Possible Effect of Hydrogen Bonding on Methylation of Pyrimidine and Pyridone Nucleosides

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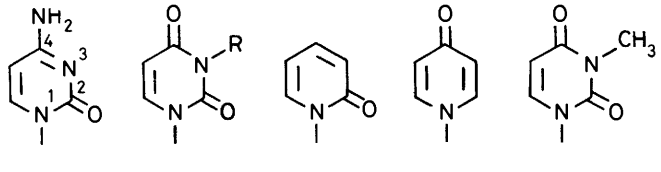
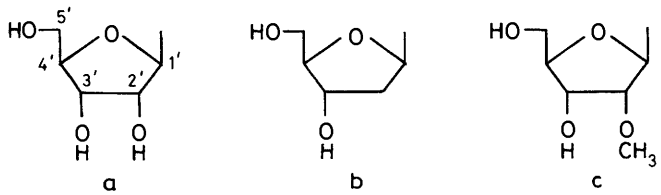
Methylation of 1-(β-D-ribofuranosyl)-2-pyridone and -4-pyridone and 3-methyluridine with trimethylsulphonium hydroxide suggested that pyrimidine ribonucleosides (cytidine and uridine) differed in reactivity from the corresponding deoxyribonucleosides because of hydrogen bonding between the 2′-OH and C(2)=O groups.

Alkylation of nucleic acids and their components (nucleosides and nucleotides) has been the subject of many chemical and biological studies.[1] Although the various classes of methylating agent differ in the extent to which they methylate particular sites on multifunctional compounds, DNA and its components are generally more reactive than the corresponding ribose analogues.[2–5] Physicochemical differences have also been reported in a phenomenological manner: (1) unlike deoxycytidine (**1b**) and deoxyuridine (**2b**; R = H), cytidine (**1a**) and uridine (**2a**; R = H) exhibited a bathochromic shift in their u.v. spectra at pH >12.[6,7] (2) The N-3 atom of (**1b**) is more basic than that of (**1a**).[6,8] (3) The rates of photochemical hydration of cytosine nucleosides[9] and the rates of hydrolysis of oligonucleotides,[10–13] differ. Such differences arise, it has been speculated, because of (i) an inductive effect of the O-2′ atom of the ribose residues[8,10,12] or (ii) intramolecular hydrogen bonding between the 2′-OH and the base moieties.[5,6,9,10]

Here we describe methylations of 1-(β-D-ribofuranosyl)-2-pyridone (**3a**) (expressed as 2-Pyd) and -4-pyridone (**4a**) (4-Pyd) and 3-methyluridine (**5a**) (3-MeUrd) in order to study the reactivity difference observed previously[5] between the ribonucleosides (**1a**) and (**2a**) (R = H) and the corresponding deoxyribonucleosides (**1b**) and (**2b**) (R = H).



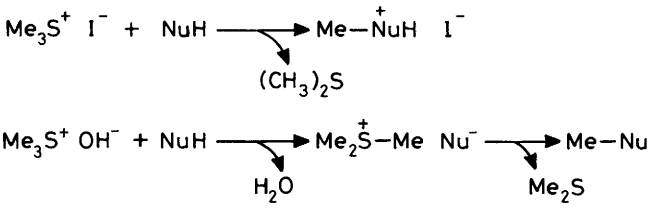
(1) (2) (3) (4) (5)



a b c

Results and Discussion

Methylations were conducted by stirring a mixture of the nucleoside and 1.1—2.0 equiv. of trimethylsulphonium iodide (Me_3SI) or trimethylsulphonium hydroxide (Me_3SOH) in dimethylformamide (DMF) at a specific temperature in the range



Scheme.

65—75 °C under nitrogen. The Me_3S^+ ions of the reagents attack the nucleophilic sites of the substrates in an S_N2 fashion. In addition, Me_3SOH is capable of methylating the NH(C=O) and sugar hydroxy groups to activate them as nucleophiles (see Scheme).[14] Typical results for our present and previous studies are listed in Tables 1 and 2.

Thus, with Me_3SI, (**1a**) and (**1b**) were transformed exclusively into 3-methylcytidine and 3-methyldeoxycytidine, respectively, in yields of >80% after 30 min. Me_3SI was inactive towards (**2a**; R = H) and (**2b**; R = H), but Me_3SOH converted them smoothly into the corresponding 3-methyl derivatives (Table 1, entry 4). As judged from the second-order rate constants listed in Table 2 (entries 1, 2, 4, and 5), (**1b**) and (**2b**) (R = H) were more reactive than (**1a**) and (**2a**) (R = H), respectively. In methylations with Me_3SOH, (**1b**) was not only more reactive than (**1a**), but differed both in the sites attacked and the extent of attack (Table 1, entries 1 and 2). Methylation of (**3a**), (**4a**), and (**5a**) with Me_3SOH also furnished noteworthy results (Table 1, entries 5—7). Thus, the extent of methylation at 2′-OH, 3′-OH, and 5′-OH was, respectively, (**3a**) 48:9:0(%) and (**5a**) 43:10:4(%). By contrast, (**4a**) was methylated rather randomly at the ribose hydroxy positions (35: 12: 13% respectively). The use of a protic solvent diminished the reactivity difference between the ribose hydroxy groups; for instance, a reaction of (**5a**) with Me_3SOH in DMF–methanol (2:1, v/v) gave 2′-*O*-methyl- and 3′-*O*-methyl derivatives (16 and 8%, respectively).

These results are best explained by postulating hydrogen bonding such as shown in the Figure. Such an interaction should decrease the basicity of the N-3 atoms of (**1a**) and the anionic form of the uridine (**2a**; R = e) to retard the methyl-

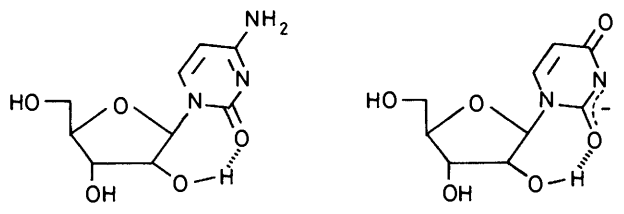


Figure.

Table 1. Methylation of nucleosides with Me_3SOH[a,b]

Entry[c]	Nucleoside	Site and extent (%)[d] of methylation: Base	Sugar	Product distribution (%)
1	Cyd (**1a**)	N-3 13	2′(3′)-OH 35	3-MeCyd (13), 2′-MeCyd (28) and 3′-MeCyd (7)
2	dCyd (**1b**)	N-3, 4-NH_2 42	3′(5′)-OH 10	3-MedCyd (25), 4-N-MedCyd (17) and 3′(or 5′)-MedCyd (10)
3	2′-MeCyd (**1c**)	N-3, 4-NH_2 55	3′-OH 8	3,2′-Me_2Cyd (34), 4-N,2′-Me_2Cyd (21) and 2′,3′-Me_2Cyd (8)
4	Urd (**2a**; R = H) dUrd (**2b**; R = H) 2′-MeUrd (**2c**; R = H)	N-3 87—97	X′-OH trace	3-MeUrd (**5a**, 87), 3-MedUrd (90) and 3,2′-Me_2Urd (97), respectively.
5	2-Pyd (**3a**)	—	2′(3′)(5′)-OH 57	2-Me-2-Pyd (48), 3′-Me-2-Pyd (9) and 5′-Me-2-Pyd (0)
6	4-Pyd (**4a**)	—	ditto 60	2′-Me-4-Pyd (35), 3′-Me-4-Pyd (12) and 5′-Me-4-Pyd (13)
7	3-MeUrd (**5a**)	—	ditto 57	2′,3-Me_2Urd (43), 3′,3-Me_2Urd (10) and 5′,3-Me_2Urd (4)

[a] Abbreviations: Cyd, cytidine (**1a**); dCyd, deoxycytidine (**1b**); Urd, uridine (**2a**; R = H); 2-Pyd, 1-(β-D-ribofuranosyl)-2-pyridone (**3a**); 2′-MeCyd, 2′-*O*-methylcytidine; 4-*N*-MedCyd, 4-*N*-methyldeoxycytidine; 3,2′-Me_2Cyd, 3,2′-*O*-dimethylcytidine. Similar abbreviations for other nucleosides. [b] Reaction conditions. Nucleoside–Me_3SOH–DMF (mmol/mmol/ml): 1.0:2.0:4.0 (entry 1—3); 1.0:1.1:4.0 (entry 4); 1.0:1.5:4.0 (entries 5—7). Reaction temperature, 70 °C; time, 1 h. [c] The entries 1—4 are cited from ref. 5. [d] For instance, the extent of methylation on the cytosine ring of (**1b**) was calculated as follows; 25% (3-MedCyd) + 17% (4-*N*-MedCyd) = 42%.

Table 2. The second-order rate constant (k) of 3-*N*-methylation of pyrimidine nucleosides (**1a—c**) and (**2a—c**) (R = H)[a]

Entry	Nucleoside[b]	Reagent	Temp. (°C)	Product[b]	10^2k (mol^{-1} min^{-1})
1	Cyd (**1a**)			3-MeCyd	3.9 ± 0.5
2	dCyd (**1b**)	Me_3SI	70	3-MedCyd	6.1 ± 1
3	2′-MeCyd (**1c**)			2′,3-Me_2Cyd	6.8 ± 1
4	Urd (**2a**; R = H)			3-MeUrd	13 ± 1
5	dUrd (**2b**; R = H)	Me_3SOH	65	3-MedUrd	38 ± 6
6	2′-MeUrd (**2c**; R = H)			2′,3-Me_2Urd	30 ± 3

[a] Reaction condition: (**1a—c**)–Me_3SI–DMF = 1.0:2.0:4.0 (mmol:mmol:ml); (**2a—c**; R = H)–Me_3SOH–DMF = 1.0:0.75:4.0 (mmol:mmol:ml). [b] See Table 1, footnote *a* for abbreviations for the nucleosides.

ation rate [the uracil nucleosides (**2**; R = H) are deprotonated easily by the OH^- ions of Me_3SOH to furnish the anionic form (**2**; R = e) which are then attacked by Me_3S^+ ions in a rate-determining step]. It is unlikely that the C(2)=O group with its inductive effect led to the selective 2′-*O*-methylation in ribonucleosides [(**1a**) and (**2a**) (R = H), (**3a**), and (**5a**)] since (**3a**) gave the 3′-*O*-methyl and 5′-*O*-methyl derivatives in yields even smaller than (**4a**) (Table 1, entries 5—7). The comparable reactivity of (**5a**) with (**3a**) suggested a minor role for the C(4) of (**5a**) in the ribose *O*′-methylations. However, even in (**4a**), 2′-OH was methylated preferentially over 3′-OH. Thus, the electron-withdrawing N-1 atoms of (**1a**) and (**2a**) (R = H) activated the ribose hydroxy group also, particularly the nearest 2′-OH groups toward methylation. The significant 5′-*O*-methylation in (**4a**) may be due to a stereochemical preference of Me_3S^+ ions for attacking the least hindered 5′-OH among the ribose hydroxy groups.

The possibility of a hydrogen bonding interaction was advocated further by methylation of the 2′-*O*-methyl ribonucleosides [(**1c**) and (**2c**) (R = H)]; *viz.*, the extents and sites of methylation in (**1c**) with Me_3SOH were almost identical with those of (**1b**) but different from those of (**1a**) (Table 1, entry 1 *vs.* 3). The second-order rate constants of 3-*N*-methylation of (**1c**) and (**2c**) (R = H) were also similar to those of (**1b**) and (**2b**) (R = H), respectively (Table 2, entries 2 and 3; 5 and 6). The masking of the 2′-OH groups by methylation destroys the hydrogen bonding interaction, and thereby makes the reactivity of (**1c**) and (**2c**) (R = H) equivalent to (**1b**) and (**2b**) (R = H), respectively.

In order to complement the methylation results, we also examined the i.r. spectra of the nucleosides as KBr mulls since the polar compounds were sparingly soluble in solvents such as $CHCl_3$ and CS_2. Not all the nucleosides gave supporting data for the interaction, but the carbonyl bands of cytidine (**1a**) (1 752 cm^{-1}) and uridine (**2a**; R = H) (1 675—1 680 cm^{-1} were lower by 10—20 cm^{-1} than those of the corresponding deoxyribose analogues. Similarly, the 2-pyridone nucleoside (**3a**) showed a carbonyl absorption at 1 654 cm^{-1} which was *ca.* 10 cm^{-1} lower than that of the 2′, 3′, 5′-*O*-tribenzoyl derivative, while 4-pyridone nucleoside (**4a**) and its 2′,3′,5′-*O*-tribenzoyl derivative showed a similar absorption at the same position (1 648 cm^{-1}) (experimental error ± 1 cm^{-1}). Such shifts to lower wavelength are in accord with the reported intramolecular hydrogen bonding of carbonyl/hydroxy compounds.[15]

In summary, hydrogen bonding between the 2′-OH and C(2)=O groups may account for the different reactivity of pyrimidine ribonucleosides from the corresponding deoxynucleosides. The selective 2′-*O*-methylation in the ribonucleosides has been ascribed to a combined effect of the interaction and the inductive effect of the N(1) atoms.

Experimental

Nucleosides (**3a**), (**4a**), and (**5a**) were synthesized by literature procedures;[16,17] Me_3SI and Me_3SOH have been prepared previously.[5] T.l.c. was carried out on silica gel precoated sheet (Merck Art. 5735) and aluminium oxide precoated sheet (Merck type T, 5551) using the solvent systems: (A) (chloroform–

methanol, 10:1, v/v); (B) (chloroform–methanol, 9:2, v/v) and (C) (chloroform–methanol–acetone–water, 120:30:11:5, v/v). Mobilities in t.l.c. are expressed as R_F values. High pressure liquid chromatography (h.p.l.c.) was performed using a Toyo-soda 803 chromatograph packed with silica gel (TSKgel 410T) in a stainless steel column (3.7 mm × 60 cm) and a mixture of water, acetonitrile, triethylamine, and acetic acid (1 000:70:3:3, v/v) as eluting solvent. Elution of nucleosides was monitored by means of a Toyo-sada model UV 8 ultraviolet photometer. I.r. spectra were recorded as KBr pellets by means of a Jasco A 202 spectrometer, where absorption bands were calibrated with 2 850 and 1 603 cm^{-1} bands of polystyrene film. U.v. and electron-impact mass spectra were recorded with a Shimazu UV-240 and a JEOL HX-100 spectrometers, respectively. ^1H N.m.r. spectra were measured on a JEOL GX-400 spectrometer using a dilute solution in D_2O or $CDCl_3$–CD_3OD (2:1, v/v).

General Methylation Procedure.—A mixture of nucleoside (0.5 g or 1.0 mmol) and *ca.* 0.7M methanolic solution of Me_3SOH (1.1—2.0 equiv.) was concentrated under reduced pressure < 20 °C. The resulting residue was dissolved in DMF (4 ml $mmol^{-1}$ of the nucleosides) and the solution was heated at a specific temperature with magnetic stirring under nitrogen. Alternatively, a mixture of nucleoside (1 mmol), Me_3SI (1.2 equiv.), and DMF (8 ml) was stirred magnetically at 70 °C under nitrogen. Product yields or the time course of methylation reactions were determined by a u.v.–t.l.c. method [for methylation of (**1a**—**c**) and (**2a**—**c**) (R = H)][5] or from the area ratio of product peaks in h.p.l.c. [for methylation of (**3a**), (**4a**), and (**5a**)]. Electron impact mass spectrometry was convenient for estimating the methylation sites in unknown products of (**3a**) and (**4a**) since the 2′-*O*-methylribonucleoside exhibited a base +58 peak ($BHCH{=}CHOCH_3$) of significant intensity while the 3′-*O*-isomer give a base + 44 peak ($BHCH{=}CHOH$).[17] In addition, 400 MHz ^1H n.m.r. spectra were used to identify the unknown products. Known compounds derived from (**1a**—**c**) and (**2a**—**c**) (R = H) and (**5a**) were identified by comparison of their m.p.s and u.v. and ^1H n.m.r. spectra with those of authentic samples or literature values.[1,5,17] The unknown compounds and their physical constants are as follows.

2′-O-*Methyl*-1-(β-D-*ribofuranosyl*)-2-*pyridone*. M.p. 111—115 °C (recrystallized from hexane–acetone); $[\alpha]_D^{20}$ +159° (*c* 0.49 in MeOH) [Found: M^+ (e.i.), 241.0927. $C_{11}H_{15}NO_5$ requires *M*, 241.0950]; R_F [silica gel, solvent (A)] 0.37; retention time in h.p.l.c., 58 min: $\nu_{max.}$ 3 350br (OH), 1 652vs (C=O), 1 565vs (conj. C=C), and 1 120 cm^{-1} (C–O); $\lambda_{max.}$(water) 300 nm (ε 5 800); $\delta_H(D_2O)$ 3.573 (3 H, s, CH_3), 4.006 (1 H, dd, $J_{1'2'}$ 2.5 Hz, $J_{2'3'}$ 5.37 Hz, 2′-H), 6.250 (1 H, d, 1′-H), 6.61 (1 H, m, 4-H), 6.64 (1 H, m, 3-H), 7.62 (1 H, m, 5-H), and 7.97 (1 H, m, 6-H); *m/z* 241 (M^+, 100%), 152 (B + 58, 17), 147 (sugar, 90), 124 (BHCHO, 17), 96 (B + 2, 25), and 95 (B + 1, 10).

3′-O-*Methyl*-1-(β-D-*ribofuranosyl*)-2-*pyridone*. Viscous oil; $[\alpha]_D^{21}$ +150° (*c* 0.05 in MeOH) [Found: M^+ (e.i.), 241.0927. $C_{11}H_{15}NO_5$ requires 241.0950]; R_F [silica gel, solvent (A)] 0.37; R_t (h.p.l.c.), 66 min; $\nu_{max.}$ 3 375br (OH), 1 640vs (C=O), 1 560m (conj. C=C), and 1 120m cm^{-1} (C–O); $\lambda_{max.}$(water) 300 nm (ε 6 000); $\delta_H(D_2O)$ 3.464 (3 H, s, CH_3), 4.485 (1 H, m, 3′-H), and 6.44 (1 H, d, $J_{1'2'}$ 2.5 Hz, 1′-H); *m/z* 241 (M^+, 22%), 146 (sugar − 1, 50), 138 (B + 44, 11), 135 (58), 124 (BHCHO, 10), 96 (B + 2, 100), and 95 (B + 1, 70).

2′-O-*Methyl*-1-(β-D-*ribofuranosyl*)-4-*pyridone*. Viscous oil; $[\alpha]_D^{21}$ −69° (*c* 0.1 in MeOH) [Found: M^+ (e.i.), 241.0926. $C_{11}H_{15}NO_5$ requires 241.0950]; R_F 0.27 [silica gel, solvent (B)], 0.79 [aluminium oxide, solvent (C)]; R_t (h.p.l.c.), 28 min; $\nu_{max.}$ 3 340br (OH), 1 638vs (C=O), 1 555vs (conj. C=C), and 1 190m cm^{-1} (C–O); $\lambda_{max.}$(water) 262 nm (ε 14 000); $\delta_H(D_2O)$ 3.43 (3 H, s, CH_3), 3.911 (1 H, dd, $J_{1'2'}$ 6.35 Hz, $J_{2'3'}$ 4.67 Hz, 2′-H), 5.456 (1 H, d, 1′-H), 6.480 (2 H, dd, $J_{2,3}$ 7.6 Hz, 3-H), and 7.976 (2 H, d, 2-H); *m/z* 241 (M^+, 100%), 152 (B + 58, 25), 147 (sugar, 90), 124 (BHCHO, 20), and 96 (B + 2, 70).

3′-O-*Methyl*-1-(β-D-*ribofuranosyl*)-4-*pyridone*. Viscous oil; $[\alpha]_D^{21}$ −60° (*c* 0.1 in MeOH) [Found: M^+ (e.i.), 241.0925. $C_{11}H_{15}NO_5$ requires 241.0950]; R_F 0.27 [silica gel, solvent (B)], 0.79 [aluminium oxide, solvent (C)]; R_t (h.p.l.c.), 35 min; $\nu_{max.}$ 3 400br (OH), 1 640vs (C=O), 1 542vs (conj. C=C), and 1 170m cm^{-1} (C–O); $\lambda_{max.}$(water) 264 nm (ε 13 000); $\delta_H(CDCl_3$–CD_3OD, 2:1, v/v), 3.43 (3 H, s, CH_3), 3.911 (1 H, dd, $J_{1'2'}$ 6.4 Hz, $J_{2'3'}$ 4.8 Hz, 2′-H), 4.387 (1 H, dd, $J_{3'4'}$ 2.9 Hz, 3′-H), and 5.46 (1 H, d, 1′-H); *m/z* 241 (M^+, 55%), 147 (sugar, 50), 138 (B + 44, 14), 124 (BHCHO, 7), 96 (B + 2, 51), and 95 (B + 1, 25).

5′-O-*Methyl*-1-(β-D-*ribofuranosyl*)-4-*pyridone*. Viscous oil; $[\alpha]_D^{21}$ −74° (*c* 0.03 in MeOH) [Found: M^+ (e.i.), 241.0926. $C_{11}H_{15}NO_5$ requires 241.0950]; R_F 0.35 [silica gel, solvent (B)], 0.51 [broad, aluminium oxide, solvent (C)]; R_t (h.p.l.c.), 57 min; $\nu_{max.}$ 3 400br (OH), 1 638vs (C=O), 1 555vs (conj. C=C), and 1 190m cm^{-1} (C–C); $\lambda_{max.}$(water) 264 nm (ε 13 000); $\delta_H(CDCl_3$–CD_3OD, 2:1, v/v) 3.425 (3 H, s, CH_3), 3.552 (1 H, dd, $J_{4'5'}$ 2.2 Hz, $J_{5'5''}$ −10.7 Hz, 5′-H), 3.679 (1 H, dd, $J_{4'5'}$ 2.4 Hz, 5″-H), and 5.250 (1 H, d, 1′-H); *m/z* 241 (M^+, 100%), 147 (sugar, 25), 129 (sugar $-H_2O$, 27), 124 (BHCHO, 5), 96 (B + 2, 20), and 95 (B + 1, 25).

References

1 B. Singer and M. Kröger *Prog. Nucleic Acid Res. Mol. Biol.*, 1979, **23**, 151; B. Singer, *ibid.*, 1975, **15**, 219 and references cited therein.
2 B. Singer and H. Fraenkel-Conrat, *Biochemistry*, 1975, **14**, 772.
3 L. Sun and B. Singer, *Biochemistry*, 1975, **14**, 1795.
4 B. Singer, *Biochemistry*, 1975, **14**, 4353.
5 K. Yamauchi, T. Nakagima, and M. Kinoshita, *J. Chem. Soc., Perkin Trans. I*, 1980, 2787.
6 J. J. Fox, L. F. Cavalieri, and N. Chang, *J. Am. Chem. Soc.*, 1953, **75**, 4315; J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, 1952, **9**, 369.
7 S. Lewin and D. A. Humphreys, *J. Chem. Soc. B*, 1966, 210.
8 J. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, 1961, **83**, 4755.
9 K. L. Wierzchowski and D. Shugar, *Biochim. Biophys. Acta*, 1957, **25**, 355.
10 H. Witzel in *Prog. Nucleic Acid Res. Mol. Biol.*, 1963, **2**, 233.
11 H. Witzel, *Ann. Chem.*, 1960, **635**, 182; H. B. Kaltrider and J. F. Scott, *Biochim. Biophys. Acta*, 1962, **55**, 379.
12 M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Am. Chem. Soc.*, 1968, **90**, 1042; P. O. P. Ts'o, *Ann. N. Y. Acad. Sci.*, 1969, **153**, 785.
13 N. K. Kochetkov and E. I. Budovskii, 'Organic Chemistry of Nucleic Acids,' Plenum, New York, 1971, p. 153, part A.
14 K. Yamauchi, T. Tanabe, and M. Kinoshita, *J. Org. Chem.*, 1979, **44**, 638; K. Yamauchi, Y. Hisanaga, and M. Kinoshita, *J. Am. Chem. Soc.*, 1983, **105**, 538.
15 K. Nakanishi, 'Infrared Absorption Spectroscopy,' Holden-Day, San Francisco, 1962, p. 32, 42, and 190.
16 U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1974, **39**, 3668.
17 M. J. Robins, S. R. Naik, and A. S. K. Lee, *J. Org. Chem.*, 1974, **39**, 1891.

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