(MeOH) 213 nm (ε 10300), 264 (5300), 284 (5500); ^{1}H NMR (Me_2SO-d_6) δ 0.92 (d, 6 H, J = 7 Hz), 2.4 (s, 3 H), 3.5–4.8 (m, 5 H), 5.2 (m, 1 H), 6.55 (d, 1 H, J = 4 Hz), 8.25 (s, 1 H). Anal. ($C_{16}H_{24}N_4O_5 \cdot H_2O$) C, H, N.

Compound 10g: 75% yield; mp 163–165 °C; $[\alpha]^{22}_D$ −73.42° (c 1.05, DMF); IR (KBr) ν_{max} 3350, 1690, 1595, 1520 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 3.5 (m, 1 H), 3.95 (m, 1 H), 4.3 (m, 1 H), 4.7 (m, 2 H), 5.15 (m, 1 H), 6.55 (d, 1 H, J = 4 Hz), 7.4 (s, 5 H), 8.55 (s, 1 H). Anal. ($C_{19}H_{22}N_4O_5$) C, H, N.

Compound 10h: 80% yield; mp 151–152 °C; $[\alpha]^{22}_D$ −77.12° (c 1, DMF) IR (KBr) ν_{max} 3350, 1695, 1615, 1585, 1520 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.35 (s, 3 H), 3.65 (s, 3 H), 3.7–4.2 (m, 3 H), 4.35 (m, 1 H), 4.6 (m, 1 H), 5.0 (s, 2 H), 6.65 (d, 1 H, J = 4.1 Hz), 6.9 (d, 2 H, J = 9 Hz), 7.2 (d, 2 H, J = 9 Hz), 7.85 (s, 1 H). Anal. ($C_{19}H_{22}N_4O_6$) C, H, N.

Cell Cultures. Vero cells were routinely grown in monolayers in Eagle's basal medium (EBM) supplemented with 10% bovine serum (BS) and maintained in a maintenance medium consisting of EBM containing 2% BS.

Virus and Virus Titration. MP strain of herpes simplex virus type 1 (HSV-1), a DNA virus that causes fusion of infected cells,[16] was propagated in Vero cells at 34 °C and stored at −80 °C.

Plaque titrations were performed on Vero cell monolayers that were maintained after infection in EBM medium containing 1% BS and 0.2% human γ-globulin. Foci of infected wells were counted after fixation of the monolayers with methanol and staining with Giemsa. The infectious titer was expressed in plaque-forming units (pfu) per millimeter.[17] Phosphate-buffered saline (PBS) supplemented with 1% serum was used for dilutions and culture washing.

Antiviral Drugs: Determination of the ED_{50}. A stock solution of each drug was prepared by dissolving 10 mg of the compound in 1 mL of dimethyl sulfoxide, and appropriate dilutions were made in the maintenance medium. For 50% effective dose (ED_{50}) titrations, all titrations were done at least in duplicate. Confluent Vero cells in 25-cm^2 flasks were infected with approximately 200 pfu and incubated at 36 °C for 48 h in the maintenance medium containing 0.2% human immune serum globulin and graded concentration of the drugs. One set of control cultures were maintained without drug. Plaques were read after fixation and staining. The number of plaques emerging in the presence of each drug concentration was calculated as a percent of the control as follows: (average number of plaques with drug/average number of plaques without drug) × 100. Each drug titration was plotted on semilogarithmic graph paper to give dose–response lines from which ED_{50} values were calculated. The ED_{50} concentration was that amount of drug per milliliter of medium that inhibited the plaques numbers by 50% compared with the no-drug controls. 5-Iodo-2′-deoxyuridine (IDU), the compound most frequently used in clinical practice, was used as positive control of anti-herpes activity.

Crystal data: $C_{18}H_{20}N_4O_5$; space group $P2_12_12_1$; a = 5.442 (1), b = 13.387 (2), c = 23.759 (4) Å; Z = 4, d_{calcd} = 1.43 g cm^{-3}. Intensity data were collected on an Enraf-Nonius CAD-4 automatic four-circle diffractometer, Mo Kα radiation with $\omega/2\vartheta$ scan technique ($\vartheta \leq 26$). 1986 independent reflections were collected, out of which 1154 having $I > 2\sigma(I)$ were considered observed. The structure was solved by direct methods by means of MULTAN-82. The structure was refined by full-matrix least squares, assuming anisotropic temperature factors for all atoms, except H's, which were refined isotopically. Final values of the discrepancy indices were R = 0.039 and R_w = 0.041.

Registry No. 4, 80030-73-5; **5a**, 89889-56-5; **5b**, 89889-57-6; **5c**, 89889-58-7; **6a**, 89889-59-8; **6b**, 89889-60-1; **6c**, 89889-61-2; **7a**, 89889-62-3; **7b**, 89889-63-4; **7c**, 89889-64-5; **7d**, 80030-90-6; **7e**, 80043-70-5; **7f**, 80030-87-1; **7g**, 80030-88-2; **7h**, 86927-78-8; **8a**, 89889-65-6; **8b**, 89889-66-7; **8c**, 89889-67-8; **8d**, 89908-13-4; **8e**, 89889-68-9; **8f**, 89889-69-0; **8g**, 89889-70-3; **8h**, 89889-71-4; **9a**, 89889-72-5; **9b**, 89889-73-6; **9c**, 89889-74-7; **9d**, 89889-75-8; **9e**, 89889-76-9; **9f**, 89889-77-0; **9g**, 89889-78-1; **9h**, 89889-79-2; **10a**, 89889-80-5; **10b**, 89889-81-6; **10c**, 89889-82-7; **10d**, 89889-83-8; **10e**, 89889-84-9; **10f**, 89889-85-0; **10g**, 89889-86-1; **10h**, 89889-87-2; *p*-chloroaniline, 106-47-8; *p*-chlorobenzylamine, 104-86-9; *p*-toluidine, 106-49-0; β-D-ribofuranosetetraacetyl, 13035-61-5.

β-Lapachone: Synthesis of Derivatives and Activities in Tumor Models

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In order to find a 3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-5,6-dione more potent than the naturally occurring 2,2-dimethyl derivative [β-lapachone (**10a**)], we synthesized a series of analogous compounds with modifications at position 2 of the pyran ring or at positions 8 and 9 of the benzene ring. Of the compounds tested in vitro for inhibition of RNA-dependent DNA polymerase and in mice infected with Rauscher leukemia, all retained good enzyme activity. Inhibition of the reverse transcriptase activity of the 2,2-substituted derivatives **10b–e** was as strong as **10a**. However, only the 2-methyl-2-phenyl derivative **10e** proved to be about as potent as the 2,2-dimethyl reference compound **10a** in prolonging the mean survival time of mice with Rauscher leukemia virus induced leukemia.

From a number of substances inhibiting the activity of retrovirus reverse transcriptase only β-lapachone (3,4-dihydro-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5,6-dione) was found to prolong the survival time of chickens infected intraperitoneally with Rous sarcoma virus.[1] Molecular biological studies have revealed that the drug inhibits reverse transcriptase, as well as eukaryotic DNA polymerase α.[2,3] In other studies, β-lapachone, which can be isolated from various tropical trees[4] or synthesized chemically from lapachol following the procedures of Hooker,[5] was shown to inhibit the growth of certain bacteria in vitro[4,6] and the cellular production of Friend virus.[7] The activity of β-lapachone in vitro and possibly in in vivo systems[1] prompted us to synthesize a number of derivatives in order to compare their activity to inhibit reverse transcriptase in vitro and to prolong the survival of mice

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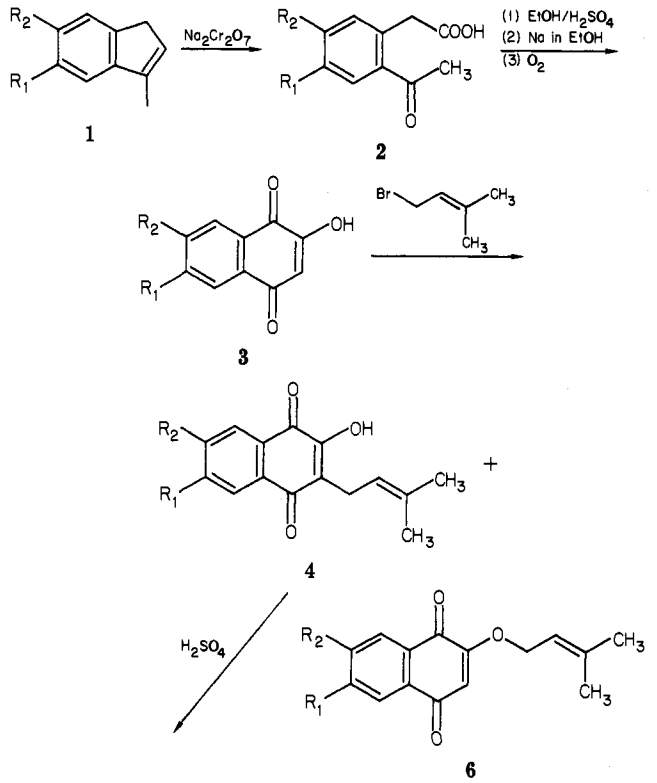
0022-2623/84/1827-0990$01.50/0

Scheme I[a]



[a] a: $R_1 = R_2 = H$. b: $R_1 = Cl$; $R_2 = H$. c: $R_1 = CH_3$; $R_2 = H$. d: $R_1 = R_2 = OCH_3$.

infected with Rauscher leukemia virus. These results are described in the present report.

Results and Discussion

Synthesis of β-Lapachones 5a–d Carrying Substituents on the Benzene Ring. Three compounds were synthesized in this series by using the naphthalenediones **3** as intermediates. Of these, the dimethoxy derivative **3d** has previously been synthesized[8] starting from ethyl homoveratrate.[9] For the preparation of the naphthoquinones **3a–c**, the methylindenes **1a–c** were synthesized from the corresponding 1-indanones and oxidized with dichromate to the phenylacetic acids **2**.[10,11] Cyclization of the corresponding ethyl esters of **2** as shown in Scheme I gave the intermediate naphthalenediones **3**, of which **3a** is commercially available. Alkylation of the potassium salts of **3** with 1-bromo-3-methyl-2-butene[12] gave the lapachols **4**, together with varying amounts of the enol ethers **6**, which on treatment with sulfuric acid gave back the starting material **3**, as exemplified by the conversion of **6a** back to **3a**. Sulfuric acid treatment of **4** in a manner analogous to the well-known lapachol (**4a**) to β-lapachone (**5a**) conversion[5] gave the desired β-lapachones carrying substituents on the benzene ring. Yields and analytical data of the intermediates and products are given in Table I. The corresponding IR and UV data are found in Table II, while NMR data of all β-lapachones described in this paper are found in Table III.

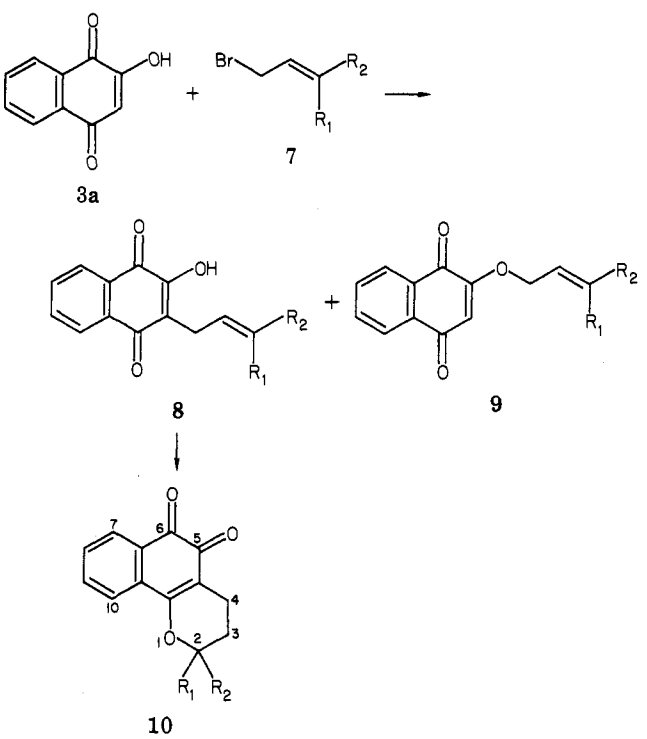
Synthesis of β-Lapachones 10a–i Carrying Various Substituents in Position 2. In this series, eight com-

Table I. Yield and Analytical Data of the Compounds Shown in Scheme I

no.	mp or bp (Torr), °C	yield,[a] %	formula	anal.
1a[c]	122 (20)[b]			
1b	131 (22)	48	$C_{10}H_9Cl$	C, H, Cl
1c	52 (0.06)	66	$C_{11}H_{12}$	C, H
2a	160–161[d]	53	$C_{10}H_{20}O_3$	
2b	144–148	63	$C_{10}H_9ClO_3$	C, H, Cl
2c	152–153	44	$C_{11}H_{12}O_3$	C, H
2d[c]	96–98			
3a[c]	192–195			
3b	209–211	54	$C_{10}H_3ClO_3$	C, H, Cl
3c	202–203	86	$C_{11}H_8O_3$	C, H
3d	221–222[e]	52	$C_{12}H_{10}O_5$	
4a	136–137[f]	48	$C_{13}H_{14}O_3$	
4b	162–166	15	$C_{15}H_{13}ClO_3$	C, H, Cl
4c	132–133	25	$C_{16}H_{16}O_3$	C, H
4d	166–168	11	$C_{17}H_{18}O_5$	C, H
5a	153–154[g]	97	$C_{15}H_{14}O_3$	C, H
5b	190–191	68	$C_{15}H_{13}ClO_3$	C, H, Cl
5c	169–171	95	$C_{16}H_{16}O_3$	C, H
5d	209–211	78	$C_{15}H_{18}O_5$	C, H

[a] For **1a–c**, yields are based on the corresponding indan-1-ones. For compounds **4a–d**, recovered starting material and O-alkylation product **6** were not taken into account. [b] Literature[10] bp 205–206 °C. [c] Commercially available. [d] Literature[11] mp 165–166 °C. [e] Literature[14] mp 221–222 °C. [f] Literature[5] mp 135.4 °C. [g] Literature[5] mp 153–154 °C.

Scheme II[a]



[a] a: $R_1 = R_2 = CH_3$. b: $R_1 = R_2 = CH_2CH_3$. c: $R_1 = CH_3$; $R_2 = CH_2CH_3$. d: $R_1 = H$; $R_2 = C_6H_5$. e: $R_1 = CH_3$; $R_2 = C_6H_5$. f: $R_1, R_2 = (CH_2)_4$. g: $R_1, R_2 = (CH_2)_5$. h: $R_1, R_2 = (CH_2)_6$. i: R_1, R_2 = adamantylidene.

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Table II. Spectral Data of the Compounds Shown in Scheme I

no.	IR, cm^{-1}	UV λ_{max}, nm (log ϵ)
1a[a]	1620, 1580[b]	
1b	1620, 1580[b]	
1c	1620, 1580[b]	
2a[a]		
2b	3460, 1735, 1710, 1685[c]	
2c	1720, 1680, 1580	
2d[a]	1710, 1640, 1590	
3a[a]	1670, 1620, 1590, 1560	
3b	3320, 1680, 1650, 1580	
3c	3400, 1660, 1660[c]	
3d[a]	3250, 1670, 1650	
4a[a]	1700, 1645, 1620, 1580[c]	267 sh, 284 (3.516), 332 (2.931), 430 (2.970)
4b	3350, 1670, 1640, 1580	259 (4.356), 285 (4.222), 324 (3.462), 396 (3.164)
4c	3440, 1670, 1620[c]	258 (4.381), 288 (4.232), 332 (3.472), 390 (3.108)
4d	3320, 1650, 1590	225 sh, 268 sh, (4.151), 275 (4.180), 315 (3.836)
5a	1700, 1645, 1610, 1575[c]	255 (3.721), 262 sh, 287 (3.292), 340 (2.642), 450 (2.517)
5b	1705, 1650, 1620, 1590, 1575[c]	259 (4.124), 265 sh, 285 sh (3.664), 335 (3.133)
5c	1705, 1660, 1600[c]	258 (4.437), 266 (4.422), 288 (3.040), 343 (3.474), 434 (3.176)
5d	1685, 1645, 1600, 1575	228 sh, 272 sh, 279 (4.228), 324 (3.667)

[a] Known compounds; compare Table I for references. [b] Liquid film. [c] Methylene chloride solution.

Table III. NMR Data for the Lapachones 5a–d and 10a–i

no.	chemical shifts, δ (J in Hz)
5a (=10a)	1.40 (s, 6-CH_3), 1.82 (t, J = 7, 2 H 3), 2.57 (t, J = 7, 2H-4), 7.20–8.40 (m, H-7 to H-10)
5b	1.53 (s, 6-CH_3), 1.87 (t, J = 7, 2 H3), 2.60 (t, J = 7, 2 H-4), 7.50 (dd (8+2), H-8), 7.77 (d, J = 2, H-10), 8.02 (d, J = 8, H-7)
5c	1.47 (s, 6-CH_3), 1.83 (t, J = 7, 2 H-3), 2.57 (t, J = 7, 2 H-4), 7.30 (dd, (8+2), H-8), 7.61 (d, J = 2, H-10), 7.98 (d, J = 8, H-7)
5d	1.48 (s, 6-CH_3), 1.83 (t, J = 7, 2 H-3), 2.53 (t, J = 7, 2 H-4), 3.98 (s, 3-OCH_3), 7.25 (s, H-10), 7.56 (s, H-7)
10b	0.97 (t, J = 7, 6-CH_2CH_3), 1.80 (q, J = 7, 4-CH_2CH_3), 1.83 (t, J = 7, 2 H-3), 2.53 (t, J = 7, 2 H-4), 7.30–8.15 (H-7 to H-10)
10c	1.03 (t, J = 7, 3-CH_2CH_3), 1.40 (s, 3-CH_3), 1.80 (q, J = 7, 2-CH_2CH_3), 1.83 (t, J = 7, 2 H-3), 2.55 (t, J = 7, 2 H-4), 7.30–8.15 (H-7 to H-10)
10d[a]	1.90–2.80 (m, 4 H-3 + H-4), 5.25 (dd, (10+4), H-2), 7.43 (s 5-Ph H), 7.30–8.17 (m, H-7 to H-10)
10e[a]	1.77 (s, 3-CH_3), 1.90–2.75 (m, 4 H-3 + H-4), 7.15–8.13 (m, H-7 to H-10), 7.33 (s, 5-Ph H)
10f[a]	1.40–2.20 (m, 10-$(CH_2)_4$ + 2 H-3), 2.59 (t, J = 7, 2 H-4), 7.28–8.20 (m, H-7 to H-10)
10g	1.35–2.15 (m, 12-$(CH_2)_5$ + 2 H-3), 2.59 (t, J = 7, 2 H-4), 7.30–8.25 (m, H-7 to H-10)
	1.30–2.30 (m, 14-$(CH_2)_6$ + 2 H-3), 2.53 (t, J = 7, 2 H-4), 7.28–8.15 (m, H-7 to H-10)
10i[a]	1.50–2.50 (m, 14-adamantyl H + 2 H-3), 2.52 (t, J = 7, 2 H-4), 7.40–8.15 (m, H-7 to H-10)

[a] Recorded on Varian Model HA100. All others were recorded on a Model A-60.

pounds, **10b–i** (Scheme II), were synthesized, starting with lawsone (**3a**). The bromides **7**, which were required to alkylate the potassium salt of **3a**, were prepared in the same way as 1-bromo-3-methyl-2-butene (**7a**), i.e., from the corresponding allylic alcohols.[5]

In order to avoid allylic rearrangement of the bromides **7**, we made no attempts to distill the crude compounds. After thoroughly drying, they were reacted without undue delay. As in the case described for the synthesis of the lapachols **4**, varying amounts of starting material **3a** and O-alkylated products **9** were isolated from the reaction mixture after alkylation. [If desired, the enol ethers **9** can be reconverted to lawsone (**3a**), as shown for the conversion **6a** to **3a**.] Cyclization of the lapachols **8b–i** with concentrated H_2SO_4 by the method of Hooker[13] gave the corresponding β-lapachones in the yields given in Table IV.

Antitumor Activity. Prolongation of Mean Survival Time in Tumor Models in Vivo by β-Lapachone and Related Compounds. In chickens infected intraperitoneally with Rous sarcoma virus S-R, well tolerated doses (31.3 or 15.6 mg/kg po) of β-lapachone caused a significant prolongation of the mean survival time (Table V).

The administration of 125 mg/kg po of β-lapachone for 5 days a week for 4 weeks to female Balb/c mice infected with Rauscher leukemia virus yielded significant prolongation of the mean survival time in various experiments; 62.5 mg/kg was inactive, with a tendency of shortened survival time; 250 mg/kg po, however, caused a significant shortening of the mean survival time [$\bar{x}$ = 31.5 days (controls 41.0) days), $p \leq 0.01$], along with a corresponding increase of the leukemic blood cells (not shown). This was not the case when the same daily dose was administered in the drinking water. Herewith, the prolongation of the mean survival time was about comparable with that of a continuous daily administration (7 days per week) of 125 mg/kg by stomach tube [$\bar{x}$ = 51.1 and 48.5 days, respectively (controls 34.1 days), $p \leq 0.01$].

Of the analogues of β-lapachone, only compound **10e** proved to be more effective at the same dosage than the parent compound in prolongation of survival in mice infected with Rauscher leukemia virus (Table VI) in repeated experiments; compound **5b** was about equally active. Of all the derivatives tested, only compound **10c** showed a significantly reduced MTD (Table VI) and, therefore, was not yet examined in Rauscher leukemia. Not enough substance for examining compound **10f** was available.

Inhibition of Reverse Transcriptase Activity. Of the three compounds active in Rauscher leukemia, two could be tested in vitro (**10a,e**), and these strongly inhibited RDDP (RNA-dependent DNA polymerase), RLV (Rauscher leukemia virus), and AMV (Avian myeloblastosis virus) [EC50 $\leq$ 2 μg/mL (Table VI)]. The two that inhibited the RDDP of AMV at the same level (**10b,d**) were not significantly active in mice with Rauscher leu-

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Table IV. Yields and Analytical Data of the Compounds Shown in Scheme II

no.	mp,[a] °C	yield,[b] %	formula	anal.[c]
7a[d]		90	C_5H_9Br	
7b		97	$C_7H_{13}Br$	
7c		87	$C_6H_{11}Br$	
7d[e]			C_9H_9Br	
7e		91	$C_{10}H_{11}Br$	
7f		72	$C_7H_{11}Br$	
7g		52	$C_8H_{13}Br$	
7h		88	$C_9H_{15}Br$	
7i		96	$C_{12}H_{17}Br$	
4a[d]	136–137[f]	48	$C_{15}H_{14}O_3$	
8b	84–85	22	$C_{17}H_{18}O_3$	C, H
8c	103–105	19	$C_{16}H_{16}O_3$	C, H
8d[d]	162–164[g]	20	$C_{19}H_{14}O_3$	
8e	147–150	15	$C_{20}H_{16}O_3$	C, H
8f	145–146	7	$C_{17}H_{16}O_3$	C, H
8g	107–108	11	$C_{18}H_{18}O_3$	C, H
8h	91–94	19	$C_{19}H_{20}O_3$	C, H
8i	109–110	19	$C_{22}H_{22}O_3$	C, H
5a[d]	153–154[h]	97	$C_{15}H_{14}O_3$	
10b	106–109	29	$C_{17}H_{28}O_3$	C, H
10c	106–107	85	$C_{16}H_{16}O_3$	C, H
10d[d]	165–167[i]	93	$C_{19}H_{14}O_3$	C, H, O
10e	114–117	60	$C_{20}H_{16}O_3$	C, H
10f	97–98	66	$C_{17}H_{16}O_3 \cdot 1/2H_2O$	C, H
10g	148–150	63	$C_{18}H_{18}O_3 \cdot 1/10H_2O$	C, H
10h	124–125	51	$C_{19}H_{20}O_3$	C, H
10i	158–159	47	$C_{22}H_{22}O_3$	C, H, O

[a] Compounds 7a–i were not purified. [b] Compounds 8a–i represent only isolated C-alkylation products without taking into account recovered starting material and O-alkylation product. [c] Only purified products were analyzed. [d] Known compounds. [e] Commercially available. [f] Literature[5] mp 136–137 °C. [g] Literature[15] mp 170 °C. [h] Literature[5] mp 153–154 °C. [i] Literature mp 167 °C.

Table V. Activity of β-Lapachone in Peritoneal Infection of Chickens with Rous Sarcoma Virus S-R

dose, mg/kg po (b.i.d.)	mean survival time, days: T/C[b]
6 × 31.3[a]	17.6/10.8[c]
6 × 31.3	17.4/8.8[c]
6 × 15.6	12.0/8.8
12 × 31.3	18.4/8.8[c]
12 × 15.6	16.9/8.8[c]

[a] Treatment was started 30 min before infection. [b] Treated/controls. [c] Cox test: $p \leq 0.001$.

kemia. Since these were not yet tested against the RDDP of RLV and no pharmacokinetic data of these substances for comparison are available, any conclusion of a probable correlation between in vitro and in vivo results is not yet possible.

Experimental Section

Chemistry. Melting points were determined on a Tottoli apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer Model 221 spectrophotometer, and, unless otherwise stated, were recorded in Nujol mulls. UV spectra were recorded on a Cary-15 apparatus utilizing ethanol solutions. NMR spectra were taken on a Varian A-60 or Varian Model HA-100 (100 MHz) spectrophotometer with Me_4Si (δ 0.00) as internal standard and $CDCl_3$ solutions (s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet). Chemical shifts are expressed as δ values (parts per million) from tetramethylsilane. Petroleum ether refers to the fraction boiling at 60–80 °C.

Preparation of β-Lapachones Carrying Substituents in the Benzene Ring. General Procedure for the Preparation of the Lapachols 4a–d. The phenylacetic acids **2b,c** were prepared by the same method as for the preparation of **2a**.[10] The ethyl esters of **2a–c** were prepared as follows. The phenylacetic acid (0.44 mol) was slowly added to a solution of H_2SO_4 (31 g) in absolute EtOH (270 mL), and the mixture was stirred at room temperature for 3 days. The clear mixture was poured into 1 N $NaHCO_3$ (800 mL) at 0–10 °C and thereafter stirred for 1 h. The esters were extracted with chloroform and, after removal of the solvent in vacuo, dried to constant weight at 0.01 Torr. They were used for the preparation of the hydroxynaphthoquinones **3a–d** without further purification. The ethyl ester **2d** was prepared by the published procedure.[14]

The hitherto unknown hydroxynaphthoquinones **3b,c** were prepared by the procedure published for the preparation of **3d**, starting from the ethyl esters of **2b** and **2c**.

The lapachols **4a–d** were prepared as follows. The potassium salts of **3a–d** (0.153 mol), prepared by the addition of 1 equiv of 1 N KOH, followed by evaporation, trituration of the residue with absolute EtOH, and drying in vacuo at 50 °C, were dissolved in hexamethylphosphorous triamide (HMPT) (500 mL). Potassium iodide (22 g) was added, and the clear solution was stirred at room temperature while adding a solution of 1-bromo-3-methyl-2-butene (22 g, 0.148 mol) in HMPT (20 mL). After the mixture was stirred for 16 h at 45 °C, it was cooled to 5 °C and poured into a mixture of concentrated HCl (60 mL) in ice–water (2 L). The organic matter was extracted with toluene, and, in turn, the toluene layer was extracted successively with 1 N $NaHCO_3$ (3 × 150 mL) and 2 N NaOH (3 × 150 mL). By acidification of the NaOH solutions and extraction with toluene, the crude lapachols **4a–d** were obtained. Recrystallization from 2-propanol gave the pure compounds listed in Table I. Acidification of the $NaHCO_3$ solution and extraction with CH_2Cl_2 yielded the unchanged starting materials. Evaporation of the original toluene layer yielded the O-alkylated products **6**.

Conversion of 6a to Lawsone (3a). The enol ether **6a** (10 g, 0.041 mol) was added in small portions to concentrated H_2SO_4 (50 mL) with gentle stirring at room temperature. After the dark brown–red solution was stirred for an additional 10 min, it was poured into ice–water (500 mL) to yield a granular precipitate. This was dissolved in ethyl acetate and extracted with 2 N Na_2CO_3. After acidification of the aqueous phase, a yellow solid was obtained, which was collected and dried in vacuo to give lawsone (**3a**): yield 6 g (83%); mp 192–194 °C; IR identical with that of commercially available **3a**.

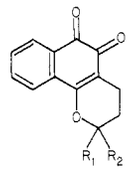
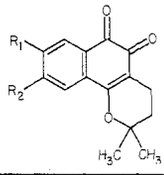
General Procedure for the Preparation of the Lapachols (8b–i). The bromides **7b–i** were prepared as follows. A solution of pyridine (4.1 mL) and phosphorous bromide (7.6 mL) in petroleum ether (100 mL) was cooled to −15 °C. A solution of the corresponding allyl alcohols (0.25 mol) in ether (100 mL) was added during 4.5 h while stirring the mixture. After stirring for as further hour at −15 °C, the mixture was allowed to reach room temperature. Water was added, and the organic phase was separated. It was washed with water, dried over Na_2SO_4, and evaporated in vacuo to give a light pink oil, which was dried in vacuo to constant weight. The lapachols **8b–i** were prepared in a fashion analogous to the one described for **4a–d** using the commercially available lawsone and the bromides **7a–i**. The yields and analytical data are given in Table IV.

General Procedures for the Preparation of the β-Lapachones 5a–d and 10b–i. The corresponding lapachols **4a–d** and **8b–i** (0.03 mol) were added in portions at 20 °C while stirring to concentrated H_2SO_4 (80 mL). After the addition, the dark mixture was stirred for an additional 15 min and then poured into ice. The mixture was extracted with toluene. The organic phase was washed with water, dried over Na_2SO_4, and evaporated in vacuo to yield the crude products. Recrystallization from 2-propanol or ether–petroleum ethers gave the pure products described in Table III and Table IV.

Biology. In Vivo Experiments. In experiments with Rauscher leukemia 4 **O** (NIH), Balb/c mice (28–34 days old) were infected ip with 0.2 mL of a suspension of virus material (dilution 10^{-1} in Hank's solution) from spleens of syngeneic mice that had been infected 4–6 weeks before. The stock material was prepared by homogenizing enlarged spleens (2.5–3.0 g) in a medium consisting of 15% glycerol puriss and 10% calf serum in Hanks' solution in a 1:10 dilution with a "VIRTIS" homogenizer for 3 min and centrifugation at 1200 rpm for 5 min. The supernatant was stored in ampules of 2 mL each at −85 °C.

(14) Burnett, A. R.; Thompson, R. H.; *Chem. Ind. (London)* **1968**, 1771.

Table VI. Biological Effects of β-Lapachone Derivatives **5** and **10**

structure	derivative[a]	R_1	R_2	MTD,[b] mg/kg po	inhibn of R leukemia[c]	inhibn of RDDP[d] RLV	inhibn of RDDP[d] AMV
	**10a**	CH_3	CH_3	1250	expt 1: 34.8/26.0 expt 2: 48.5/31.6[e] expt 3:51.1/34.1[f]	<2	<2
	10b	CH_3CH_2	CH_3CH_2	2500	61.7/46.0	nt	2
	10c	CH_3	CH_3CH_2	125	nt	nt	<2
	10d	H	C_6H_5	>2500	66.8/58.8	nt	<2
	10e	CH_3	C_6H_5	>2500	expt 1: 55.8/26.0[f] expt 2: 78.0/58.8[f]	2	2
	10f	$-(CH_2)_4-$		>2500	nt	nt	3
	10g	$-(CH_2)_5-$		>2500	46.8/46.0	nt	3
	10h	$-(CH_2)_6-$		nt	nt	nt	15
	10i	adamantyl		>2500	69.0/58.8	nt	5
	**5a**(=**10a**)	H	H				
	5b			>2500	45.8/31.6[f]	nt	nt
	5c			>2500	38.9/31.6	nt	nt
	5d	CH_3O	CH_3O	>1250	39.6/46.0	nt	5

[a] Lapachol and a number of other related compounds were marginally active or inactive. [b] Maximum tolerated dose; approximate determination, one administration, and 7 days observation. [c] Mean survival time (days) of treated/controls; treatment began 30 min before and then once daily with 125 mg/kg po for 4 weeks. [d] Micrograms per milliliter yielding 50% inhibition (ED50). [e] $p \leq 0.05$ (Student's t test). [f] $p \leq 0.01$ (Student's t test).

The original virus material caused 100% mortality after the 4th passage in female Balb/c mice with 0.2 mL of the dilution of 10^{-1} intraperitoneally. The dilution of $10^{-1.2}$ caused 40–50% mortality; $10^{-1.4}$ and $10^{-1.8}$ dilutions caused 10–20% mortality. The mean survival time after infection with 0.2 mL of 10^{-1} dilution was about 30 days. [In male Balb/c mice, 0.2 mL of 10^{-1} dilution also caused 100% mortality, but the mean survival time was much longer (>70 days).]

The hematological and pathological investigation of 12 mice each 3, 4, 6, and 8 weeks after infection revealed an initial erythoblastosis, followed by leukemia from the 4th week onwards.

β-Lapachone or its analogues were suspended in distilled water with 0.5% CMC Tylose C 600 (Hoechst, Germany). The inidividual doses were given in 0.5 mL/20 g of mouse. The suspension medium alone showed no effect.

The approximate maximum tolerated dose (MTD) of the β-lapachone analogues was determined by using NMRI male mice and an observation period of 7 days. Those compounds with an MTD > 500 mg/kg ip were tested in Rauscher leukemia (Table VI). The MTD of β-lapachone was about 1.250 mg/kg po in mice and >1.250 mg/kg po in chickens.

In Vitro Experiments. Inhibition of reverse transcriptase activities was measured as previously described[2] with poly(rA)·oligo(dT) or poly(rC)·oligo(dG) as template primer (20 μg/mL) and [^{3}H]dTTP or [^{3}H]dGTP as substrates (20 μM, 0.5 Ci/nmol). For inhibition studies, reference values were obtained by using the equivalent amount of solvent (Me_2SO) without inhibitor. The results are expressed as EC_{50} values, i.e., the effective concentration of drug producing 50% inhibition of the enzyme activity.

Acknowledgment. We thank E. Kuettel, B. Poncioni, and J. L. Crespo for their skilled technical assistance and A. H. Kirkwood for revising the English manuscript.

Registry No. **1a**, 767-60-2; **1b**, 90149-70-5; **1c**, 52310-59-5; **2a**, 36073-90-2; **2a** ethyl ester, 3469-05-4; **2b**, 90149-71-6; **2b** ethyl ester, 90149-72-7; **2c**, 90149-73-8; **2c** ethyl ester, 90149-74-9; **2d**, 38210-84-3; **2d** ethyl ester, 90149-75-0; **3a**, 83-72-7; **3a**·K, 36417-18-2; **3b**, 74237-21-1; **3b**·K, 90149-76-1; **3c**, 58472-21-2; **3c**·K, 90149-77-2; **3d**, 17173-25-0; **3d**·K, 90149-78-3; **4a**, 84-79-7; **4b**, 90149-79-4; **4c**, 90149-80-7; **4d**, 90149-81-8; **5a**, 4707-32-8; **5b**, 90149-82-9; **5c**, 90149-83-0; **5d**, 90149-84-1; **6a**, 42164-69-2; **7a**, 870-63-3; **7b**, 21378-06-3; **7c**, 869-72-7; **7d**, 4392-24-9; **7e**, 90149-85-2; **7f**, 931-42-0; **7g**, 932-86-5; **7h**, 90149-86-3; **7i**, 90149-87-4; **8b**, 90149-88-5; **8c**, 90149-89-6; **8d**, 90149-90-9; **8e**, 90149-91-0; **8f**, 36417-19-3; **8g**, 36417-21-7; **8h**, 90149-92-1; **8i**, 90149-93-2; **10b**, 90149-94-3; **10c**, 90149-95-4; **10d**, 90149-96-5; **10e**, 90149-97-6; **10f**, 90149-98-7; **10g**, 90149-99-8; **10h**, 90150-00-8; **10i**, 90150-01-9; $(CH_3)_2C{=}CHCH_2Br$, 870-63-3; $HOCH_2CH{=}C(CH_2CH_3)_2$, 39821-65-3; $HOCH_2CH{=}C(CH_3)CH_2CH_3$, 2747-48-0; $HOCH_2CH{=}C(Ph)CH_3$, 1504-54-7; $HOCH_2CH{=}\overline{C(CH_2)_4}$, 931-43-1; $HOCH_2CH{=}\overline{C(CH_2)_5}$, 932-89-8; $HOCH_2CH{=}\overline{C(CH_2)_6}$, 4448-83-3; 2-(2-hydroxyethanylidene)adamantane, 90150-02-0.

(15) Fieser, L. F. *J. Am. Chem. Soc.* **1926**, *48*, 3201.