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NITRIC ACID ESTERS OF PYRIMIDINE NUCLEOSIDES

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In view of the fact that sugar nitrates are a well known class of compounds(1) it is very surprising that nucleoside nitrates have never been described. While limiting ourselves to pyrimidine deoxyriboside nitrates we wish to report the first synthesis of this species. We also wish to demonstrate in an example the usefulness of these nucleosides, whose hydroxyls are blocked by nitro groups, as intermediates for transformations in the sugar moiety.

Johnson had shown (2) that 5-bromo- and 5-chlorouracil, as well as thymine, when treated with fuming nitric acid, add HNO_3 to the 5,6 double bond thus affording 5-halo (or methyl)-5-nitro-6-hydroxyhydrouracil, while uracil passing through an analogous intermediate undergoes nitration in 5-position. Accordingly we found that 5-fluorouracil when reacted for 3 hours with fuming HNO_3 (d = 1,49) until disappearance of the u.v. absorption taken in 0,1 N HCl gave a 57% yield of 5-fluoro-5-nitro-6-hydroxyhydrouracil, m.p. 176°. Calcd. for $C_4H_4FN_3O_5$: C, 24.90; H, 2.09; N, 21.76. Found: C, 25.14; H, 2.12; N, 21.61.

In contrast to the behavior of the pyrimidine bases the corresponding deoxyribosides did not lose their u.v. absorption upon treatment with HNO_3. Instead of addition to the double bond esterification of the sugar hydroxyls was

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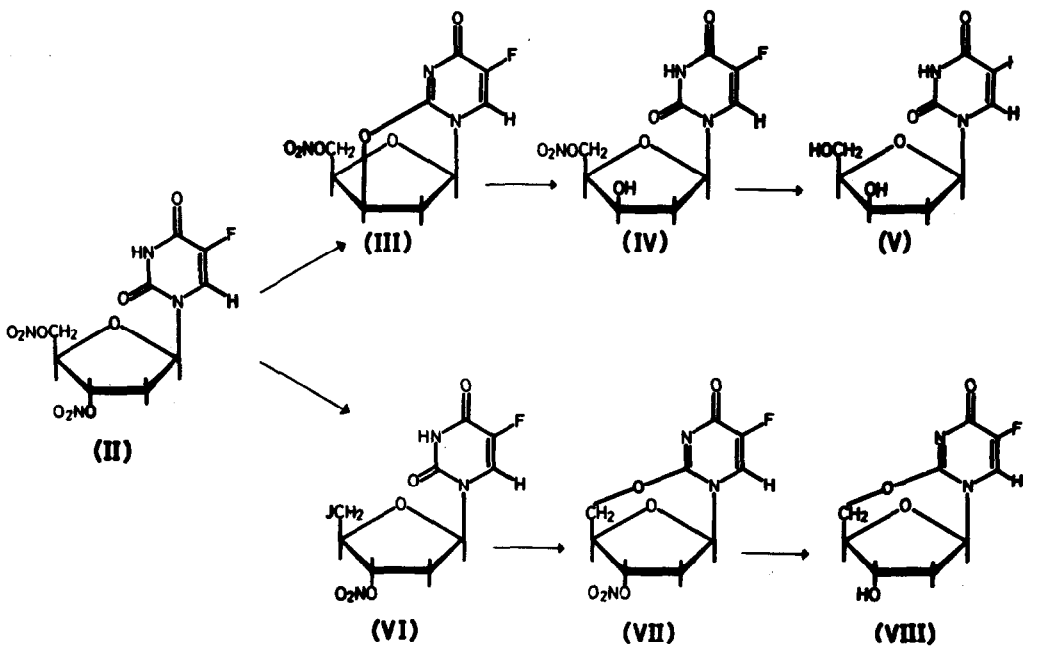
TABLE I

PYRIMIDINE 2'-DEOXYRIBOSIDE 3',5'-DINITRATES.

3',5'-Dinitrate of	m.p.*	Formula	Calcd.%			Found %		
			C	H	N	C	H	N
2'-Deoxyuridine	129°	$C_9H_{10}N_4O_9$	33.97	3.17	17.61	33.88	3.10	17.39
5-Fluoro-2'-deoxyuridine	156°	$C_9H_9FN_4O_9$	32.28	2.69	16.64	32.48	2.81	16.79
α-Anomer of above	123°	$C_9H_9FN_4O_9$	32.28	2.69		32.36	2.80	
5-Bromo-2'-deoxyuridine	156°	$C_9H_9BrN_4O_9$	27.45	2.26	14.10	27.48	2.31	14.08
Thymidine	125°	$C_{10}H_{12}N_4O_9$	36.15	3.64	16.87	36.41	3.66	16.62
5-Fluoro-2'-deoxycytidine	163°	$C_9H_{10}FN_5O_8$	32.25	3.01	20.89	32.20	3.06	20.92

* melting with decomposition (Tottoli apparatus).

CHART I



observed. Thus 24,6 g of 5-fluoro-2'-deoxyuridine (I) was dissolved in 50 ml of HNO_3 (d = 1,49) cooled in an ice bath and kept there for one hour. Upon addition of 1700 g of ice and water 30,2 g (90%) of the crystallized 5-fluoro-2'-deoxyuridine 3'5'-dinitrate (II) was isolated in practically pure state, $[\alpha]_D$ + 19° (c = 1 in ethylacetate), λ_{max}^{EtOH} 266 mμ (ε 9600). The failure of HNO_3 addition to the double bond can be explained by the dilution of the reaction mixture with water liberated in the course of the esterification of the sugar hydroxyls, since HNO_3 does produce disappearance of the absorption when reacted with dry isolated dinitrate. Other nucleoside 3'5'-dinitrates (see table I) were prepared similarly, but in the case of 5-fluoro-2'-deoxycytidine ca. 75 mg of urea was added per ml of HNO_3 used, in order to prevent deamination by HNO_2, which might have been present. Yields of 80 to 90% were obtained. The progress of the reaction and the uniformity of the obtained compounds were checked by TLC on Silicagel HF_{254} (Merck) using solvents such as ethyl acetate, methanol, ethanol, acetone and combinations thereof. The u.v. and i.r. spectra (absence of OH peak at ca. 3400 cm^{-1}) as well as the n.m.r. spectra of the compounds were compatible with those to be expected for nucleoside esters. Moreover hydrogenolytic removal (vide infra) of the nitro groups from II regenerated I, thus definitely establishing the assumed structure.

Chart I illustrates the versatility of nitrate esters as intermediates in two series of reactions, i.e. A) the conversion of a deoxyriboside into a deoxylyxoside via a 2,3'-anhydronucleoside (II ⟶ V) and B) the synthesis of a 2,5'-anhydronucleoside (II ⟶ VIII).

A) To a solution of 20,17 g of II in 720 ml of ethanol kept refluxing, aqueous 0,5 N KOH was added in such a manner that the pH always stayed at 9,5 or below. The drifting of the pH stopped after 2.5 hrs with an uptake of 117 ml of KOH indicating the removal of one mole HNO_3. The solution deposited upon cooling 9 g (55%) crystals, m.p. 209-210°, $[\alpha]_D$ + 59° (c = 0,1 in 50% aqu. EtOH). Calcd. for $C_9H_8FN_3O_6$: C, 39,57; H, 2,95; N, 15,38. Found: C, 39,47; H, 2,89; N, 15,25. The i.r. spectrum (absence of OH and appearance of a peak at 1550 cm^{-1} probably due to C=N stretching vibrations) the u.v. spectrum exhibiting λ_{max}^{pH7} 225-230 (S),

253 mμ (ε 6800, 7800) and n.m.r. spectrum were in accord with the structure of the 2,3'-anhydro-5-fluoro-2'-deoxyuridine 5'-nitrate (III). Thus a nitrate ester group - similar to tosyl and mesyl (3) - underwent an intra-molecular nucleophilic attack resulting in an inversion at C-3'. It has been reported that mildly alkaline solutions do not remove nitrate groups from sugars (4). This also seems to hold true for the nucleoside nitrates, provided that the 2-carbonyl of the pyrimidine nucleus, for sterical reasons, is incapable of approaching from the rear the carbon carrying the nitrate group. Accordingly we have found that the α-anomer of II, where the pyrimidine is located unfavorably, did not undergo loss of HNO_3 when maintained at pH 9.5. For reopening of the 2,3'-anhydro bond compound III (1,09 g) was reacted at 60° with 80 ml of 0,05 N KOH (in 50% EtOH). Upon neutralization with HCl and evaporation to dryness and crystallization of the obtained residue from water 0,512 g (43%) of 1-(2'-deoxy-β-D-lyxofuranosyl)-5-fluorouracil 5'-nitrate (IV), m.p. 193°, $[\alpha]_D$ + 25° (c = 1 in MeOH) was obtained. Calcd. for $C_9H_{10}FN_3O_7$: C, 37,11; H, 3,71; N, 14,37. Found: C, 37,04; H, 3,62; N, 14,37. Reappearance of the i.r. peak at 3650 cm^{-1} (OH) and the u.v. maximum at 268 - 269 mμ (ε 9100) in 0,1 N HCl decreasing to 6800 in 0,1 N KOH was characteristic for the "open" nucleoside. The nitro group was removed by hydrogenation at 25° of an ethanolic solution of IV using a Lindlar catalyst (5% Pd on $CaCO_3$, 5% Pb) which left the double bond and the attached fluorine intact. It proceeded according to the following equation (5): $2RONO_2 + 5H_2 \longrightarrow 2ROH + 4H_2O + N_2$ i.e. with an overall uptake of 2 moles of gas per NO_2 group. 1-(2'-Deoxy-β-D-lyxofuranosyl)-5-fluorouracil (V) was obtained by crystallization from ethanol-ether in 59% yield and identified by analysis, mixture m.p. (197 - 199°) and i.r. spectrum with an authentic specimen which had been synthesized and kindly supplied by Fox and Miller (6).

B) The synthesis of 2,5'-anhydro-5-fluoro-2'-deoxyuridine (VIII) was undertaken in pursuing our search for a latent form of the parent "open" nucleoside which could offer advantages as a chemotherapeutic agent (7,8).

A solution of 30 g of II and 24,1 g of NaI in 200 ml of acetone was refluxed for 28 hrs. After removal of the separated $NaNO_3$ by filtration and evaporation of the filtrate to dryness, the obtained residue was taken up with 100 ml of ethyl acetate which was washed with water and NaCl solution and dried over Na_2SO_4. Evaporation and dissolution of the residue in 15 ml ethyl acetate followed by addition of 35 ml of ether produced 22,84 g (58%) of 2'5'-dideoxy-5-fluoro-5'-iodouridine 3'-nitrate (VI) (assumed to contain ca. 8% of impurities, difficult to remove), m.p. 81°. This product was used in the following step. Calcd. for $C_9H_9FIN_3O_6$: C, 26,95; H, 2,26; N, 10,48; I, 31,64; Found: C, 28,94; H, 2,81; N, 9,85; I, 29,35; EtO, 3,44. It was expected from the behavior of sugar nitrates (9) and born out by the results of the ensuing steps that predominantly the nitrate group on the primary carbon is replaced by iodine. Following the method described by Brown et al. (10) compound VI (0,348 g) was cyclized by refluxing for 30 min. with 0,159 g of silver acetate dissolved in 150 ml of methanol, to yield 0,142 g (66%) of 2,5'-anhydro-5-fluoro-2'-deoxyuridine 3'-nitrate (VII), m.p. 215° (dec), $[\alpha]_D$ + 22° (c = 0,1 in acetonitrile), which after separation from the precipitated AgI crystallized upon cooling the reaction mixture. Calcd. for $C_9H_8FN_3O_6$: C, 39,57; H, 2,95; N, 15,38. Found: C, 39,86; H, 3,17; N, 15,41. The u.v. spectrum exhibiting λ_{max}^{EtOH} 246 mμ (ε 12200), the i.r. spectrum showing the characteristic peak at 1540 cm^{-1} and the n.m.r. spectrum were in accord with structure VII, but distinctly different from the spectra of the 2,3'-anhydro compound III. Hydrogenolysis of VII (1,22 g) in 120 ml of methanol with 1,55 g of Lindlar catalyst at 25°, was accomplished in 75 min. After addition of 120 ml of water and separation of the catalyst by filtration, the solution was evaporated at room temperature to a small volume yielding 0,813 g (79%) of compound VIII, m.p. 181° (dec), $[\alpha]_D$ + 70,5° (c = 0.4 in H_2O). Calcd. for $C_9H_9FN_2O_4$: C, 47,38; H, 3,98; N, 12,28. Found: C, 47,24; H, 3,83; N, 12,20. The i.r. spectrum exhibited the characteristic peak at 1520 cm^{-1} and the n.m.r. and mass spectra were found to be compatible with structure VIII.

Examination of the u.v. spectrum indicated that the compound is unstable in acid and alkali. It could not be recrystallized from hot water (unless heating was limited to a few seconds) without undergoing a profound change. The u.v. values found at pH 7 (phosphate buffer) of λ_{max} 250 mμ (ε 11200) and λ_{min} 218 mμ (ε 5060) are similar to those reported for 2,5'-anhydrothymidine by Michelson and Todd (11). The instability of 2,5'-anhydronucleosides is probably the reason why only this one single unblocked species carrying a free hydroxyl group has been isolated in a pure state. Therefore, the nitrate ester approach which permits deblocking, under very mild conditions, should make this interesting class of compounds more accessible.

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