Antiviral Compounds. 1. Structure–Activity Relationship of Some Antiviral Enediones Derived from Aldehydo Sugars

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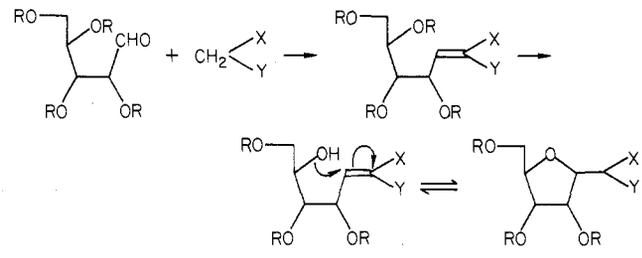
A series of aldehydo sugars was subjected to condensation reactions with active methylene compounds. Acetylacetone was condensed with 2,4-*O*-benzylidene-3,5-*O*-dibenzoyl-D-ribose (**1**), 2,4:3,5-*O*-dibenzylidene-D-ribose (**6**), 2,3,4,5-tetraacetyl-D-ribose (**7**), and 2,3,4,5,6-pentaacetyl-D-glucose (**9**) to yield 3-ylidene-2,4-pentanedione derivatives **2**, **11**, **12**, and **13**, respectively. Sugar derivatives **1** and **6** were also condensed with benzoylacetone to give **14** and **18**, with acetoacetanilide to give **16** and **19**, with malononitrile to give **17** and **20**, and with α-(γ-butyrolactonylidene)triphenylphosphorane to give **21** and **22**, respectively. Condensation of **1** with dibenzoylmethane gave **15**. The double bond in compounds **2** and **11** was saturated by hydrogenation to give **23** and **24**. All α,β-unsaturated carbonyl compounds obtained exhibited antiviral activity and cytotoxicity. Compound **11** was found to have the most significant and selective antiviral activity against herpes simplex virus.

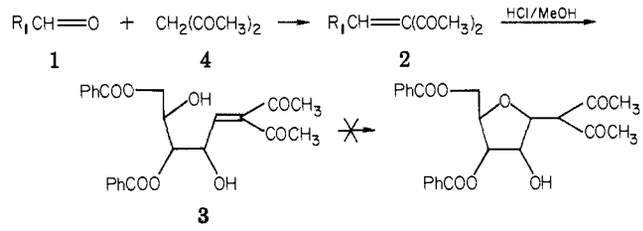
The discovery of C-nucleosides in nature and their interesting antiviral and anticancer properties stimulated a large volume of research directed toward the synthesis of their analogues.[1] We have initiated a research program aimed toward developing a convenient general route for the synthesis of C-nucleoside analogues based on a Knoevenagel-type condensation between an active methylene compound and an appropriately protected ribose molecule. Such a condensation would lead to a ribosylidene derivative which might be expected to undergo cyclization to a ribofuranoside, after deprotection of the 4-hydroxy group (Scheme I). Groups X and Y would serve as synthetic handles for the construction of various heterocyclic rings.

A survey of the literature revealed that the Knoevenagel reaction has sporadically been employed previously in sugar chemistry;[2] however, no report exists involving the condensation of ribose derivatives. Zinner and co-workers reported that condensation of 2,3:4,5-di-*O*-isopropylidene-D-arabinose with a series of β-dicarbonyl compounds leads to the expected unsaturated derivatives.[2c] A recent reexamination of this reaction with methyl acetoacetate showed that the reaction is more complicated and that it leads to a mixture of three products.[2a] In our hands, the Knoevenagel reaction of 2,3:4,5-di-*O*-isopropylidene-D-arabinose under the conditions reported gave erratic results. In one instance, we isolated a product that appears to be a bis-condensate from its molecular weight.[3] Consequently, we sought more reliable reaction conditions. After having examined piperidine, diethylamine, potassium fluoride combined with 18-crown-6, triethylamine, and ammonium acetate as potential catalysts for the condensation of acetylacetone with aldehydo sugars, we found that titanium tetrachloride, which has been introduced recently for catalyzing Knoevenagel reactions,[4] is an efficient catalyst for these reactions. This method, however, has the limitation that the sugar has to be protected as an acetal or ester. A trityl protecting group has been found to undergo cleavage under the reaction conditions. Partially unprotected sugars, such as D-ribose-2,3-acetonide or 4,6-*O*-benzylidene-D-glucose, could not be used, since we were not able to recover either product or starting material from reactions of those, presumably due to water solubility. However, aldehydo sugars fully protected as acetals, esters, or mixed acetals–esters could be reacted with β-dicarbonyl compounds to yield, reproducibly, the corresponding unsaturated condensates.

Scheme I



Scheme II



Thus, reaction of 2,4-*O*-benzylidene-3,5-*O*-dibenzoyl-D-ribose (**1**) with acetylacetone (**4**) gave enedione **2** (Scheme II). This compound was subjected to acid hydrolysis, which gave the partially deprotected compound **3**. This did not undergo spontaneous cyclization to the furanoside **4**, contrary to such precedents in the literature. Other analogous compounds that did not cyclize spontaneously were converted to the ribofuranoside by base catalysis.[5]

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(1) (a) S. Hanessian and A. G. Pernet, *Adv. Carbohydr. Chem. Biochem.*, *33*, 111 (1976); (b) G. Doyle Daves and C. C. Cheng, *Prog. Med. Chem.*, **13**, 302 (1976).
(2) (a) F. J. Lopez Herrera, *Tetrahedron Lett.*, **21**, 4963 (1980); (b) F. J. Lopez Aparicio, F. J. Lopez Herrera, and F. Garcia Gonzalez, *An. R. Soc. Esp. Fis. Quim., Ser. B*, **54**, 705 (1958); (c) H. Zinner, E. Wittemburg, and G. Rembarz, *Chem. Ber.*, **92**, 1614 (1959); (d) N. K. Kochetkov and B. I. Dmitriev, *Chem. Ind. (London)*, 2147 (1962); (e) F. Michael and W. Moeller, *Ann. Chem.*, **670**, 63 (1963); (f) I. Alonso Cermeno, A. M. Gonzalez Nogal, and F. J. Lopez Aparicio, *An. R. Soc. Esp. Fis. Quim., Ser. B*, **68**, 285 (1972); (g) F. J. Lopez Aparicio and F. J. Lopez Herrera, *ibid.*, **72**, 931 (1976); (h) F. J. Lopez Aparicio, M. Gomez Guillen, and I. Izquierdo Cubero, *ibid.*, **72**, 938 (1976); **73**, 1168 (1977); (i) F. J. Lopez Aparicio, F. J. Lopez Herrera, and J. Sanches Ballesteros, *Carbohydr. Res.*, **69**, 55 (1979).
(3) D. Melumad, Ph.D. Thesis, The Hebrew University of Jerusalem, 1979.
(4) W. Lehnert, *Synthesis*, 667 (1974); *Tetrahedron*, **30**, 301 (1974); **29**, 635 (1973); **28**, 663 (1972).

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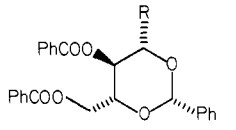
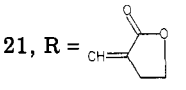
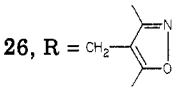
Chart I

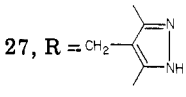


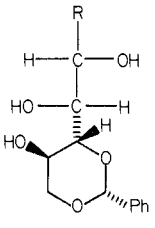
1, R = CHO
2, R = CH=C($COCH_3$)$_2$
14, R = CH=C(COPh)-$COCH_3$
15, R = CH=C(COPh)$_2$
16, R = CH=C($COCH_3$)-CONHPh
17, R = CH=C(CN)$_2$



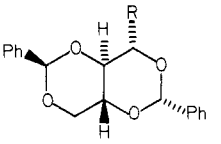
23, R = $CH_2CH(COCH_3)_2$
25, R = $CH_2CH[C(=NOH)CH_3]_2$



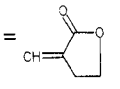




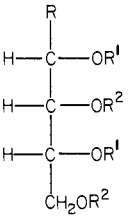
8, R = CHO
20, R = CH=C(CN)$_2$



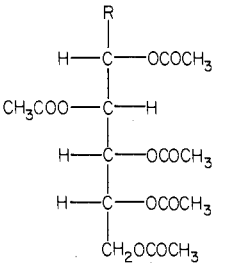
6, R = CHO
11, R = CH=C($COCH_3$)$_2$
18, R = CH=C(COPh)-$COCH_3$
19, R = CH=C(COPh)-CONHPh



24, R = $CH_2CH(COCH_3)_2$



3, R = CH=C($COCH_3$)$_2$; R^1 = H; R^2 = CO
7, R = CHO; R^1 = R^2 = $COCH_3$
10, R = CH=C($COCH_3$)$_2$; R^1 = $COCH_3$; R^2 = COPh
12, R = CH=C($COCH_3$)$_2$; R^1 = R^2 = $COCH_3$



9, R = CHO
13, R = CH=C($COCH_3$)$_2$

Accordingly, we treated **3** with potassium *tert*-butoxide and, in a separate experiment, with boron trifluoride etherate but did not observe any change in starting material **3**, as indicated by its NMR spectrum and comparison of other properties.

It is widely recognized that many compounds with α,β-unsaturated carbonyl functions may act as biological alkylating agents.[6] Consequently, it was of interest to subject compound **2** to biological testing. When preliminary results indicated (see section on virology) that it possessed significant activity, we synthesized a series of

Table I. Semiquantitative Evaluation of Toxicity and Antiviral Activity

group	no.	cytotoxicity, mm	antiviral act., mm: HSV-1	vaccinia	Semliki Forest
2	1	7	1	8	0
	2	4	5	5	0
	3	5	3	7	3
	6	8	8	12	6
	10	2	4	6	4
	13	8	1	3	0
	14	2	0	8	0
	15	4	2	6	1
	16	4	2	9	0
	19	11	9	9	0
	21	0	2	4	3
	22	3	0	14	0
3	11	10	11	5	0
	12	13	11	0	0
	18	14	12	8	0

condensates of other related sugar aldehydes with **4** to determine the structure–activity relationship in this series. The sugar aldehydes that reacted with **4** were **6**, **7**, and **9**. Following this, additional condensation reactions were carried out with β-dicarbonyl compounds: benzoylacetone, dibenzoylmethane, acetoacetanilide, as well as malononitrile with **1** and **6**. These two sugar derivatives were also reacted with the Wittig reagent derived from α-bromo-γ-butyrolactone to give the unsaturated lactone derivatives **21** and **22**, respectively. The products of these reactions were characterized by UV, IR, NMR, and mass spectrometry. The spectra obtained were fully consistent with the structures indicated.

In order to determine the role of the carbon–carbon double bond in the biological activity of this series, compounds **2** and **11** were subjected to catalytic hydrogenation to yield the corresponding saturated β-diketones, **23** and **24**. Compound **23** was also reacted with hydroxylamine to give dioxime **25** and isoxazole **26**. Reaction of **23** with hydrazine gave pyrazole **27**. Compounds **26** and **27** represent analogues of "acyclic nucleosides". Compounds of this type were recently the subject of considerable interest; their ability to mimic the natural sugar nucleosides or to modify the solubility properties and transport behavior of the attached heterocyclic ring in the biological systems was evaluated.[7]

Antiviral Activity. Toxicity and antiviral activity of the compounds were determined by a semiquantitative method. The drug, placed in the center of the agar overlay of the infected cultures, diffuses from the disk, thus forming a gradient of concentrations—higher in the center of the culture (location of the disk) and gradually decreasing toward the periphery. The toxicity was determined for BSCl and Vero cells, which were originated from green monkey kidney, while antiviral activity was studied with Semliki Forest (RNA-containing virus) and herpes simplex type 1 (HSV-1) and vaccinia viruses (DNA-containing viruses). (HSV-1 is considered today as one of the most important candidates for antiviral chemotherapy.) The compounds tested in the present study are divided

(5) (a) J. G. Buchanan, A. R. Edgar, M. J. Power, and P. D. Theaker, *Carbohydr. Res.*, **38**, C22 (1974); (b) S. Hanessian, T. Ogawa, and Y. Cuindon, *ibid.*, **38**, C12 (1974); (c) C. K. Chu, I. Wenipen, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, **41**, 2793 (1976).

(6) (a) K.-H. Lee, T. Ibuka, S.-H. Kim, B. R. Vestal, I. H. Hall, and E.-S. Huang, *J. Med. Chem.*, **18**, 812 (1975), and references cited therein; (b) S. M. Kupchan, *Pure Appl. Chem.*, **21**, 231 (1970); (c) S. Roy-Burman, Y. H. Huang, and D. W. Visser, *Biochem. Biophys. Res. Commun.*, **42**, 445 (1971).

(7) (a) D. Horton, D. C. Baker, and S. S. Kokrady, *Ann. N.Y. Acad. Sci.*, **255**, 131 (1975); (b) M. Fuertes, M. T. Garcia-Lopez, G. Garcia-Munoz, and R. Madronero, *J. Carbohydr. Nucleosides, Nucleotides*, **2**, 277 (1975); (c) R. Bognar, Z. Gyorgydeak, L. Szilagyi, G. Horvath, G. Czira, and L. Radics, *Ann. Chem.*, 450 (1976); (d) I. Farkas, I. F. Szabo, and R. Bognar, *Carbohydr. Res.*, **48**, 159 (1976), and previous papers in this series.

into three groups, according to their antiviral activity: (1) compounds that do not affect the growth of the viruses examined, (2) compounds that exhibit an equal or higher level of antiviral activity toward vaccinia virus than to HSV-1, and (3) compounds that show higher inhibitory activity toward HSV-1 than to vaccinia virus. In the first group are included compounds **8**, **17**, **20**, **23**, **24**, **26**, and **27**. The antiviral activities of compounds of the second and third groups are presented in Table I. All α,β-unsaturated carbonyl compounds belong to groups 2 and 3, displaying biological activity. Moreover, that activity is seemingly associated with the α,β-unsaturated carbonyl function. This can be inferred from the observation that saturation of the double bond is accompanied by total loss of both the cytotoxic and the antiviral activity. Both hydrogenated derivatives **23** (from **2**) and **24** (from **11**) belong to group 1. Lack of activity in these β-diketones is in contrast to the series of antiviral β-diketones, reported by Diana and co-workers, which are active against both RNA and DNA viruses.[8]

Two other compounds that were prepared in this work, containing a polar double bond, are the ylidenemalononitriles **17** and **20**. However, this function does not appear to possess the activity associated with the α,β-unsaturated carbonyl function; compound **20** showed no activity (group 1), while the toxicity of **17** can be attributed to its lower solubility and its precipitation on the cell surface. The two acyclic sugar nucleoside dervatives **26** and **27** were found to be biologically inactive. It is remarkable that some of the protected sugar derivatives were found to be active. Thus, the two ribose derivatives 2,4-*O*-benzylidene-3,5-*O*-dibenzoylribose (**1**) and 2,4:3,5-*O*-dibenzylideneribose (**6**) were found to be both cytotoxic and antiviral (group 2). The data (Table I) show that compounds **11**, **12**, and **18** are highly active against HSV-1 but less active against vaccinia virus. Of these, the lowest cytotoxicity is shown by compound **11**. This compound was chosen for further investigation of the nature of its antiviral activity.

First, we determined the optimal concentration of this compound necessary for the inhibition of the growth of HSV-1. The virus was grown for 22 h in the presence of different concentrations of the compound. Compound **11** at a concentration of 68 μg/mL is toxic, and partial toxic effects were visible at 34 μg/mL. Therefore, most of the decrease in virus infectivity caused by these two concentrations can be attributed to the toxicity to the cells. In the presence of lower concentrations, no toxic effects were visible. A concentration of 17 μg/mL inhibited virus growth by 99.33%, while 8.5 μg/mL was ineffective. The results indicated that the range of concentrations of compound **11**, which is inhibitory to HSV-1, is quite narrow and is around 17 μg/mL.

Specific antiviral activity against a particular virus can be shown with this virus in combination with at least one other virus, when they are capable to to grow under similar conditions, in the same host cells. Compound **11**, at a concentration of 17 μg/mL, was included in the agar overlay of BSCl cultures infected with serial dilutions of HSV-1 and vaccinia virus. The number of plaques was counted 4 days after infection and compared to that of infected untreated cultures. The results showed that plaque formation of HSV-1 was inhibited by more than 730-fold by compound **11**, while vaccinia virus was hardly affected.

Table II. Growth of HSV-1 in BSCl in the Presence of Several Inhibitors

time, h	treatment	concn, μg/mL	concn, μM	virus titer (pfu/mL)	inhibn, %
0				1.2×10^4	
22				1.5×10^9	0
22	11	17.0	42.2	6.7×10^7	95.5
22	28	0.4	1.1	4.3×10^8	71.3
22	30	20.0	82.3	5.3×10^7	96.5
22	29	0.7	2.9	9.7×10^7	93.6

Table III. DNA Synthesis of HSV-1 and BSCl Cells in the Presence of Several Inhibitors[a]

treatment	concn, μg/mL	DNA synthesis, % viral	DNA synthesis, % cellular
		100.00	100.00
11	17.0	50.86	80.66
28	0.8	31.05	21.47
30	20.0	0	86.79
29	1.4	0	38.28

[a] The DNA was analyzed as described under Experimental Section. Radioactivity of the fractions included in the viral and cellular DNA peaks was summarized, and their ratio (%) relative to the radioactivity in infected untreated culture was calculated.

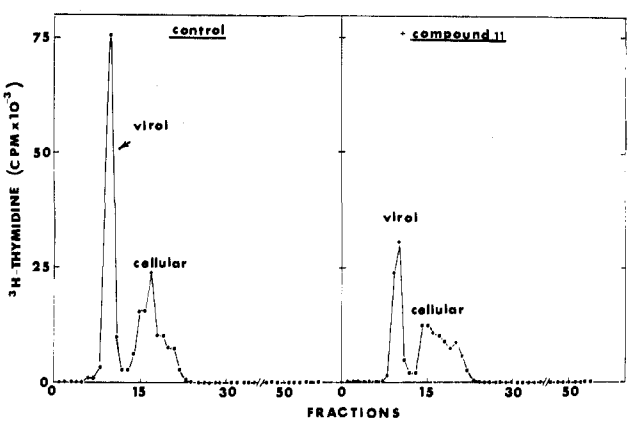


Figure 1. Cellular and HSV-1 DNA synthesized in the absence and in the presence of compound 11 (17 μg/mL), separated in CSCl gradients.

The effect of compound **11** was then compared with several drugs known to inhibit the growth of HSV-1: 5-iodo-2′-deoxyuridine (**28**),[9] cytosine arabinoside (**29**),[10] and adenosine arabinoside (**30**).[11] In order to be at the range of concentrations that exerts minimal toxic effects on the host cell, we chose concentrations that inhibit the growth of the virus between 71.3 and 96.5% (Table II). The results indicate that **28** and **29** are effective at much lower molar concentrations as compared to **30** and **11**. However, the concentrations of **30** and **11** needed for establishing a similar level of inhibition are quite similar one to another (Table II).

(8) (a) G. D. Diana, U. J. Salvador, E. S. Zalay, R. E. Johnson, J. C. Collins, D. Johnson, W. B. Hinshaw, R. R. Lorenz, W. H. Thielking, and F. Pancic, *J. Med. Chem.*, **20**, 750 (1977); (b) G. D. Diana, U. J. Salvador, E. S. Zalay, P. M. Carabateas, G. L. Williams, J. C. Collins, and F. Pancic, *ibid.*, **20**, 757 (1977); (c) G. D. Diana, P. M. Carabateas, U. J. Salvador, G. L. Williams, E. S. Zalay, F. Pancic, B. A. Steinberg, and J. C. Collins, *ibid.*, **21**, 689 (1978); (d) G. D. Diana, P. M. Carabateas, R. E. Johnson, G. L. Williams, F. Pancic, and J. C. Collins, *ibid.*, **21**, 889 (1978).

(9) A. Diwan and W. H. Prusoff, *Virology*, **34**, 184 (1968).

(10) S. S. Cohen, *Prog. in Nucleic Acid Res. Mol. Biol.*, **5**, 1–88 (1966).

(11) M. Privat-de Garilhe and J. de Rudder, *C. R. Hebd. Seance Acad. Sci.*, **259**, 2725 (1964).

Table IV. Products for Titanium Tetrachloride Catalyzed Knoevenagel Reactions of Aldehydo Sugar Derivatives

no.	reaction time, h	yield, %	mp, °C (solv of recrystn)[a]	$[\alpha]^{20}_D$, deg	TLC solvent[b]	formula	anal.
2	72	65	128 (A)	−10.2	C	$C_{31}H_{28}O_8$	C, H
11	72	44	148 (B)	−66.0	C	$C_{24}H_{24}O_6$	C, H
12	72	25		0	D	$C_{18}H_{24}O_{10}$	C, H[d,g]
13	48	15		0	D	$C_{21}H_{28}O_{12}$	C, H[e,g]
14[c]	72	35	130 (B)	+8.2	C	$C_{36}H_{30}O_8$	C, H
15	72	10		+32.0	C	$C_{41}H_{32}O_8$	C, H[f,g]
16[c]	72	25	82 (B)	+35.9	C	$C_{36}H_{31}NO_8$	C, H, N
18[c]	48	33	90 (B)	−67.9	C	$C_{29}H_{26}O_6$	C, H
19[c]	72	68	147 (B)	−47.7	D	$C_{29}H_{27}NO_6$	C, H, N

[a] Solvent of recrystallization: A, dilute methanol; B, chloroform–hexane. [b] TLC developer solvent: C, 1% methanol in chloroform; D, 2% methanol in chloroform. [c] The formation of only one of the two possible geometrical isomers was observed. The stereochemistry around the double bond is not established. [d] Calcd: C, 54.00; H, 6.00. Found: C, 53.20; H, 6.81. [e] C: calcd, 53.88; found, 52.18. [f] C: calcd, 75.46; found, 74.38. [g] This compound could not be crystallized; it was purified by preparative thick-layer chromatography. Its structure assignment is based upon UV, IR, NMR, and mass spectra. Its homogenity was established by thin-layer chromatography.

Since Semliki Forest virus (an RNA-containing virus), as well as other viruses that belong to this category (influenza virus, Victoria strain, Newcastle disease, poliomyelitis type 1 and Sindbis viruses, data not shown), was not inhibited by compound **11**, we suggest that a function involved in the DNA synthesis of HSV-1, and not RNA synthesis, may be involved in the inhibition caused by this compound. In order to examine this point further, we labeled infected cells with [^{3}H]thymidine and determined the synthesis of viral and cellular DNA. Since HSV-1 DNA has a different density as compared to host-cell DNA, it is possible to determine the effect of the compound on each species of DNA separately.[12] Figure 1 shows that the inhibition of the synthesis of viral DNA by compound **11** is more effective than that of host-cell DNA. A quantitative comparison of these findings is presented in Table III. Compound **11** shows higher selectivity than **28** in the inhibition of viral DNA, as compared to cellular DNA. The selectivity of **30** and **29** is greater than that exhibited by the other two compounds; these two drugs inhibit completely the DNA synthesis of HSV-1, while their inhibitory effect on host-cell DNA synthesis is much lower. Compound **30** shows the best antiviral selectivity of all four compounds tested here.

Conclusion

Among the new series of compounds included in this study, compound **11** displayed the best inhibitory effect on the growth of HSV-1. It appears to be selective in its antiherpes virus activity, since it affects other viruses, including vaccinia virus, to much lesser degrees. Although its selectivity is lower than that of **30** and **29** in inhibiting the synthesis of HSV-1 DNA as compared to host-cell DNA, it is significantly better than that of **28** at comparable concentrations.

Experimental Section

General Procedure for Titanium Tetrachloride Catalyzed Condensations. A solution of 1.1 mL of titanium tetrachloride in 2.5 mL of dry carbon tetrachloride is added to 20 mL of dry tetrahydrofuran; precipitation is observed. To the resulting mixture is added a solution containing 5 mM active methylene compound and 5 mM protected sugar derivative in 12.5 mL of tetrahydrofuran, followed by the slow addition (1 h, with stirring at 0 °C) of a solution of 1.6 mL (20 mM) of pyridine in 3.5 mL of dry tetrahydrofuran. The reaction mixture is left to be stirred for the period stated in Table I at 0 °C, while protected from moisture. After that, 50 mL of water is added, and the reaction mixture is extracted by chloroform. The organic solution is washed with a solution of sodium chloride and dried over sodium sulfate. After the removal of the solvent, the residue is separated by thick-layer chromatography. Physical constants, yields, and other characteristics of the products of these reactions are listed in Table IV.

3-(3′,5′-*O*-Dibenzoyl-D-ribosylidene)-2,4-pentanedione (3). Compound **2** (0.528 g) was dissolved in a mixture of 50 mL of methanol and 0.5 mL of concentrated hydrochloric acid, and the solution was left to stand at ambient temperature for 48 h. After the solvent was stripped, the residue was separated by thick-layer chromatography, developed by 1% methanol in chloroform, yielding 220 mg of product as a viscous oil: $[\alpha]^{20}_D$ +28.8°; IR (neat film) 1000, 1270, 1450, 1660, 1720 cm^{-1}; UV max (MeOH) 245 nm (ϵ 25 500); ^{1}H NMR δ 2.18 (s, 3 H), 2.36 (s, 3 H), 5.8 (s, 1 H), 6.66 (d, 1 H, J = 8 Hz), 7.1–7.8 (m, 11 H), 7.8–8.2 (m, 4 H); MS, m/e 528 (M$^+$), 486, 380, 258. Anal. Calcd for $C_{24}H_{24}O_8$: C, 65.48; H, 5.45. Found: C, 64.77; H, 6.34.

3-(2′,4′-*O*-Diacetyl-3′,5′-*O*-dibenzoyl-D-ribosylidene)-2,4-pentanedione (10). Compound **3** (440 mg, 1 mM) was dissolved in 3 mL of pyridine and treated in the cold with 3 mL of acetic anhydride. After 24 h at ambient temperature, the mixture was diluted with water, and the oily layer was taken up in chloroform, which was washed successively with 1 N sulfuric acid and saturated sodium bicarbonate solutions and then dried over sodium sulfate. The chloroform was removed after drying over sodium sulfate, and the residue was purified by thick-layer chromatography over silica gel developed by 1% methanol in chloroform to yield 185 mg of viscous oil: $[\alpha]^{20}_D$ +14°; IR (neat film) 1270, 1450, 1680, 1720, 1740 cm^{-1}; UV max (MeOH), 245 (ϵ 17 100); ^{1}H NMR δ 2.00, 2.66 (4 s, 12 H), 7.00–7.66 (m, 6 H), 7.83–8.16 (m, 4 H); MS, m/e 404 (M$^+$ − 2CH$_3$CO$_2$H). Anal. Calcd for $C_{28}H_{28}O_{10}$: C, 64.12; H, 5.34. Found: C, 63.48; H, 5.96.

Condensation of Compounds 1 and 8 with Malononitrile. To a solution containing 2 mM malonitrile and 2 mM protected sugar aldehyde in 50 mL of absolute methanol is added 40 mg of ammonium acetate. After 48 h at room temperature, the solvent is removed and the product is isolated.

2,4-*O*-Benzylidene-3,5-*O*-dibenzoyl-D-ribosylidenemalononitrile (17) crystallized out spontaneously from the reaction mixture in a yield of 55%: mp 129–130 °C (from methanol); $[\alpha]^{20}_D$ −105.3°; IR (KBr) 1040, 1110, 1270, 1450, 1590, 1620, 1720, 2240 cm^{-1}; UV max (CHCl$_3$) 240 nm (ϵ 10 000); ^{1}H NMR δ 6.03 (s, 1 H), 7.0 (s, 1 H), 7.30–7.83 (m, 11 H), 7.83–8.06 (m, 4 H); MS, m/e 250 (M$^+$ − 2PhCO$_2$H). Anal. ($C_{29}H_{22}N_2O_6$) C, H, N.

4,6-*O*-Benzylidene-D-glucosylidenemalononitrile (20) was isolated by thick-layer chromatography developed by 9% methanol in chloroform in 35% yield: mp 69–71 °C (chloroform–hexane); $[\alpha]^{20}_D$ −16.0°; IR (KBr) 1040, 1080, 1400, 1450, 1600, 1680, 2200, 3400 cm^{-1}; UV max (MeOH) 250 nm (ϵ 8890); ^{1}H NMR (Me$_2$SO-d_6) δ 5.60 s, 1 H), 6.26 (s, 1 H), 7.26–7.56 (m, 5 H); MS, m/e 316 (M$^+$). Anal. ($C_{16}H_{16}N_2O_5$) C, H. N.

Wittig Reaction of Compounds 1 and 6 with α-(γ-Butyrolactonylidene)phosphorane. A solution of 860 mg (2.5 mM) of Wittig reagent and 2.5 mM sugar derivative in 30 mL of dichloromethane was stirred at room temperature for 24 h.

(12) W. C. Russell and L. V. Crawford, *Virology*, **21**, 353 (1963).

After concentration of the solution, the product was isolated by thick-layer chromatography developed by 1% methanol in chloroform.

α-(2′,4′-*O*-Benzylidene-3′,5′-*O*-dibenzoyl-D-ribosylidene)-γ-butyrolactone (21) was obtained in 20% yield: mp 84–85 °C (chloroform–hexane); $[\alpha]^{20}_D$ −38.6°; IR (KBr) 1030, 1110, 1270, 1450, 1600, 1720, 1760 cm^{-1}; UV max ($CHCl_3$) 245 nm (ε 7000); ^1H NMR δ 2.83–3.33 (m, 4 H), 5.8 (s, 1 H), 6.81 (br, 1 H), 7.10–7.73 (m, 11 H), 7.76–8.16 (m, 4 H); MS, *m/e* 514 (M^+). Anal. ($C_{30}H_{26}O_8$) C, H.

α-(2′,4′:3′,5′-*O*-Dibenzylidene-D-ribosylidene)-γ-butyrolactone (22) was obtained in 32% yield: mp 144–145 °C (chloroform–hexane); $[\alpha]^{20}_D$ −74.1°; IR (KBr) 1020, 1110, 1300, 1450, 1760 cm^{-1}; UV max ($CHCl_3$) 244 nm (ε 2550); ^1H NMR 2.73–3.23 (m, 4 H), 5.60 (s, 1 H), 5.80 (s, 1 H), 6.81 (br, 1 H), 7.16–7.66 (m, 10 H); MS, *m/e* 394 (M^+). Anal. ($C_{23}H_{22}O_6$) C, H.

3-(2′,4′-*O*-Benzylidene-3′,5′-*O*-dibenzoyl-D-ribityl)-2,4-pentanedione (23). Enedione **2** (1 mM) was hydrogenated in 50 mL of ethyl acetate in the presence of 100 mg of 5% palladium on charcoal in a Parr hydrogenation apparatus under a pressure of 14 psi at room temperature for 48 h. The product **23** was obtained in a quantitative yield: mp 130 °C (chloroform–hexane); $[\alpha]^{20}_D$ −3.5°; IR (KBr) 1030, 1120, 1270, 1450, 1600, 1720 cm^{-1}; UV max ($CHCl_3$) 285 nm (ε 16 000); ^1H NMR 2.2 (s, 6 H), 5.62 (s, 1 H), 7.2–7.8 (m, 11 H), 8.0–8.2 (m, 4 H); Mass spectrum, *m/e* 530 (M^+) 302, 180. Anal. ($C_{31}H_{30}O_8$) C, H.

3-(2′,4′:3′,5′-*O*-Dibenzylidene-D-ribityl)-2,4-pentadiene (24). This experiment was carried out following the previous one to give product **24** in a quantitative yield: mp 126 °C (chloroform–hexane); $[\alpha]^{20}_D$ −29.8°; IR (KBr) 1020, 1110, 1410, 1600, 1700, 1720 cm^{-1}; UV max ($CHCl_3$) 290 nm (ε 7700); ^1H NMR ($CDCl_3$) 2.1 (s, 6 H), 5.50 (s, 1 H), 5.57 (s, 1 H), 7.12–7.5 (m, 10 H); mass spectrum, *m/e* 410 (M^+). Anal. ($C_{24}H_{26}O_6$) C, H.

4-(2′,4′-*O*-Benzylidene-3′,5′-*O*-dibenzoylribityl)-3,5-dimethylpyrazole (27). A solution containing 106 mg (2 mM) of **23**, 10 mg of hydrazine hydrate, and 2 μl of acetic acid in 5 mL of methanol was refluxed for 3 h. After the solvent was removed, the product **27** was isolated by thick-layer chromatography on silica gel, developed with 1% methanol in chloroform: mp 130 °C (chloroform–hexane); $[\alpha]^{20}_D$ +2.0°; IR (KBr) 1030, 1120, 1270, 1450, 1600, 1730 cm^{-1}; UV max ($CHCl_3$) 245 nm (ε 15 300); ^1H NMR 2.0 (s, 3 H), 2.06 (s, 3 H), 5.40 (s, 1 H), 7.0–7.5 (m, 11 H), 7.7–8.0 (m, 4 H); mass spectrum, *m/e* 526 (M^+). Anal. ($C_{31}H_{30}N_2O_6$) C, H, N.

3-(2′,4′:3′,5′-*O*-Dibenzylidene-D-ribityl)-2,4-pentanedione Dioxime (25) and 4-(2′,4′-*O*-Benzylidene-3′,5′-*O*-dibenzoylribityl)-3,5-dimethylisoxazole (26). A solution of 530 mg of **23**, 690 mg of hydroxylamine hydrochloride, and 3 mL of pyridine in 50 mL of chloroform is refluxed with stirring for 3 h. The solution is then washed with 1 N sulfuric acid and with a saturated solution of sodium bicarbonate and water. After the solution is dried and the solvent is removed, the residue is separated by thick-layer chromatography on silica gel developed by 1% methanol in chloroform, to yield two products, each in an approximate yield of 20%.

Dioxime 25: mp 150 °C (chloroform–hexane); $[\alpha]^{20}_D$ +2.8°; IR (KBr) 1650, 1730, 3600 cm^{-1}; UV max ($CHCl_3$) 245 nm (ε 12 000), ^1H NMR δ 1.5 (s, 6 H), 5.5 (s, 1 H), 7.00–7.58 (m, 1 H), 7.62–8.00 (m, 4 H); mass spectrum, *m/e* 560 (M^+). Anal. ($C_{31}H_{32}N_2O_8$) C, H, N.

Isoxazole 26: mp 150 °C (chloroform–hexane); $[\alpha]^{20}_D$ −2.0°; IR (KBr) 1030, 1120, 1270, 1450, 1600, 1630, 1730, 2980 cm^{-1}; UV max ($CHCl_3$) 245 nm (ε 21 000); ^1H NMR 2.2 (s, 6 H), 5.5 (s, 1 H), 7.1–7.5 (m, 11 H), 7.8–8.0 (m, 4 H); mass spectrum, *m/e* 527. Anal. ($C_{31}H_{29}NO_7$) C, H, N.

Viruses and Cells. Herpes simplex type 1 (HSV-1; HF strain) and vaccinia WR strain were grown in BSCl cells, and Semliki Forest virus was grown in Vero cells. BSCl cells were cultured in M199 and Vero cells in RPMI, supplemented with 10% inactivated calf serum in 50-mm diameter plastic petri dishes. The cultures were incubated at 37 °C in a humidified atmosphere supplied with 5% CO_2.

Infection Procedure. Cell monolayers were infected with virus at a multiplicity of 1 pfu (plaque forming unit) per cell. After 1 h at 37 °C, the cells were washed, and 5 mL of medium containing 2% calf serum was added.

Plaque Assay. Cell monolayers were infected with 0.3 mL of virus dilution. After 1 h at 37 °C, the cells were overlaid with Eagle's medium containing 1% Agar Noble (difco Laboratories, Detroit, MI) and 5% inactivated calf serum and incubated at 37 °C. After 3 days for Semliki Forest and 4 days for HSV-1 and vaccinia, the cultures were fixed and stained with crystal violet, and plaques were counted.

Semiquantitative Determination of Toxicity and Antiviral Activity. Cell monolayers were infected with a dilution of virus suspension in order to give confluency of plaques. When the agar-containing overlay was solidified, a disk of Whatman 3MM paper (5-mm in diameter) was immersed in a solution containing the examined compound (5 mg/mL in dimethyl sulfoxide) and layered on the agar in the center of the culture. The cultures were incubated at 37 °C for 3 to 4 days and then fixed and stained.

The diameters of the central area, in which the cell monolayer was destroyed by the compound and as a result lost the vital stain, and the external area, in which the cells were alive but plaques were not formed, were measured. The first diameter reflects toxicity, while the second minus the first serves as the semiquantitative value for antiviral activity.

DNA Synthesis and CsCl Gradient Analysis. Infected BSCl cells were labeled with 2 μCi of [^3H]thymidine per milliliter starting at 3 h after infection. The cells were harvested 22 h postinfection, washed, and suspended in 0.15 M NaCl, 0.01 M sodium citrate, pH 7.2. Sodium docecyl sulfate and Pronase were added at a final concentration of 1% and 0.3 mg/mL, respectively. After incubation for 5 h at 37 °C, the DNA was centrifuged in a CsCl density gradient in buffer containing 0.01 M tris(hydroxymethyl)aminomethane, 0.001 M ethylenediaminetetraacetic acid, pH 8.0, in a 50 Ti rotor at 35 000 rounds per minute at 20 °C for 48 h. Fractions were collected from the bottom of the tube, and radioactivity, after trichloroacetic acid precipitation, was determined.