Synthesis and Antiviral Activities of Arabinofuranosyl-5-ethylpyrimidine Nucleosides. Selective Antiherpes Activity of 1-(β-D-Arabinofuranosyl)-5-ethyluracil

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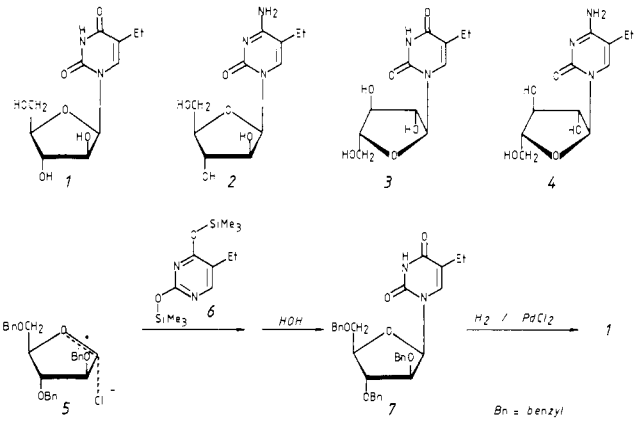
Several procedures are described for the synthesis of arabinofuranosyl-5-ethylpyrimidine nucleosides: (a) condensation of benzylated α-chloroarabinofuranose (**5**) with 2,4-bis-*O*-(trimethylsilyl)-5-ethylpyrimidine (**6**) in the presence of $SnCl_4$ or molecular sieves yielded exclusively the β-anomer **7** and proved to be the best pathway to 1-(β-D-arabinofuranosyl)-5-ethyluracil (**1**); (b) condensation of benzoylated α,β-bromoarabinose **9** with **6** led to a mixture of anomeric nucleosides **10** and **11** in the ratio 5:2; (c) condensation of benzoylated α-bromoarabinose **8** with **6** led exclusively to the α-anomer **10** which, on thiation and amination, gave 1-(α-D-arabinofuranosyl)-5-ethylcytosine (**4**); (d) condensation of benzylated α-halogenoarabinose with 2,4-diethoxy-5-ethylpyrimidine (**15**) in the presence of $SnCl_4$ or molecular sieves gave exclusively the β anomer of the 4-ethoxy nucleoside **16** which, on amination and reduction, was the most convenient procedure for the synthesis of 1-(β-D-arabinofuranosyl)-5-ethylcytosine (**2**). The mechanisms of the various condensation methods are compared and discussed. CD and NMR data are presented for **1** and **2** and their corresponding α anomers. Antiviral activities of **1** and **2** and their α-anomers **3** and **4** were tested in primary rabbit kidney cells and in human skin fibroblasts. Herpes simplex virus was inhibited by **1** at a concentration as low as 2 μg/mL, whereas concentrations exceeding 200 μg/mL were required to inhibit vaccinia virus replication or normal cellular metabolism. Compound **2** was significantly less active as an antiherpes agent than compound **1**. The relative lack of activity of **2** could be attributed to its low susceptibility to enzymatic deamination by mammalian cytosine nucleoside deaminase. The α anomers of **1** and **2** were totally inactive as antiviral agents.

In contrast to the class of 5-substituted pyrimidine deoxyribonucleosides, which includes several compounds with potent antiviral properties,[1] there are only a few examples of arabinofuranosyl 5-substituted pyrimidine nucleosides with appreciable antiviral activity, viz., the 5-halogenated derivatives of *ara*-C (1-β-D-arabinofuranosylcytosine) and *ara*-U (1-β-D-arabinofuranosyluracil), which are active against herpes simplex virus.[2-7] More recently, the 5-(thiocyano), 5-nitro, 5-hydroxy, 5-cyano, and 5-(propynyloxy) derivatives of *ara*-U were evaluated as antiviral agents; two of these, 5-(thiocyano)-*ara*-U and 5-(propynyloxy)-*ara*-U, exhibited anti herpes activity.[8,9] The 5-methyl derivative of *ara*-C has also been reported to inhibit herpes virus replication but only in cells with a substantial level of deoxycytidine deaminase, leading to intracellular conversion of 5-Me-*ara*-C to the active 5-Me-*ara*-U (*ara*-T).[10] The latter has been shown to inhibit replication of herpes simplex and varicella-zoster viruses at doses far below those which are cytotoxic.[11-13] Even the parent *ara*-U, long considered inert as an antimetabolite, has been found to suppress herpes virus replication, albeit to a substantially lower extent than *ara*-T.[14] The foregoing, in conjunction with the known antiviral activity of 5-ethyl-2′-deoxyuridine,[15] pointed to the utility of an examination of the antiviral behavior of 5-Et-*ara*-U. We describe here the synthesis and antiviral properties of 5-Et-*ara*-U (**1**), 5-Et-*ara*-C (**2**), and their corresponding α-anomers **3** and **4**.

Chemistry. The two most convenient condensation procedures leading to the β anomers of cytosine and uracil arabinosyl nucleosides are the Hilbert–Johnson reaction of a 2,3,5-tri-*O*-benzyl-1-halogenoarabinose with the appropriate 2,4-dialkoxypyrimidine[16-19] and the Lewis acid catalyzed (Friedel–Crafts catalysts) condensation of the appropriate bis-*O*-(trimethylsilyl)pyrimidine with the foregoing halogenobenzylarabinose in a solvent of intermediate polarity, e.g., dichloroethane.[20] By contrast, preparation of the corresponding α anomers required the use of a halogenose with a 2-(acyloxy) substituent ("participating group") in a "fusion" reaction, leading to a mixture of the α and β anomers.[21,22]

Scheme I



Purine arabinonucleosides have been prepared by fusion or reaction of the free base in the presence of molecular sieves in low or high polar solvents, e.g., toluene and acetonitrile,[23,24] leading to a mixture of the two anomers, with the α anomer predominating, or exclusively to the α anomer.

The procedure of Vorbruggen,[20] which provides excellent yields with acylated ribose and deoxyribose, provided only 42% of the β anomer of 6-aza-*ara*-U with the use of the halogenobenzylarabinose.[20] With a view to clarification of this anomaly, it was decided to examine it in more detail, partly with the hope of developing a more effective procedure for 5-Et-*ara*-U (**1**) and 5-Et-*ara*-C (**2**), as well as their α-anomers **3** and **4**.

The β anomer of 5-Et-*ara*-U (**1**) was prepared by condensation of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**5**) with 2,4-bis-*O*-(trimethylsilyl)-5-ethylpyrimidine (**6**) in dichloroethane in the presence of molecular sieves (method A) or $SnCl_4$, 0.29 equiv/base (method B) (see Scheme I). In both instances, TLC demonstrated formation of a single product, 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-5-ethyluracil (**7**) as a syrup, with yields of 75 and 85%, respectively. Following workup, the overall yield of crystalline products was 31%; the

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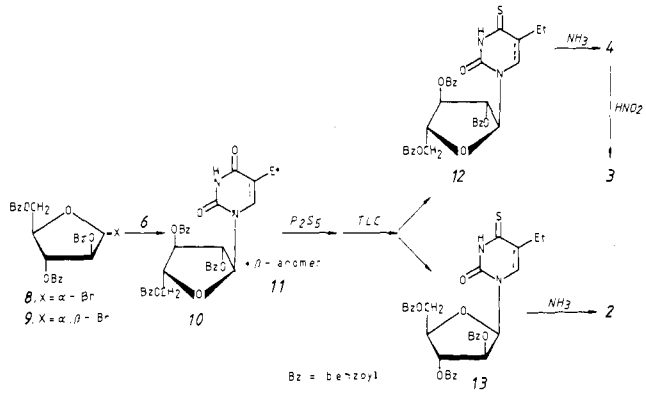
Table I. Thin-Layer Chromatography of Arabinosyl-5-ethylpyrimidine Nucleosides

compd	R_f values in solvent systems						
	A	B	C	D	E	F	G
7	0.73	0.83	0.74	0.35	0.23	0.89	0.98
3	0.66	0.73	0.72	0.00	0.00	0.75	0.56
1	0.66	0.74	0.73	0.00	0.00	0.76	0.56
10	0.74	0.85	0.80	0.30	0.21	0.90	0.95
12	0.71	0.83	0.73	0.73	0.59	0.88	0.95
13	0.75	0.87	0.77	0.77	0.59	0.89	0.97
4	0.50	0.55	0.57	0.00	0.00	0.53	0.28
2	0.52	0.57	0.59	0.00	0.00	0.55	0.30
16[a]	0.75	0.87	0.78	0.42	0.18	0.91	0.94
17[a]	0.71	0.82	0.75	0.00	0.00	0.86	0.95

[a] Compounds 16 and 17 are 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-4-ethoxy-5-ethyl- and 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-4-amino-5-ethyl-2(1*H*)-pyrimidinone, respectively.

Scheme II



remainder could not be readily crystallized. In fact, catalytic reduction of the entire crude product led in both instances to pure 5-Et-*ara*-U (1), as shown by TLC, NMR, and CD (Tables I–III) in 80% yield.

To obtain 5-Et-α-*ara*-U (3) and the 5-ethyl derivatives of *ara*-C, the bromide of 2,3,5-tri-*O*-benzoyl-α-D-arabinose (8, with its "participating group") was condensed with 6 in dichloroethane in the presence of $SnCl_4$, 0.60 equiv/base, yielding exclusively 1-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-5-ethyluracil (10) in 82% yield. Thiation of 10 with P_2S_5 in dioxane[25,26] under reflux gave 1-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-5-ethyl-4-thio-2(1*H*)-pyrimidinone (12) in 80% yield. By contrast, condensation of an anomeric mixture of the bromide 9 with 6 in the presence of molecular sieves gave a mixture of the anomers of the benzoylated nucleosides 10 and 11 in the ratio 5:2 in 71% yield. Fractionation of such a mixture by TLC is difficult, but the anomers of the corresponding 4-thio derivatives are more readily resolved.[27] The mixture of 10 and 11 was therefore thiated as above to give a mixture of the anomeric 4-thio nucleosides 12 and 13 (α/β = 5:2) in 65% yield, which exhibited sufficient differences in R_f values on silica gel (Table I) for isolation. Subsequent amination of each of these led to 5-Et-α-*ara*-C (4) and 5-Et-β-*ara*-C (2) in yields of 74 and 72%. Deamination of 4 with HNO_2 at elevated temperature gave 5-Et-α-*ara*-U (3) in 43% yield (Scheme II).

In view of the low yield of the β anomer relative to the α in the foregoing reaction, 1-bromo-2,3,5-tri-*O*-benzyl-arabinose (14) was reacted with an excess of 2,4-diethoxy-5-ethylpyrimidine (15) in the presence of molecular sieves (with 100% excess 15) or $SnCl_4$ (with 25% excess 15) and led to 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-4-ethoxy-5-ethyl-2(1*H*)-pyrimidinone (16) in 50%

Table II. Proton Chemical Shifts[c] and Coupling Constants[d] of Anomeric Arabinosyl-5-ethylpyrimidine Nucleosides[e]

compd	CH_3	CH_2	H_6	NH_2	H_1'	H_2', H_4'	H_3'	H_5', H_5''	2'-OH	3'-OH	5'-OH	$J_{CH_3CH_2}$	$J_{1'-2'}$	$J_{2'-3'}$	$J_{3'-4'}$
1	1.07	2.26	7.55		6.06	4.04	3.76	3.65	*a*	*a*	*a*	7.4	4.5	*b*	*b*
	(t)	(q)	(s)		(d)	(unres br m)	(q)	(ctr of unres band)							
2	1.06	2.27	7.45	6.90	6.09	3.96	3.71	3.65	5.35		5.03	7.3	4.0	*b*	*b*
	(t)	(q)	(s)	(br s)	(d)	(unres br m)	(q)	(ctr of unres band)	(br m)		(br q)				
3	1.07	2.28	7.57		5.78	4.13	3.99	3.55	*a*	*a*	*a*	7.4	4.5	4.5	4.5
	(t)	(q)	(s)		(d)	(q; H_2', H_4')	(q)	(ctr of unres band)							
4	1.04	2.24	7.43	6.90	5.73	4.08	3.90	3.50	5.56	5.34	4.89	7.5	3.8	4.0	4.0
	(t)	(q)	(s)	(br s)	(d)	(unres br m)	(q)	(ctr of unres band)	(d)	(d)	(q)				

[a] Strong overlapping of OH signals due to exchange with traces of water in Me_2SO-d_6. [b] $J_{2',3'}$ and $J_{3',4'}$ could not be determined because of overlapping of signals due to H_3', H_5', and H_5''. [c] In ppm, vs. internal DSS in Me_2SO-d_6. [d] In Hz. [e] Abbreviations used are: br, broad; unres, unresolved; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Table III. Circular Dichroism Data for Anomeric Arabinofuranosyl-5-ethylpyrimidine Nucleosides in Water (pH 7)

compd	band B_{2u} λ (nm)	band B_{2u} $\theta \times 10^{-3}$
1	270	+15.9
2	278	+15.3
3	275	−5.6
4	276	−7.1

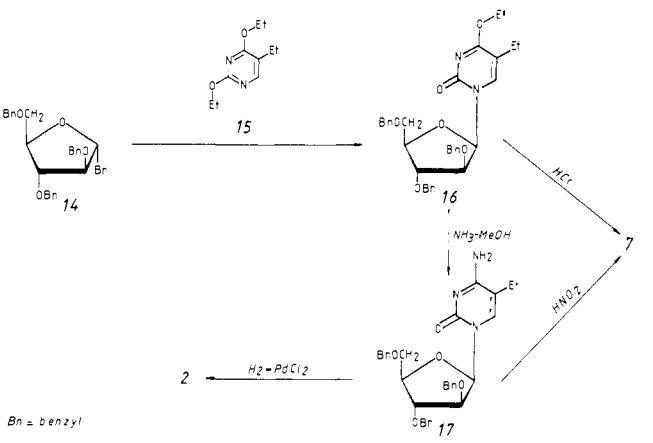
yield. This was isolated by TLC and aminated to give an 80% yield of 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-4-amino-5-ethyl-2(1*H*)-pyrimidinone (**17**) as a syrup. Reduction of the latter on palladium, followed by column chromatography on Dowex 2 (HCO_3^-) led to crystalline 5-Et-*ara*-C (**2**) in 70% yield. Consequently, the sequence **14** → **16** → **17** → **2** (Scheme III) is the most convenient pathway to **2**. Furthermore, the *O*′-benzylated 4-amino nucleoside **17** could be deaminated with HNO_2 in aqueous methanol to the keto nucleoside **7** in 70% yield. The latter was also obtained quantitatively by hydrolysis of **16** with HCl in anhydrous $CHCl_3$.

^{1}H NMR Spectra. The data for the various compounds, assembled in Table II, fully confirm the structures proposed. The doublets of the anomeric protons H(1′) for the β-anomers **1** and **2** are shifted to lower field by about 0.3 ppm with respect to the corresponding doublets for the H(1′) of **3** and **4**. A change in configuration from α to β in arabinofuranosyl nucleosides is accompanied by a change in orientation to H(1′) relative to the 2′-OH from cis to trans. This leads to deshielding of H(1′) by 0.28 ppm for uracil nucleosides and 0.36 ppm for cytosine nucleosides, in agreement with the change observed in passing from cytidine (with a *cisoidal* system relative to the 2′-OH) to *ara*-C (*transoidal* system), 0.33 ppm.[28]

The values of the coupling constants for the α-nucleosides **3** and **4** (Table II) indicate a C(2′)-endo ⇌ C-(3′)-endo conformational equilibrium for the sugar ring with comparable populations of the two forms. The inability to accurately measure the coupling constants for the β-nucleosides **1** and **2** and the fact that $J_{1',2'}$ is in these cases a *cisoidal* coupling constant make it difficult to assign sugar ring conformations for these systems.

CD Spectra. The CD spectra of both α-anomers **3** and **4** exhibited the expected negative Cotton effects, with a B_{2u} band at 275–276 nm. The corresponding β-anomers **1** and **2** possessed more intense positive bands in the range 270–279 nm (Table III). These results are fully consistent with the assigned structures.

Scheme III



The foregoing results show that condensation reactions leading to 2,4-dioxopyrimidine arabinonucleosides may be subjected to stereochemical control. Condensations with 2,4-bis-*O*-(trimethylsilyl)pyrimidines (such as **6**) or 2,4-diethoxypyrimidines (such as **15**) with α-halogenobenzylarabinoses (**5** or **14**) follow the mechanism proposed by Watanabe et al.,[29] according to which there is formation of a "*close* ion pair" in which nonbonded interaction of the chloride counterion with a bulky vicinal cis substituent is absent. With the chloride ion in the position α and a distributed positive charge in the position β (see Scheme I), there is a nucleophilic attack from the position β, leading to the exclusive formation of a β-nucleoside. On the other hand, the tri-*O*-benzoyl-α-D-arabinofuranosyl bromide (**8**), which contains a "participating group" on C-2, gives with bases such as **6** and **15**, in accordance with the "trans" rule of Baker, exclusively α-nucleosides. This appears to be the first reported case of selective formation of the α anomer of an arabinosyl nucleoside of a 2,4-dioxopyrimidine, and its mechanism is being subjected to further study. Finally, it should be noted that application in the condensation reactions of a mixture of the α- and β-halogenobenzoylarabinoses, **9**, gives, in accordance with the "trans" rule, a mixture of the anomeric nucleosides **10** and **11**.

Biological Activity. Antiviral Activity. In both primary rabbit kidney (PRK) and human skin fibroblast

Table IV. Antiviral and Antimetabolic Activities of 5-Et-*ara*-U and 5-Et-*ara*-C

assay system	min inhib concn,[a] μg/mL 5-Et-*ara*-U	5-Et-*ara*-C	ara-T	ara-U	*ara*-C
Antiviral Activity					
vesicular stomatitis/PRK	>200	>200	>200	>200	4-40
vaccinia/PRK	>200	>200	4	>200	0.02
vaccinia/HSF	>200	>200	2	>200	0.02
herpes simplex-1 (KOS)/PRK	2	100	0.2	20	0.03
herpes simplex-1 (KOS)/HSF	2	20	0.1	20	0.02
herpes simplex-2 (LYONS)/PRK	10		0.3	20	0.04
herpes simplex-2 (196)/PRK	20		0.4	20	0.03
Antimetabolic Activity					
[*methyl*-^{3}H]dThd incorp/PRK	>200		300	300	0.1
[2-^{14}C]dUrd incorp/PRK	>200	>200	300	>400	0.05

[a] Required to inhibit virus-induced cytopathogenicity or [*methyl*-^{3}H]dThd incorporation or [2-^{14}C]dUrd incorporation by 50%. The compounds were added immediately after virus adsorption or together with the radiolabeled DNA precursors. Virus input was 100 $CCID_{50}$ (dose infecting 50% of the cell cultures) per cell culture. Input of the radiolabeled precursors was (0.12 μCi/0.01 nmol)/10^5 cells for [*methyl*-^{3}H]dThd and (14 μCi/250 nmol)/10^5 cells for [2-^{14}C]dUrd. Viral cytopathogenicity was recorded as soon as it reached completion in the control (virus infected but untreated) cell cultures (generally at 2-3 days after virus infection). Incorporation of the radiolabeled precursors was determined after a 16-h incubation period.

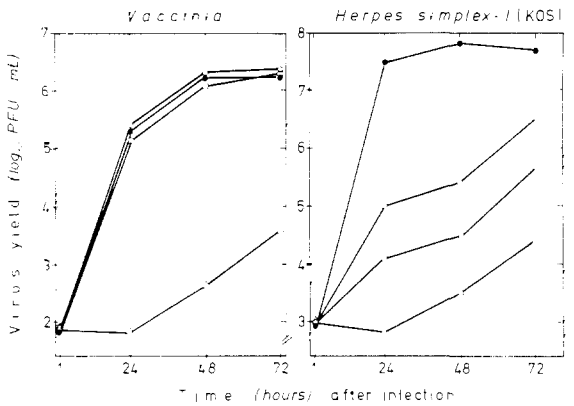


Figure 1. Effect of 5-Et-*ara*-U, *ara*-T, and *ara*-U on the growth of vaccinia virus and herpes simplex-1 (KOS) virus in PRK cell cultures. The compounds were added at 100 μg/mL immediately after virus adsorption. Virus input was 4.5 $\log_{10}$ plaque forming units (PFU) per mL. Virus yields were determined by plaque formation in either PRK (vaccinia) or VERO (herpes simplex) cell cultures: (●) control; (○) *ara*-U; (△) *ara*-T; (□) 5-Et-*ara*-U.

(HSF) cells, 5-Et-*ara*-U inhibited the cytopathic effect (CPE) of herpes simplex virus (HSV, type 1, strain KOS) at a concentration as low as 2 μg/mL (Table IV). Herpes CPE was also inhibited by 5-Et-*ara*-C, but only at a 10- to 50-fold higher concentration than required for 5-Et-*ara*-U. It is conceivable that 5-Et-*ara*-C is not active per se but that it is deaminated intracellularly to the active 5-Et-*ara*-U; its low activity, relative to 5-Et-*ara*-U, would then be accounted for by its low rate of deamination by the cellular cytidine deaminase (see below), due to steric hindrance of the 5-ethyl side chain.

Neither 5-Et-*ara*-U nor 5-Et-*ara*-C inhibited the multiplication of vaccinia or vesicular stomatitis virus (Table IV). The corresponding α anomers showed no antiviral activity, with either herpes or vaccinia virus, at 200 μg/mL, the highest concentrations tested (data not shown).

That the inhibitory effects obtained with 5-Et-*ara*-U in the herpes CPE assay (Table IV) actually reflected an inhibition of virus replication was ascertained by measuring the influence of the nucleoside on HSV growth. As shown in Figure 1, 5-Et-*ara*-U (at 100 μg/mL) led to a significant reduction in herpes virus yield (more than 3 $\log_{10}$ when assayed 24 or 48 h after virus inoculation). As might have been anticipated from the vaccinia CPE assay (Table IV), 5-Et-*ara*-U did not inhibit vaccinia virus growth (Figure 1). The parent *ara*-U behaved much like 5-Et-*ara*-U in that it was inhibitory to HSV but not to vaccinia virus. However, the antiherpes activity of *ara*-U was less pronounced than that of 5-Et-*ara*-U (Table IV, Figure 1).

In contrast to 5-Et-*ara*-U, *ara*-T exhibited a marked inhibitory effect on vaccinia CPE (Table IV) and growth (Figure 1), so that the former could be considered as a more selective antiherpes agent. It should be emphasized, however, that, at approximately equimolar concentrations, *ara*-T was about 10–50 times more effective against HSV than 5-Et-*ara*-U (Table IV). Since it is known that the activated species are the phosphorylated nucleosides, it will be of interest to examine the substrate properties of these two nucleosides for cellular and/or viral kinases.

Of the various pyrimidine arabinosides listed in Table IV, the most potent antiviral activity was exhibited by *ara*-C. However, this activity was not very specific, since the nucleoside inhibited host cell DNA synthesis at a concentration only slightly more elevated than that required to inhibit viral replication. In contrast, 5-Et-*ara*-U did not affect cellular DNA synthesis (as monitored by either dThd or dUrd incorporation) at a concentration as high as 200 μg/mL (Table IV). Hence, the antiviral index of 5-Et-*ara*-U (ratio of minimal toxic dose required to inhibit cellular DNA synthesis by 50% to minimal effective dose required to inhibit HSV-1-KOS CPE) can be estimated as >100.

The minimal effective dose (2 μg/mL or $\sim 6 \times 10^{-6}$ M) of 5-Et-*ara*-U required to inhibit HSV-1-KOS replication is similar to that for several other active nucleoside analogues, e.g., the deoxyribosides of 5-ethyl-, 5-propyl-, and 5-(propynyloxy)uracil.[30-32] All of these exhibit a significantly greater safety margin (at least in PRK cell cultures) than the established antiviral drugs 5-iodo-2′-deoxyuridine, *ara*-C, and *ara*-A.[30-32] 5-Et-*ara*-U differs from 5-ethyl- and 5-(propynyloxy)-2′-deoxyuridine in that the latter are also active vs. vaccinia virus and more closely resembles 5-propyl-2′-deoxyuridine[30] in that both of these exhibit only antiherpes activity, absence of antivaccinia activity, and lack of toxic effects on the host cell.

Consequently, 5-Et-*ara*-U is an additional example of a 5-substituted nucleoside analogue with antiviral activity limited to HSV. The growing list of specific antiherpes agents now includes such nucleoside analogues as *ara*-T;[11-13] 5-propyl-,[30] 5-(propynyloxy),[32] 5-(methylamino)-[33] and 5-(methoxymethyl)-2′-deoxyuridine;[34] 5-iodo- and 5-bromo-2′-deoxycytidine;[35] 5-iodo-5′-amino-2′,5′-dideoxyuridine,[36] 9-(2-hydroxyethoxymethyl)guanine;[37] (*E*)-5-(2-bromovinyl)- and (*E*)-5-(2-iodovinyl)-2′-deoxyuridine;[38] 5,6-dihydro-5-aza-2′-deoxythymidine;[39] *erythro*-9-(2-hydroxy-3-nonyl)adenine;[40a] and 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine.[40b]

Susceptibility of 5-Alkyl-*ara*-C to Enzymatic Deamination. The active antimetabolite *ara*-C is rapidly deaminated intracellularly to the less active *ara*-U by deoxycytidine deaminase. It was earlier shown that the 5-methyl and 5-ethyl derivatives of cytidine and deoxycytidine are deaminated more slowly than the parent nucleosides by the deoxycytidine deaminase of *S. typhimurium*.[41] It has now been shown[42] that the rate of deamination of 5-Me-*ara*-C by deaminase extracts from *B. subtilis*, *S. typhimurium* and human transformed kidney cells is about twofold lower than for *ara*-C. For 5-Et-*ara*-C the rate of deamination by the bacterial enzymes is fourfold lower than for *ara*-C and the rate of deamination by the human enzyme is about 25-fold lower.[42] Neither 5-Me- nor 5-Et-*ara*-C is deaminated by extracts of human leukocytes.[42] One may speculate, therefore, that the relative lack of activity of 5-Et-*ara*-C (Table IV) is related to its low susceptibility to deamination by cellular cytosine nucleoside deaminase.

Experimental Section

Melting points (uncorrected) were measured on a Boetius microscope hot stage. UV spectra were recorded on a Pye Unicam SP-500 instrument, using 10-mm path-length cuvettes. Spectral titrations employed the same instrument, using acetate buffers in the pH range 3–4 and phosphate buffers in the range 6–8.4. Extremes of pH made use of standard solutions in HCl and NaOH. A Radiometer PHM-22 with glass electrode was employed for pH measurements. ^{1}H NMR spectra were recorded on a Varian HA-100 instrument. CD spectra were obtained with a JASCO UV–ORD–CD Model 5 instrument, using 10-mm path-length cuvettes. Mass spectra were run on a Varian MAT Model 111 equipped with a combination field desorption–field ionization electron-impact ion source. Analytical TLC made use of Merck silica gel 60 F_{254} sheets with solvents A–F and cellulose F sheets with solvent G. Preparative chromatography was carried out with 20 × 20 cm plates covered with a 2-mm layer of Merck silica gel PF_{254}. Solvent systems (v/v) were: (A) $CHCl_3$–MeOH, 85:15; (B) $CHCl_3$–MeOH, 7:3; (C) $CHCl_3$–MeOH, 8:2; (D) $CHCl_3$–Et_2O, 93:7; (E) C_6H_6–EtOAc, 7:3; (F) $CHCl_3$–MeOH, 6:4; (G) upper phase of *s*-BuOH–H_2O at 20 °C. *ara*-C was a gift from Upjohn (Puurs,

Belgium), while *ara*-T and *ara*-U were obtained from Terra-Marine (La Jolla, Calif.) and Sefochem Fine Chemicals (Emek Hayarden, Israel), respectively. The molecular sieves used in condensation reactions were types 3 Å, $^1/_8$ in. from BDH (London, England). Measurements of antiviral and antimetabolic activities made use of the same methodology recently applied for this purpose to 5-propyl-2′-deoxyuridine.[30]

2,4-Bis-*O*-(trimethylsilyl)-5-ethyluracil (6). A mixture of 10 g (71 mmol) of well-dried 5-ethyluracil,[43] 250 mL of hexamethyldisilazane, and 100 mg of $(NH_4)_2SO_4$ was heated under reflux, with protection from moisture, for 48 h. Excess HMDS was removed under vacuum (14 mmHg, 80 °C). The oily residue was subjected to fractional distillation under vacuum, with maintenance of anhydrous conditions, and collection of the fraction at 137–139 °C (14 mmHg), lit.[42] 135–138 °C (14 mmHg), yield 14.3 g (56 mmol, 79%).

1-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-5-ethyluracil (7). Method A. To a solution of 15.4 g (35 mmol) of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**5**)[45] in 320 mL of 1,2-dichloroethane was added a solution of 10.7 g (42 mmol) of **6** in 200 mL of dichloroethane, and 12 g of molecular sieves, and the mixture was stirred for 24 h at room temperature. Following addition of 200 mL of dichloroethane, the mixture was shaken with 500 mL of a saturated, aqueous, solution of $NaHCO_3$ and then extracted with water. The resulting emulsion was filtered through a thin layer of Hyflo Super Gel. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum to a pale yellow syrup of **7** (13.6 g, 75%), which was subjected to reduction without further purification. The syrup (7.95 g) was taken up in anhydrous Et_2O and precipitated with petroleum ether. The resulting gummy precipitate was washed with anhydrous Et_2O, collected by filtration, and dried under vacuum over P_2O_5 to yield 2.46 g (31%). Crystallization from EtOH gave an analytical sample: mp 99–99.5 °C; UV λ_{max} (MeOH) 265 nm (ε 9830); λ_{min} (MeOH) 234 nm (ε 2160); MS *m/e* 542 (M^+). Anal. ($C_{32}H_{34}N_2O_6 \cdot 0.5H_2O$) C, H, N.

Method B. To 0.44 g (1 mmol) of the chloride **5** in 10 mL of dichloroethane was added a solution of 0.30 g (1.2 mmol) of **6** in 6 mL of dichloroethane. To this solution, cooled to 0 °C, was added a solution of 40 μL of $SnCl_4$ (0.34 mmol) in 200 μL of dichloroethane, and the whole solution was stirred for 16 h at ambient temperature. Subsequent workup was as in method A, to yield 460 mg (85%) of a syrup, **7**.

Method C. A solution of 47 mg (0.082 mmol) of **16** in 5 mL of $CHCl_3$ containing 50 mg of $(CH_3CO)_2O$ and saturated with HCl at 0 °C was left for 24 h at ambient temperature. The reaction mixture was brought to dryness under reduced pressure and the residue taken up in MeOH, yield 790 OD_{280} (MeOH) units (98%). Crystallization from EtOH–hexane gave **7** with melting point and spectral characteristics as in method A.

Method D. To 4 OD_{280} (MeOH) units of **17** in 2 mL of MeOH–2 N CH_3COOH (1:1) in a cooled tube was added 20 mg of $NaNO_2$. The tube was sealed and heated at 80 °C for 1.5 h. The reaction mixture was neutralized with 1 N NaOH, brought to dryness under reduced pressure, and extracted with $CHCl_3$. The chloroform extract was brought to small volume, deposited on a preparative silica gel GF_{254} plate, and the latter developed with solvent A to give a single UV-absorbing band. This was eluted with solvent B, and the eluate was brought to dryness and extracted with $CHCl_3$. The chloroform extract was brought to dryness and the residue taken up in MeOH [yield 3.4 OD_{265} (MeOH) units, 70%]. The chromatographic and spectral properties of the product, **7**, were identical with those of method A.

1-(β-D-Arabinofuranosyl)-5-ethyluracil (1). A suspension of 2.2 g of $PdCl_2$ in 500 mL of anhydrous MeOH was shaken in a 1-L flask with H_2 at a pressure of 20 mmHg. To this catalyst was added 3.4 g of **7** in 300 mL of anhydrous MeOH and the mixture again saturated with H_2 until consumption of the latter ceased (15 min). TLC with solvent B demonstrated virtually quantitative reduction of **7** to the free nucleoside **1**. This procedure was repeated three times to reduce the total 13.6 g of **7**, in a total volume of 2.5 L, which was percolated through a 40 × 3 cm column of Dowex 1 × 2 (HCO_3^-) (100–200 mesh). The column was washed with 5 L of MeOH, and the combined eluates were brought to dryness. The amorphous residue was taken up in anhydrous toluene, brought to dryness several times, and finally dried over P_2O_5. The dried product (**1**) was dissolved in a mixture of 800 mL of EtOAc and 100 mL of anhydrous EtOH. About 200 mL of the solvent was removed by distillation to leave a faintly turbid solution. Storage of this overnight at −30 °C yielded 3 g of colorless needles of **1**, mp 179.5–181 °C. The mother liquors yielded two additional crops of crystals, total 5.4 g (80%). An analytical sample was obtained by recrystallization: mp 180–181 °C; UV λ_{max} (pH 7) 268 nm (ε 10 520), λ_{min} 236 nm (ε 2220); λ_{max} (pH 12) 268 nm (ε 8290), λ_{min} 244 nm (ε 4590); λ_{max} (pH 14) 270 nm (ε 8390), λ_{min} 245 nm (ε 4130); CD (H_2O) $[\theta]$ $+15.9 \times 10^3$ (270 nm); MS *m/e* 272 (M^+). Anal. ($C_{11}H_{16}N_2O_6$) C, H, N.

1-(2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranosyl)-5-ethyluracil (10). To 525 mg (1 mmol) of the bromide **8** in 35 mL of dichloroethane was added a solution of 0.31 g (1.2 mmol) of **6** in 10 mL of dichloroethane and 85 μL (0.73 mmol) of $SnCl_4$ in 1.2 mL of dichloroethane. The mixture was stirred for 24 h at room temperature and the product then isolated as in the case of **7** and dried with anhydrous toluene: yield 479 mg (82%); mp 82–84 °C; UV λ_{max} (MeOH) 265 nm (ε 13 400), λ_{min} (MeOH) 253 nm (ε 10 670); MS *m/e* 584 (M^+). Anal. ($C_{32}H_{28}O_9N_2$) C, H, N.

1-(2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranosyl)-5-ethyl-4-thio-2(1*H*)-pyrimidinone (12). Method A. To a solution of 292 mg (0.5 mmol) of **10** in 17 mL of anhydrous freshly distilled dioxane was added 170 mg of P_2S_5, and the whole solution was heated under reflux for 1 h, at which point TLC with solvent C demonstrated the disappearance of **10**. The reaction mixture was concentrated to small volume, deposited on six preparative plates, and developed with solvent D. The yellow band with R_f 0.42 was eluted with solvent C, the eluant was brought to dryness, and the residue was crystallized from EtOH: yield 240 mg (80%); mp 93–95 °C; UV λ_{max} (MeOH) 275 nm (ε 2860), 336 (11 310), λ_{min} (MeOH) 289 nm (ε 1700); MS *m/e* 600 (M^+). Anal. ($C_{32}H_{28}N_2O_8 \cdot 0.5H_2O$) C, H, N.

Method B. To a solution of 525 mg (1 mmol) of the bromide of 2,3,5-tri-*O*-benzoyl-α,β-D-arabinose (**9**)[46] in 35 mL of dichloroethane was added a solution of 0.31 g (1.2 mmol) of **6** in 10 mL of dichloroethane and 0.35 g of molecular sieves. The mixture was stirred for 24 h at room temperature and the product isolated as in the case of **7** (method A), yielding 500 mg of a syrup which contained 415 mg of 1-(2,3,5-tri-*O*-benzoyl-α,β-D-arabinofuranosyl)-5-ethyluracil (**10** and **11**, in 71% yield). This was subjected to thiation without further purification as follows: 350 mg (0.5 mmol) of the syrup was thiated as in method A. The reaction mixture was then cooled to room temperature, filtered, and concentrated to an oil, which was dissolved in 5 mL of $CHCl_3$ and subjected to preparative chromatography on three plates with solvent D. The yellow, somewhat broad band at R_f ~0.4–0.5, which appeared to consist of two overlapping bands, was eluted with solvent C (195 mg, 65%) and rechromatographed with solvent D on seven plates which were developed five times. Two clearly defined bands were obtained, one with R_f 0.82 (α-anomer **12**) and the other with R_f 0.90 (β-anomer **13**) in the ratio 5:2. The band with R_f 0.82 was eluted with solvent C and crystallized from ethanol to yield 120 mg (40%) of **12**, mp 93–95 °C, with spectral properties as in method A.

1-(2,3,5-Tri-*O*-benzoyl-β-D-arabinofuranosyl)-5-ethyl-4-thio-2(1*H*)-pyrimidinone (13). The faster migrating band from the previous section was eluted with solvent C and crystallized from ethanol to yield 50 mg (17%) of **13**, mp 96–98 °C, and with UV and MS spectra as for **12**. Anal. ($C_{32}H_{28}N_2O_8S \cdot 0.5H_2O$) C, H, N.

1-(α-D-Arabinofuranosyl)-5-ethylcytosine (4). A solution of 90 mg (0.15 mmol) of **12** in 15 mL of anhydrous MeOH in a glass ampule was saturated with NH_3 at 0 °C, and the ampule was sealed and kept overnight at 115 °C. The reaction contents were brought to dryness under reduced pressure, and the residue was taken up in 8 mL of water and deposited on a column of 20 mL of Dowex 50 W (H^+) (100–200 mesh). The column was washed with water until the effluent was free of UV-absorbing material. The product was then eluted with 9 N NH_4OH, the eluate was brought to dryness, and the residue was crystallized from hot EtOH. Recrystallization from EtOH yielded 30 mg (74%) of platelets of **4**: mp 214–215.5 °C; UV λ_{max} (pH 1) 288 nm (ε 11 430), λ_{min} 245 nm (ε 934); λ_{max} (pH 12) 278 nm (ε 7650), λ_{min} 255 nm (ε 4430); λ_{max} (pH 14) 280 nm (ε 7680), λ_{min} 255 nm (ε 3450); pK_a

(protonation of ring N_3) = 4.45; CD (H_2O) [θ] -7.1×10^3 (276 nm); MS m/e 271 (M + 1). Anal. ($C_{11}H_{17}N_3O_5 \cdot 0.5H_2O$) C, H, N.

1-(β-D-Arabinofuranosyl)-5-ethylcytosine (2). Method A. A solution of 50 mg (0.083 mmol) of **13** in 15 mL of anhydrous MeOH was aminated and then subjected to workup as for **4** (preceding paragraph) and leading to 17 mg (72%) of **2** in the form of needles: mp 209.5–212.5 °C; UV λ_{max} (pH 1) 290 nm (ϵ 12 290), λ_{min} 245 nm (ϵ 1120); λ_{max} (pH 12) 278 nm (ϵ 8550), λ_{min} 255 nm (ϵ 4460); λ_{max} (pH 14) 282 nm (ϵ 9210), λ_{min} 255 nm (ϵ 3260); pK_a = 4.40; CD (H_2O) [θ] $+15.3 \times 10^3$ (279 nm), -4.8×10^3 (230 nm); MS m/e 271 (M^+). Anal. ($C_{11}H_{17}N_3O_5 \cdot 0.5\ H_2O$) C, H, N.

Method B. To 0.47 g (0.9 mmol) of the bromide of 2,3,5-tri-*O*-benzoyl-α-D-arabinose[22] in 10 mL of anhydrous CH_3CN was added 393 mg (2 mmol) of 2,4-diethoxy-5-ethylpyrimidine (**15**)[47] and 1.25 g of molecular sieves. The mixture, protected from moisture, was stored at room temperature for 5 days. The molecular sieves were removed and well washed with CH_3CN, and the combined filtrates were brought to a small volume and chromatographed on five plates of PF_{254} silica gel with solvent E. The major band (R_f 0.26) was eluted with $CHCl_3$–MeOH (1:1) and concentrated to an oil (246 mg, 49%). When the product **16** was dissolved in MeOH, spectral measurements showed the presence of 3050 OD_{283} (MeOH) units (245 mg): UV λ_{max} (MeOH) 283 nm (ϵ 6340), λ_{min} 245 nm (ϵ 1840); MS m/e 570 (M^+). This procedure was repeated several times, and the product was subjected to amination without further purification. To a solution of 411 mg (0.72 mmol) of the syrup **16** in 5 mL of anhydrous EtOH was added 10 mL of MeOH previously saturated with NH_3 at 0 °C, and the whole solution was kept at 100 °C overnight. TLC with solvent F showed the formation of only one product (R_f 0.55). The mixture was brought to dryness, taken up in anhydrous EtOH, and again brought to dryness, and the latter step was repeated twice more, to yield 310 mg (80%) of chromatographically homogeneous 1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-4-amino-5-ethyl-2(1*H*)-pyrimidinone (**17**) as a syrup: UV λ_{max} (MeOH) 279 nm (ϵ 7990), λ_{min} 260 nm (ϵ 5780); MS m/e 541 (M^+). This was used as such in the subsequent reduction step. A solution of 224 mg (0.41 mmol) of the syrup in 5 mL of anhydrous MeOH was added to 280 mg of previously reduced $PdCl_2$ in 55 mL of MeOH. The mixture was shaken in the presence of H_2 until uptake of the latter ceased (15 min), at which point TLC with solvents A and F exhibited the presence of only **2**. The catalyst was removed and the alcoholic solution deposited on a 20 × 1 cm column of Dowex 2 × 8 (HCO_3^-) (100–200 mesh) and eluted with MeOH, with collection of 15-mL fractions. Fractions 1–6, including all of **2**, were pooled and brought to dryness, and the residue was crystallized from EtOH–Et_2O to yield 78 mg (70%) of **2** in the form of minute crystals, mp 219–221 °C.

Method C. The condensation reaction was also carried out in the presence of catalyst, as follows: The bromide **14** (470 mg, 0.9 mmol) in 20 mL of anhydrous CH_3CN was reacted with the base **15** (245 mg, 1.25 mmol) in the presence of $SnCl_4$ (170 μL, 1.44 mmol) at room temperature for 6 days. The mixture was brought to dryness, the residue was taken up in 20 mL of CH_2Cl_2 and shaken with 20 mL of saturated aqueous $NaHCO_3$ and then with 20 mL of water, and the organic phase was dried over anhydrous Na_2SO_4 overnight. The mixture was filtered, and the filtrate was brought to small volume, deposited on three preparative plates of silica gel PF_{254}, and developed twice with solvent E. The major band (R_f 0.44) was eluted with $CHCl_3$–MeOH (1:1), concentrated to give **16** as an oil (120 mg, 49%). This oil was subjected to amination and reduction as in method B (above).

1-(α-D-Arabinofuranosyl)-5-ethyluracil (3). To a solution of 40 mg (0.015 mmol) of **4** in 10 mL of CH_3COOH, in an ampule immersed in dry ice, was added 200 mg of $NaNO_2$, and the ampule was sealed and heated for 1 h at 80 °C. The contents were brought to neutrality with 0.1 N NaOH and brought to dryness under reduced pressure. The residue was extracted three times with anhydrous EtOH, and the extract was brought to small volume and deposited on a large sheet of Whatman paper no. 3, which was developed with solvent G. The product (R_f 0.28) was eluted with water, the eluate was brought to dryness, and the residue was crystallized from EtOAc–Et_2O–EtOH to yield 17 mg (43%) of **3**: mp 159.5–161 °C; UV λ_{max} (pH 7) 268 nm (ϵ 10630), λ_{min} 236 nm (ϵ 2990); λ_{max} (pH 12) 268 nm (ϵ 8330), λ_{min} 244 nm (ϵ 4620); λ_{max} (pH 14) 270 nm (ϵ 8420), λ_{min} 245 nm (ϵ 4200); CD (H_2O) [θ] -5.6×10^3 (275 nm), $+4.0 \times 10^3$ (243 nm); MS m/e 272 (M^+). Anal. ($C_{11}H_{16}N_2O_6$) C, H, N.

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Studies on the Mechanism of Antiviral Action of 1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-carboxamide (Ribavirin)

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Syntheses are described for the 5′-phosphates of the 2′- and 3′-*O*-methylribavirins (**2a** and **2b**) and the methyl ester of ribavirin 5′-phosphate (**3**). The 5′-phosphate of 1-β-(D-ribofuranosyl)-1,2,4-triazole (**2d**) was obtained via the 3-carboxyl derivative of ribavirin 5′-phosphate (**2c**). Compounds **2a**, **2b**, and **2d** were inactive as inhibitors of IMP dehydrogenase under conditions where the parent ribavirin 5′-phosphate (**2**) was an effective inhibitor. Weak inhibitory activity was exhibited by **3** ($K_i \approx 200$ μM) and **2c** ($K_1 \approx 70$ μM). Under conditions where ribavirin (**1**) is effectively phosphorylated by rat liver nucleoside kinase, the 2′- and 3′-*O*-methylribavirins (**1a** and **1b**), the 3-carboxylate of ribavirin (**1c**), and the riboside of 1,2,4-triazole (**1d**) were totally inactive. The overall results are fully consistent with the lack of antiviral activity of **1a** and **1b**, while the specificity of ribavirin as an antiviral agent is further underlined by the behavior of the methyl ester **3**.

Ribavirin, 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide, originally designated as "virazole", has been shown to be a broad-spectrum antiviral agent in vitro.[1-3] Particularly striking are the inhibitory effects of this compound on influenza and parainfluenza virus replication in cell cultures and in some animals.[1,4-9] The drug has been licensed for human use in some countries and is currently undergoing clinical trials, although reports on its efficacy are somewhat conflicting.

Ribavirin 5′-phosphate has been found to competitively inhibit IMP dehydrogenase (IMP:NAD^+ oxidoreductase, EC 1.2.1.14),[10] ascribed to its structural resemblance to IMP and GMP.[11] Taken in conjunction with the fact that ribavirin is converted to the 5′-phosphate by the appropriate cellular kinase, presumably adenosine kinase,[12] this led to the proposal that the compound inhibits virus multiplication via depletion of the GTP pool.[10,13,14] Some reservations have been raised with regard to this concept,[15,16] and, at least in the case of influenza virus, the RNA polymerase of which is potently inhibited by ribavirin 5′-triphosphate,[17] it is conceivable that this may represent the major site of action of this antimetabolite, at least in this instance. It is of interest in this regard that ribavirin 5′-triphosphate is inactive in vitro against eukaryotic DNA polymerases α and β, eukaryotic RNA polymerases I and II, and eukaryotic poly(A) polymerase.[14]

Regardless of the eventual decision regarding the clinical utility of ribavirin, its broad spectrum of activity, encompassing nearly all major virus groups, suggests the utility of examining in further detail the mechanism of its in vitro activity. We previously showed that 2′- and 3′-*O*-methyl derivatives of ribavirin were virtually inactive against several viruses in cell culture notwithstanding that, unlike the parent ribavirin, the 2′(3′)-*O*-methyl derivatives did not suppress cellular DNA synthesis.[18] The lack of activity of the 2′(3′)-*O*-methylribavirins could conceivably be due to their inability to undergo phosphorylation by cellular kinase(s) or to the failure of their 5′-phosphates to inhibit IMP dehydrogenase. We have therefore synthesized the appropriate 2′(3′)-*O*-methylribavirin 5′-phosphates and examined the foregoing two possibilities. We have also prepared several additional analogues of ribavirin and its 5′-phosphate with a view to delineate more accurately its specificity of action.

Chemistry. The 2′- and 3′-*O*-methylribavirin 5′-phosphates (**2a** and **2b**) were obtained by phosphorylation of the parent nucleosides[18] according to the procedure of Yoshikawa,[19] with slight modifications.[10] This method, although convenient, is frequently capricious and not always as selective as claimed.[20] Consequently, the isolated nucleotides, following purification on a DEAE-Sephadex A-25 (HCO_3^-) column, were checked by treatment with

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