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NUCLEOSIDES XXXVI. TRANSFORMATION OF ARABINOPYRIMIDINE NUCLEOSIDES (1)

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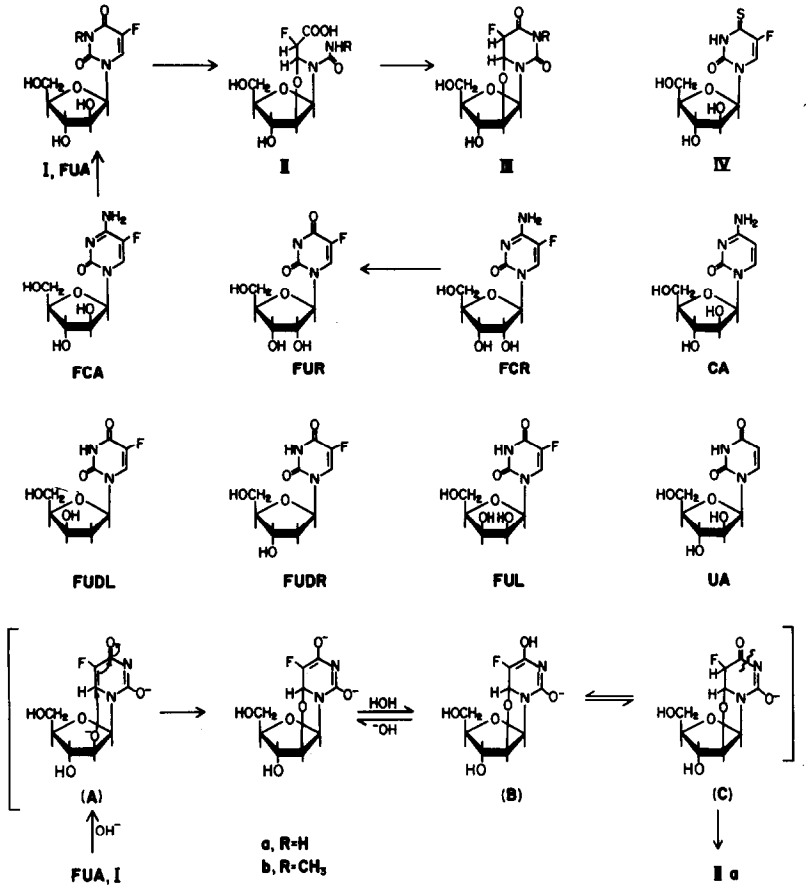
1-β-D-Arabinofuranosyl nucleosides of 5-fluorouracil, 5-fluorocytosine and cytosine have exhibited interesting anti-tumor and/or antiviral properties (2). During our investigation into the synthesis of some of these compounds, we have found a varying instability of these nucleosides to aqueous alkali. We report our studies on novel transformations which several of these nucleosides undergo (Fig. 1).

When 1-β-D-arabinofuranosyl-5-fluorouracil (FUA, Ia) is treated with 0.1 N NaOH at 60-70°, a rapid loss of selective absorption in the ultraviolet occurs which is complete within 0.5 hours. The colorless reaction mixture is placed on a column of Dowex 50 (H^+) and eluted with water. The eluate is concentrated to dryness and the crystalline residue recrystallized from 95% ethanol from which is obtained a near quantitative yield of IIa, m.p. 192-194° (eff. dec.), $[\alpha]_D^{23°}$ - 124° (H_2O). Anal., found for $C_9H_{13}FN_2O_7$: C, 38.81; H, 4.78; N, 9.85; F, 6.96. CO_2 is evolved when IIa is placed in dilute $NaHCO_3$. Compound IIa does not consume metaperiodate over a 3-day period whereas FUA shows the expected consumption of one mole of oxidant per mole within 24 hrs. (3). IIa gives a positive p-dimethylaminobenzaldehyde-HCl

test (4) for a ureide structure, gives negative aldehyde tests, and shows no selective absorption maximum in alkali above 220 mμ. These data support an open-chain ureide structure for IIa rather than the cyclic ureide structure (IIIa).

Fig. I



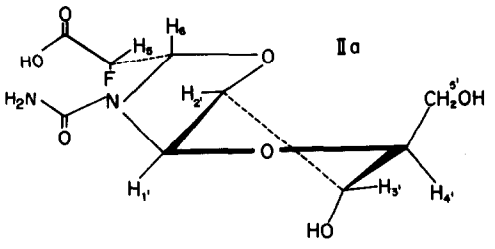
The failure of IIa to consume metaperiodate and the fact that the 5'-O-trityl derivative of FUA (Ia) is converted by alkali to the 5'-O-trityl derivative of IIa indicate that the "up" 2'-OH group is involved in the saturation of the double bond of Ia (the 3'-"down"-OH is unfavorably disposed). Moreover, when 1-β-D-ribofuranosyl-5-fluorouracil (FUR) (2), its 2'-deoxy analog (FUDR) (5a), or 1-(2'-deoxy-β-D-lyxofuranosyl)-5-fluorouracil (FUDL) (5b) are treated with 0.1 N alkali at 60-70° for 5 hrs., no reaction occurs as evidenced by the constancy of their ultraviolet spectra. These latter three nucleosides lack a 2'-hydroxyl in the "up" position. On the other hand, 1-β-D-lyxofuranosyl-5-fluorouracil (FUL) does undergo loss of its ultraviolet spectrum when similarly treated with alkali.

N.m.r. spectral studies (Table I) confirmed structure IIa as an open chain ureide with a "6 to 2'" anhydro linkage. In addition to signals listed, a peak at 6.40 δ equivalent to 2-3 protons was observed as well as a very broad peak at 6.80-5.00 δ equivalent to 1-2 protons. Since these peaks were absent when D_2O was used as solvent, they must be due to the C_3' and C_5' hydroxyl protons and the ureide NH_2 protons. The couplings involved in the protons on C_5 and C_6 in IIa confirm the saturation of the 5,6-double bond. The geminal H_5-F coupling constant of 48 c.p.s. and the vicinal H_6-F coupling constant of 23.5 c.p.s. are in line with those previously noted for such systems (6-9). In the sugar ring the $J_{H1'-H2'}$ of 4.0 c.p.s. (in DMSO-d_6) suggests that fusion of the two 5-membered rings results in some "skewing" of the furanose moiety to the C_2 conformation in accord with the findings of Abraham et al. (10) with 1,2-O-isopropylidine derivatives of hexofuranoses.

To decide between structures IIa and IIIa, FUA was methylated with diazomethane according to the procedure of Miles (11). An amorphous solid was obtained which contained starting material, 3-methyl-FUA and probably the 4-methyl ether of FUA. The mixture was placed on a silica-gel column

TABLE I

NUCLEAR NAGNETIC RESONANCE DATA FOR COMPOUND IIa



IIa

Solvent	Approximate Coupling Constants (c.p.s.)						
	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5,6}$	J_{5-F}	J_{6-F}
DMSO-d_6	4.0	2.0	2.0	6.0	1.0	48	23.5
D_2O	4.5	3.0	2.0	6.5	1.0	47.5	23

	Approximate Chemical Shifts (δ)						
	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	H_5	H_6
DMSO-d_6	5.90	4.70	4.10	3.98	3.47	5.57	5.69
D_2O	6.03	4.97	4.24	4.21	3.63	5.44	5.89

(100-200 mesh) and eluted with benzene-methanol (3:1). After elution of the O-methyl derivative, the 3-N-methyl-FUA, Ib, followed and was obtained (70%) crystalline (from EtOAc-ether), m.p. 151-152°. Anal., found for $C_{10}H_{13}FN_2O_6$: C, 43.61; H, 4.92; N, 10.14; F, 6.77. Compound Ib was very sensitive to alkali and was converted in 0.1N alkali at room t° within 3 minutes (loss of selective absorption) to IIb which, after neutralization with Dowex 50 (H^+), was obtained as a sirup. The n.m.r. spectrum of this sirup was identical with that for IIa in DMSO-d_6 except for a doublet (3H) at 2.64 δ with a splitting of 4.2 c.p.s. This doublet (which becomes a singlet in dilute

acid) proves the presence of an $-NHCH_3$ grouping which can be rationalized only with the open chain ureide for IIb and IIa. The absence of a carboxyl proton in the 7-16 δ region in the n.m.r. spectrum of IIa may be due to H-bonding which would freeze the conformation into that shown (Table I) and account for a vicinal J_{H-F} coupling higher than usually observed in acylic compounds (8a,9) and more like that found for rigid cyclic molecules (6,8b).

When FCA (2) is treated with 0.1N NaOH at 60-70° for 0.5 hrs., a complete loss of selective absorption is observed and an ~ 50% yield of IIa is obtained. This reaction may be explained by the formation of $H_2NC(=O)-N-CHCHF-C(=O)NH_2$ which undergoes hydrolysis of the amide to IIa. On the other hand some FCA may be converted directly to FUA which would then be converted to IIa. We have noted by chromatographic and ultraviolet examination that FCR (12), when similarly treated with alkali, is slowly converted to FUR as well as to some fluorescent material.

With CA and UA, which lack the fluorine atom on C_5, alkaline treatment in the same manner produces a much slower loss of selective absorption which does not go to completion. The product(s) of these reactions were not investigated. Treatment of FCA, FUA and FUL with 0.1N alkali at room t° for 72 hrs., produced a 90, 75 and 50% loss in extinction respectively.

A plausible mechanism for the formation of IIa from FUA by a 1,4-addition is shown in Fig. 1. The FUA ion (13)(A) undergoes intramolecular nucleophilic attack by the 2'-hydroxyl anion to form the 6,2'-anhydronucleoside (B) which tautomerizes to (C), the latter of which undergoes ring cleavage to produce IIa. Cleavage of 5,6-dihydro pyrimidine nucleosides in alkali at the 3,4 positions are well documented (14). The presence of a 5-fluoro atom should enhance the susceptibility of C_6 to nucleophilic attack. In support of this mechanism, 1-β-D-arabinofuranosyl-4-thio-5-fluorouracil (IV) (prepared from

its tri-O-acetate (2) by de-blocking with 50% alkali in ethanol m.p. 177-179°, found for $C_9H_{11}FN_2O_5S$: C, 38.75; H, 4.09; F, 6.95; N, 9.95; S, 11.65) was treated with 0.1N NaOH for 60-70° for 5 hrs. Unlike FUA, no reaction for IV was detected as determined by the constancy of its U.V. spectrum under these reaction conditions. In the anion of IV, the negative charge would localize mainly on the sulfur atom which would mitigate against nucleophilic attack by the 2'-OH anion on C_6. The fact that 3-methyl-FUA (Ib) reacts rapidly with alkali under mild conditions to produce IIb is to be expected since formal negative charge in the aglycon is absent, and polarization of the α,β-unsaturated ketone would be enhanced, as described by Wang (15) for 1,3-dimethyluracil.

Compound II is the first example of a "6,2'-anhydro" structure in the nucleoside area. The formation of 6,5'-episulfide (cyclic) structures from certain 5'-thiol nucleoside derivatives have been reported (16a,b,c) however their formation was reversible leading to 5'-mercaptide anions or to disulfides with regeneration of the 5,6-double bond. Similarly, Chang (16d) prepared a 6,5'-anhydro-5,5-diiodo-5,6-dihydro uridine by iodination of cytidine. This dihydro derivative splits out HI with alkali to regenerate the 5,6-double bond. Previously, Lipkin et al. (16e) synthesized a 6,5'-anhydrouridine (unsaturated at the 5,6 position) by treatment of 5-iodouridine with base in DMSO. Piskala and Sorm (16f) showed that 5-azauridine in the crystalline state also exists as a 6,5'-anhydro nucleoside. In all these cases (16) the 6,5'-anhydro or -episulfide structures contained a 7-membered ring fused to the 1,4 positions of the sugar moieties. In our case (II) double bond regeneration did not occur under our reaction conditions and 3,4-bond cleavage predominated, possibly as a result of strain introduced by the 1',2'-fused 5-membered rings on the 6-membered pyrimidine ring.

Regeneration of the double bond (II→I) was, however, achieved by

esterification of IIa. Treatment of IIa (500 mg.) with diazomethane in methanol-ether yielded a sirup which, unlike IIa, gave a neutral reaction in water and showed two ultraviolet absorbing spots by thin layer chromatography (t.l.c.) on silica gel G (benzene-methanol, 3:1). Separation of the mixture by column chromatography on silica gel (vide supra) yielded two fractions. The first of these was probably the 5,6-dihydro derivative (IIIa and/or IIIb) since it showed an ultraviolet absorption maximum at ~220 mμ which was lost rapidly by addition of alkali (14). After elution of the second (and major) fraction, 350 mg. of a crystalline product was obtained, m.p. 151-152°. This product was identical with Ib obtained above by methylation of FUA.

Conversion of IIa to an ester enhances cyclization to the cyclic amide IIIa or b. It is not clear whether the methylation of N_3 with diazomethane occurred before or after cyclization. In any case, the dihydronucleoside III, under the anhydrous conditions employed in the esterification and N-methylation reaction, eliminates the alcohol from C_5-C_6 to form Ib.

The ease with which the 2'-OH in the arabino ("up") configuration participates in the reaction of FUA and FCA with alkali is of great interest. The fact that UA and CA also lose ultraviolet extinction in alkali indicates that 6,2'-anhydro formation is probably a general characteristic of pyrimidine nucleosides bearing the aglycon and 2'-hydroxyl in a "cis" relationship. The biological importance of 6,2'-anhydro structures for this class of chemotherapeutically active nucleosides is yet to be determined.

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