, 1285, 1060 cm^{-1}; CIMS (CH_4), *m/z* (relative intensity) 333 (M + H − H_2O, 9), 315 (57); ^{13}C and ^{1}H NMR data are listed in Tables I and III, respectively.

Anal. Calcd for $C_{19}H_{26}O_6 \cdot 0.5\ H_2O$: C, 63.51; H, 7.52. Found: C, 63.41; H, 7.51.

Acetylation of CI-920 To Yield 8. CI-920 sodium salt (25 mg) was treated with acetic anhydride (0.6 mL) in the presence of pyridine 0.3 mL) for 5 h at 5 °C. Volatile components were removed in vacuo and the residue dissolved in 5% sodium bicarbonate and chromatographed over 20 mL of Diaion HP-20 resin packed in water. After washing the resin with water–methanol (75:25), **8** was eluted with water–methanol (30:70). Removal of solvent in vacuo yielded 21 mg (71%) of CI-920 diacetate, sodium salt: UV (MeOH) λ_{max} (log ϵ) 268 nm (4.54) with inflections at 259 nm and 278 nm; IR (KBr) 1735, 1380, 1245, 1120, 1070, 1025 cm^{-1}; ^{1}H NMR data are listed in Table III.

Acetylation of Dephosphorylated CI-920 (7) To Yield 10. Dephosphorylated CI-920 (**7**, 30 mg) was treated with acetic anhydride (0.6 mL) in the presence of pyridine (0.3 mL) for 5 h at 5 °C. After the volatile components were removed in vacuo, the residue was dissolved in chloroform and chromatographed

over silica gel (5 g) packed in chloroform. After washing with chloroform, **10** was eluted with chloroform–methanol (95:5) to afford the triacetate, **10**, as a colorless film (35 mg, 86%): CIMS (CH_4), m/z (relative intensity) 477 (M + H, 10), 459 (4), 417 (40), 399 (13), 357 (100), 339 (19), 313 (79), 297 (64), 279 (12), 253 (20); ^{1}H NMR data are listed in Table III.

Hydrolysis of CI-920 To Yield 12. CI-920 sodium salt (100 mg) was dissolved in water (50 mL) and the pH adjusted to 11 with dilute sodium hydroxide. After standing at 5 °C overnight, the pH (8.8) was readjusted to 11 and the solution lyophilized to yield **12** as a white amorphous solid (110 mg): UV (MeOH) λ_{max} (log ϵ) 268 nm (4.49) with inflections at 259 nm and 278 nm; IR (KBr) 3400, 1650, 1560, 1435, 1350, 1090, and 970 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 1.30 (s, H-19), 1.52 (br t, H-10A), 1.65 (br t, H-10B), 2.56–2.76 (m, 2 H, H-4), 4.16 (m, H-9), 4.20 (d, H-18), 4.3 (m, H-5), 4.95 (br t, H-11) 5.55 (t, H-12), 5.7–6.1 (m, 5 H, H-2,3,6,7,17), 6.18 (t, H-15), 6.40 (t, H-14), 6.56 (t, H-13), 6.80 (dd, H-16).

Conversion of CI-920 to 8-Methyl-1-octadecanol (13). CI-920 sodium salt (200 mg) was dissolved in 50% aqueous ethanol (40 mL) containing platinum oxide (72 mg) and the mixture stirred for 2 h in an atmosphere of hydrogen. Filtration of the reaction mixture through Celite and concentration in vacuo yielded a grey solid (208 mg) which was refluxed for 24 h in 47% hydriodic acid (15 mL) with 200 mg of red phosphorus. The reaction mixture was diluted with water (30 mL) and extracted with ether (4 × 40 mL). The yellow extracts were combined and washed successively with water (50 mL), 2% sodium thiosulfate (2 × 50 mL), and water (2 × 50 mL). The ether solution was dried over anhydrous sodium sulfate and concentrated in vacuo to afford an iodo derivative as a yellow oil (172 mg). This product was refluxed for 16 h in tetrahydrofuran (10 mL) containing 180 mg of $LiAlH_4$. Workup in the usual manner afforded a hexane-soluble oil (96 mg). This product was dissolved in hexane–ethyl acetate (2:1, 10 mL) and stirred under hydrogen with platinum oxide for 3 h. The reaction mixture was filtered and concentrated in vacuo to yield a pale yellow oil (96 mg) which was purified by preparative layer chromatography on silica gel (chloroform–ethanol, 95:5), affording the alcohol, **13**, as a colorless film (26 mg, 21%): IR ($CHCl_3$) 2940, 2870, 1470, and 1385 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.9 (6 H, t), 1.1–1.7 (32 H, m), 3.65 (2 H, t); CIMS (CH_4), m/z (relative abundance) 285 (M + H, 1) 283 (47), 267 (M + H – H_2O, 100).

Synthesis of 8-Methyl-1-octadecanol (13). (A) Preparation of 1-(Benzyloxy)-7-bromoheptane. Benzyl alcohol (50 mL) was stirred with sodium metal (1 g, 43 mmol) for 45 min and the resulting solution added slowly to 1,7-dibromoheptane (10.3 g, 40 mmol) in ether (40 mL). After refluxing for 17 h the precipitated NaBr was removed by filtration and the filtrate washed with water (3 × 100 mL), dried over sodium sulfate, and concentrated in vacuo to a colorless oil. Distillation of the product afforded the monobenzyloxy derivative, (6 g, approximately 47%), contaminated with approximately 10% of 1,7-bis(benzyloxy)-heptane: bp 225–230 °C (16 mm); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.2–1.9 (12 H, m), 3.2–3.4 (2 H, m), 4.4 (2 H, s), 7.1 (5 H, s).

Anal. Calcd for 90% $C_{14}H_{21}BrO$ + 10% $C_{21}H_{28}O_2$: C, 61.31; H, 7.54; Br, 25.03. Found: C, 61.35; H, 7.57; Br, 25.29.

(B) Preparation of 2-Dodecanone. To a solution containing undecanoic acid (7.44 g, 40 mmol) in anhydrous ether (150 mL) was added dropwise with stirring 40 mL of ethereal methyllithium (2.2 M). The mixture was stirred at room temperature for 1 h, then refluxed for an additional 2.5 h. The reaction mixture was then syphoned into a vigorously stirred solution of hydrochloric acid (0.7 M). After adjusting the pH of the resulting aqueous solution to 11, the organic layer was separated and the aqueous solution extracted once with ether (50 mL). The two ether layers were combined, washed with water (3 × 100 mL), dried over sodium sulfate, and concentrated to a yellow oil (6.2 g). This product was chromatographed over silica gel (hexane elution) to yield nearly pure 2-dodecanone as a colorless liquid which slowly solidified upon cooling (4.3 g, 58%): bp 136–137 °C (16 mm); IR (KBr) 1710 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.9 (3 H, t) 1.1–1.7 (16 H, m), 2.05 (3 H, s), 2.3 (2 H, t). [lit.[15] 2-dodecanone: mp 21 °C; bp 101 °C (3.5 mm)].

(C) Preparation of 1-(Benzyloxy)-8-methyl-8-octadecanol. A solution of 1-(benzyloxy)-7-bromoheptane (3.1 g, 10 mmol) in ether (50 mL) was added with stirring to crushed magnesium turnings (0.25 g, 10 mmol) and the mixture allowed to reflux. After 1 h, a solution of 2-dodecanone (1.8 g, 10 mmol) in ether (30 mL) was added slowly with stirring. After refluxing for 14 h, the reaction mixture was treated with ice and 3 mL of 3 N HCl. The organic layer was separated and the aqueous layer was extracted with ether (40 mL). The two ether layers were combined, washed with water (40 mL), 5% sodium bicarbonate (2 × 40 mL), and again with water (2 × 40 mL). Concentration of the ethereal solution afforded the Grignard product as a viscous, pale yellow oil (4.0 g, 92%) which was used without further purification: ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.88 (3 H, t), 1.1–1.8 (33 H, m), 3.43 (2 H, t), 4.45 (2 H, s), 7.25 (5 H, s).

(D) Preparation of 8-Methyl-1-octadecanol (13). A solution of 1-(benzyloxy)-8-methyl-8-octadecanol (3.9 g) and *p*-toluenesulfonic acid (0.78 g) in toluene (20 mL) was stirred under reflux for 1 h. Workup in the usual fashion yielded a mixture of dehydration products as a yellow oil (3.7 g). Catalytic hydrogenation of this oil with 20% palladium on carbon (1 g) in ethyl acetate (50 mL) for 4 h yielded an oily residue which was chromatographed over silica gel (50 g). After washing with hexane, the column was eluted with hexane–ether (1:1), affording 730 mg of a colorless oil. Final purification of a portion of this product was achieved by preparative-layer chromatography on silica gel (chloroform–ether, 95:5) to yield 130 mg of **13**, identical in all respects (TLC, NMR, IR, CIMS) with the degradation product obtained from CI-920: CIMS (CH_4), $m/$ (relative intensity) 285 (M + H, 4.6), 283 (49), 267 (100).

Periodate Cleavage of Dephosphorylated CI-920 (7) To Yield 14 and 15. Dephosphorylated CI-920 (**7**, 64 mg) was added to 10 mL of 0.05 M sodium periodate solution and the mixture stirred until complete dissolution had occurred (2–3 min). Immediate extraction with chloroform (2 × 20 mL) allowed the isolation of the nearly pure keto lactone, **14** (31 mg), from the chloroform soluble fraction. Preparative-layer chromatography over silica gel (chloroform–2-propanol, 8:2) afforded **14** as a colorless film: $[\alpha]_D$ +216.8° (c 1.16, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 nm (4.13); IR ($CHCl_3$) 1725, 1700, 1680, 1640, 1380, and 1360 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.3 (3 H, s), 2.45–2.65 (2 H, m), 5.0–5.3 (1 H, m), 6.1 (1 H, ddd, J = 10, 2, 1.5 Hz), 6.45 (1 H, dd, J = 14, 1.5), 6.75 (1 H, dd, J = 14, 5 Hz), 6.9 (1 H, m); EIMS, m/z (relative intensity) 166 (33), 148 (33), 123 (17), 121 (30), 95 (22), 68 (100).

Extraction of the remaining aqueous layer from above (following chloroform extraction) with ethyl acetate (2 × 20 mL) afforded, upon concentration of the organic layer, the unstable triene, **15**, as a pale yellow film (22 mg): UV (MeOH) λ_{max} (log ϵ) 268 nm (4.13) with inflections at 260 and 279 nm; ^{1}H NMR (90 MHz, $CDCl_3$) 2.7 (2 H, m), 4.25 (2 H, d), 4.9–7.0 (7 H, complex m), 9.85 (1 H, t); CIMS (CH_4), see Scheme II.

Acknowledgment. We thank Timothy Hurley, Suresh Stampwala, and Dr. John Schaumberg for assistance with various aspects of this work. We also thank Frank Parker, University of Michigan, for recording NMR spectra and Dr. Gary McClusky, Warner-Lambert/Parke-Davis Pharmaceutical Research, for FAB mass spectral data. The important contributions to this work made by the members of our fermentation, tumor biology, and physical chemistry sections are also gratefully acknowledged. This work was supported in part by the National Cancer Institute, contract NO1-CM-07379.

(15) Asinger, F. *Chem. Ber.* **1944**, *77*, 73.