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BROMINATION OF 2,2'-ANHYDROURIDINE

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Abstract—When 2,2'-anhydrouridine (1) is treated with aqueous Br_2, cleavage of the pyrimidine ring occurs and the 2-amino-oxazoline (2) as well as its corresponding 2-oxo-analogue (3) is formed, the structure of which was proved by investigating their acetylated derivatives, (4 and 5). Bromination of 1 in the absence of water yields the 2',5-dibromo-uridine (6) which can be converted into the corresponding 2,2'-anhydro-derivative (7). Structures were proved by MS, NMR and IR.

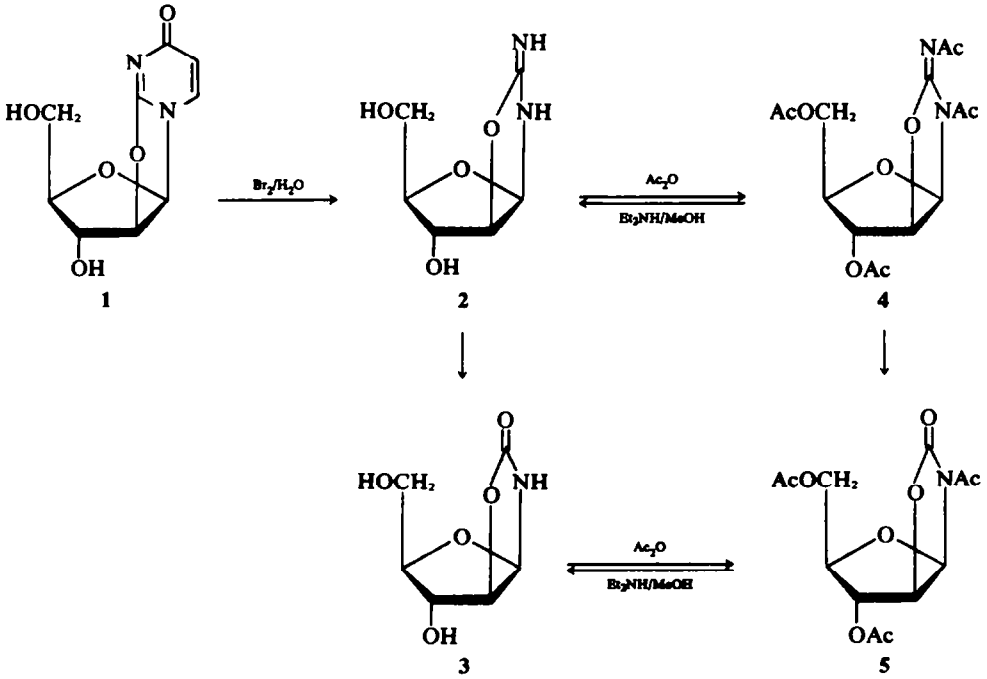
It is well known, that most of the pyrimidine nucleosides, containing a halogen at C-5 possess cytostatic or antiviral properties.[1] Recently it was shown,[2] that some of the 2,2'-anhydropyrimidine nucleosides exhibit similar biological activity. For increasing this activity, combination of these two elements, i.e. the synthesis of 2,2'-anhydro-5-bromo pyrimidine nucleosides was worked out.[3,4] In every case the corresponding 5-halogen derivatives were used as starting material and the 2,2'-anhydro bridge was formed subsequently.

The elegant and simple synthesis of Sanchez and Orgel[5] made the 2,2'-anhydropyrimidine readily available, therefore we investigated the direct bromination of these compounds. As model substance 2,2'-anhydrouridine (1) was chosen which was obtained from the 2 - amino - oxazoline 2 according to the literature.[5]

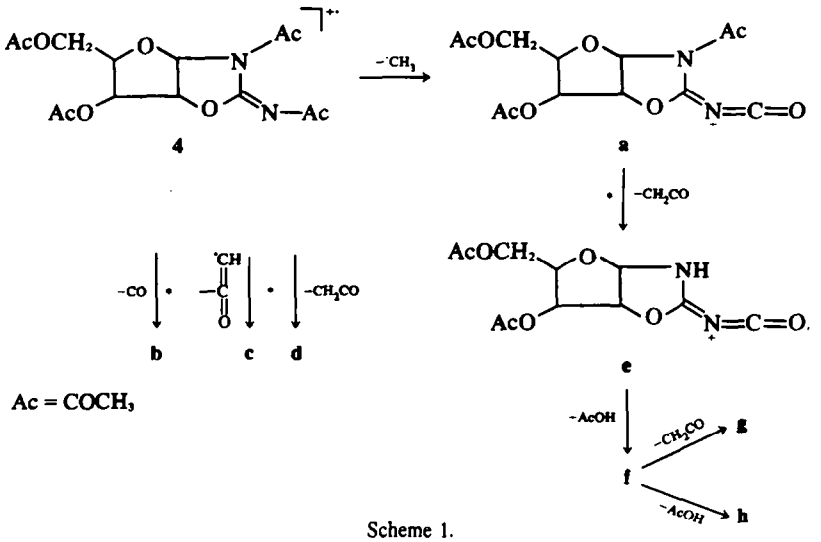
When compound 1 was treated with aqueous bromine—which reaction is generally used for the bromination of pyrimidine nucleosides at C-5—cleavage of the pyrimidine ring occurred and the 2 - amino - oxazoline 2, as well as its corresponding 2-oxo analogue 3 was formed. The latter could only be isolated from the reaction after acetylation as its triacetate 5 together with the tetraacetate 4. It is very probable, that in the bromination 2 is formed firstly via oxidative cleavage of the pyrimidine ring, and 3 is only a secondary product, formed by hydrolysis of 2 in the acidic media. The instability of 2 was proved directly, as it could be converted into 3 under the conditions applied for the bromination. The tetraacetyl derivative 4 is even more sensitive towards acids and is partly converted to 5 even by column chromatography on silicic acid.

Neither 4 or 5 could be deacetylated by sodium methoxide, therefore the acetyl groups were removed by treatment with diethylamine in methanol when 2 and 3 were formed, respectively.

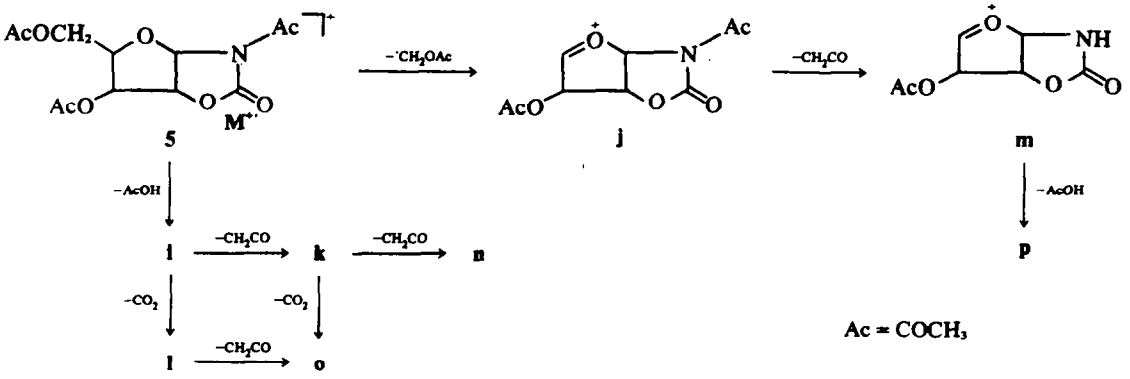
It is interesting to note, that the 2,2'-anhydro ring which can be split easily by acids remains intact during these



1 2 3 4 5



Scheme 1.



Scheme 2.

reactions, whereas the otherwise stable pyrimidine ring suffers a cleavage. A similar oxidative cleavage of the pyrimidine ring in "normal" acetylated nucleosides by permanganate was described by Gody and Walker,[6] who obtained an urea derivative which is the acyclic analogue of **2**. When 2,2' - anhydro - 3',5' - diacetyl - uridine was treated with aqueous permanganate, oxidative cleavage of the pyrimidine ring took place, and after acetylation of the crude mixture **4** and **5** could be separated as the only products. Nevertheless the mechanism, postulated for the reaction with permanganate[6] cannot be applied to the bromination, as according to GLC and MS bromoform is formed as a byproduct.

The structure of **4** and **5** obtained from **1** was established by high resolution mass spectrometry.

The fragmentation pattern of **4**, predominant both at 70 and 12·5 eV electron energy is depicted in Scheme 1, while the corresponding mass spectral data are listed in Table 1. Besides the "normal" behaviour of acetates, i.e. the loss of ketene and acetic acid, some unique processes can be observed in the mass spectrum of **4**, which we believe to be due to the presence of the acetyl-imino grouping. First of all the loss of $\cdot CH_3$, which is rarely observed in acetates, and the rearrangements leading to ions *b* and *c*, respectively. As all the processes depicted in Scheme 1 are rearrangements, except $M^{+\cdot}$ to *a*, the trends of abundances upon changing the electron energy (Table 1) are indicative for primary, secondary and subsequent processes and corroborate the sequence given in Scheme 1.

It is worth mentioning, that the mass spectrum of **4** formed from **1** was identical with that of the authentic compound prepared by the direct acetylation of **2**.

The fragmentation pattern of **5** (Scheme 2) and the data of the ions depicted (Table 2) are shown below.

The mass spectral characteristics of **5** are drastically different from those of **4**. It behaves like acetylated furanosides,[7] giving practically no molecular-ion peak and showing abundant ions for the loss of the side-chain and the consecutive ejection of stable neutral molecules (ions *j*, *m* and *p*). In the route initiated by the loss of acetic acid (ion *i*) the only fragmentation of the oxazoline-ring, the ejection of CO_2 (ion *l*) is observed, furnishing direct evidence for the structure of this moiety. The effect of electron energy on abundances can be evaluated as described for compound **4**.

It is interesting to note, that the replacement of the C=N–Ac grouping of **4** to C=O in **5** shifted the centre of fragmentation from the oxazoline- to the tetrahydrofurano-ring, presumably by increasing the local ionization potential in the former.

When bromination of **1** was carried out under anhydrous conditions, i.e. in dry chloroform, 1 - β - 2' - bromo - 2' - deoxy - D - ribofuranosil - 5 - bromouracil (**6**)

*Represents that a metastable peak was observed for the transition.

Table 1. Mass spectral data of ions of compound 4 depicted in Scheme 1

Symbol	m/e	Relative abundances (%) 70 eV	12.5 eV	Elemental composition*
$M^{+\cdot}$	342	24	27	$C_{14}H_{16}N_2O_8$
a	327	42	54	$C_{13}H_{15}N_2O_8$
b	314	23	29	$C_{13}H_{18}N_2O_7$
c	301	27	37	$C_{12}H_{17}N_2O_7$
d	300	17	22	$C_{12}H_{16}N_2O_7$
e	285	100	100	$C_{11}H_{13}N_2O_7$
f	225	4	3	—
g	183	25	5	$C_7H_7N_2O_4$
h	165	47	18	$C_7H_5N_2O_3$

* All high-resolution mass measurements were within ± 3 mmu to the calculated values.

was obtained. Location of the two bromo atoms at C-2' and C-5 in compound **6** was proved in part by the IR and NMR spectra of its diacetyl derivative **8**, and in part chemically as after hydrogenation over $Pd(OH)_2$ and subsequent acidic hydrolysis, 2'-deoxy-D-ribose could be detected as the only sugar component. For proving the "ribo" configuration of the brominated sugar, the dibromo compound **6** was treated with sodium methoxide, when 2,2' - anhydro - 5 - bromo - uridine (**7**) should be formed. The latter was described by Ponpipom and Hanessian[8] in 1972. The anhydro derivative, obtained from **6** was according to its IR, NMR and mass spectra the desired compound, but its physical data (m.p. 214°, $[\alpha]_D^{20} - 120°$) differed from those in the literature[8] (m.p. 195°, $[\alpha]_D^{20} + 20{\cdot}7°$).

Further chemical proof of the structure dibromide **6**, was provided when the anhydride **7** was treated under anhydrous conditions with HBr, yielding a dibromide, identical with **6**. On the other hand, treatment of 2' - bromo - 2' - deoxy - uridine (**9**)—which was obtained from anhydrouridine **1** according to Fox *et al.*[9]—with aqueous bromine gave a dibromo nucleoside also identical with **6**. Thus the location of the bromine at C-2' of the sugar moiety in "ribo" configuration was proved unambiguously as well as the structure of the 2,2'-anhydro derivative **7**, derived from it.

The fragmentation of **6** is in accordance with the general MS behaviour of pyrimidine-nucleosides,[10] yielding the (B + 1)-ion (*m/e* = 190, 192) as the base peak, which shows a further fragmentation analogous to that of 5 - bromo - uracil.[11] Cleavage at the glycosidic linkage in **6** yields ion *m/e* = 195, 197, representing the sugar moiety with one bromine atom. The fragmentation pattern of **7** can be deduced from that of **1** studied in detail[12] by inserting bromine in the ions containing the pyrimidine portion.

EXPERIMENTAL

M.ps are uncorrected. TLC was carried out on Kieselgel G coated microscope slides using EtOAc/EtOH 1:1 (A), 3:2 (B) EtOAc (C), EtOAc/CCl_4 1:1 (D) and 2:1 (E) for elution. Detection was effected with 0·1 N $KMnO_4$ and 2 N H_2SO_4 (1:1) and heating to 100°. NMR spectra were recorded at 60 MHz with a Varian A-60D spectrometer with TMS as internal standard. IR spectra were recorded is KBr pellets on a Perkin Elmer 457 spectrometer. Mass spectra were recorded on a Varian MAT SM-1 instrument. All evaporations were carried out in a rotary evaporator under diminished pressure, after drying the organic solutions over Na_2SO_4. Kieselgel 40 (0·063–0·200 mm) was used for column chromatography.

Bromination of 2,2'-anhydrouridine with bromine water

(a) *Isolation of* **2**. A soln of 1 (5 g) in water (70 ml) was treated with Br_2 (10 g) and was kept at room temp. for 24 hr. After removing the excess of Br_2 by a stream of air, the soln was adjusted to pH 8 with an ion exchanger and was then evaporated to dryness. The semisolid residue was filtered with EtOH and was recrystallized from water, yielding **2**; (1·6 g; 42%), m.p. 175–177°, R_f 0·35 (A), $[\alpha]_D^{20} = +61°$ (*c* 1, pyridine), $[\alpha]_D^{20} = +20°$ (*c* 1, water). Lit[5]: m.p. 175–176°.

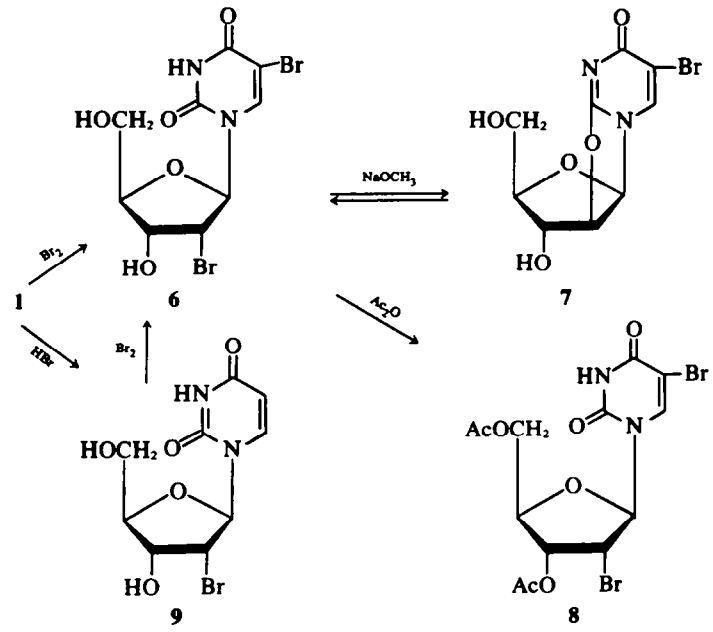
(b) *Isolation of* **4** *and* **5**. The mixture obtained after removing the excess of Br_2 was evaporated, and then several times reevaporated with EtOH. The oily residue was dissolved in pyridine (30 ml) and Ac_2O (30 ml) and was kept at room temp. overnight. After usual work up the $CHCl_3$ soln was evaporated and the solid residue was recrystallized from EtOH, yielding **4** (2 g; 27%), m.p. 123–124°, R_f 0·4 (E), $[\alpha]_D^{20} = -60{\cdot}4°$ (*c* 1, $CHCl_3$) IR: 1750, 1730, 1710 and 1670 (ester, amide), 1280, 1260, 1230, 1215, 1140, 1110 and 1065 cm^{-1} (ν_{C-O}), NMR ($CDCl_3$): δ 6·20 (H-1'), 5·10 (H-3'), 4·92 (H-2'), 2·47, 2·17, 2·08 and 2·04 ppm (2xO-Ac and 2xN-Ac), $J_{1',2'} = 5$, $J_{2',3'} = 0$, $J_{3',4'} = 2$ Hz. (Found: C, 49·30; H, 5·33; N, 8·38. $C_{14}H_{18}N_2O_8$ requires: C, 49·12; H, 5·30; N, 8·18%).

Chromatography of the mother liquor (solvent E) afforded further **4** (0·3 g; 4%) and **5** (0·5 g; 7·5%), m.p. 80–81°, R_f 0·6 (E) $[\alpha]_D^{20} = -109°$ (*c* 1, $CHCl_3$), IR: 1800, 1745, and 1710 (ester, amide), 1235, 1210, 1155, 1110 and 1075 cm^{-1} (ν_{C-O}), NMR

Table 2. Mass spectral data of ions of compound 5 depicted in Scheme 2 Relative abundances (%)

Symbol	m/e	70 eV	12.5 eV	Elemental composition*
$M^{+\cdot}$	301	0	0.05	-
i	241	3.6	7.5	-
j	226	52	100	$C_9H_{10}NO_6$
k	199	11	17	$C_8H_9NO_5$
l	197	3.6	4.7	$C_9H_{11}NO_4$
m	186	68	60	$C_7H_8NO_5$
n	157	8	7	$C_6H_7NO_4$
o	155	12	18	$C_7H_9NO_3$
p	126	100	89	$C_5H_4NO_3$

* All high-resolution mass measurements were within ± 1.5 mmu to the calculated values.



($CDCl_3$): δ 6·13 (H-1'), 5·10 (H-3'), 4·82 (H-2'), 2·46 (N-Ac), 2·09 and 2·03 ppm (2xO-Ac), $J_{1',2'} = 5$, $J_{2',3'} = 0$, $J_{3',4'} = 2$ Hz, (Found: C, 48·20; H, 5·22; N, 4·69. $C_{12}H_{15}NO_8$ requires: C, 47·85; H, 5·02; N, 4·65%).

Deacetylation of compound **4** *and* **5**. A soln of **4** (0·3 g) in MeOH (5 ml) was heated in the presence of diethylamine (0·7 g) on a steam bath for 4 hr. The crystalline residue, obtained after evaporation was recrystallized from water to yield **2** (0·1 g; 55%). Similar treatment of **5** gave after column chromatography (solvent A) **3** as colorless oil, yield 80%, $[\alpha]_D^{20} = -35·7°$ (*c* 1, pyridine), R_f 0·75 (A).

1 - *β* - D - (2' - *Bromo* - 2' - *deoxy* - *ribofuranosyl* - 5 - *bromo* - *uracil* (**6**)

(a) A slurry of **1** (5·5 g) in $CHCl_3$ (50 ml) was treated with Br_2 (4 g). The mixture was stirred at boiling temp. for 6 hr. The chloroform was decanted after cooling and the brown oily residue was triturated several times with chloroform. The sticky solid residue was dissolved in EtOH and was purified on a silica column using EtOAc as solvent. The fractions, containing **6** were evaporated and the residue was recrystallized from EtOAc (4·1 g; 42%), m.p. 193–194°, R_f 0·7 (C), $[\alpha]_D^{20} = -24·6°$ (*c* 1, pyridine), $[\alpha]_D^{20} = -18·3$ (*c* 1, water), IR: 1685, 1620 (pyrimidine-dione), 3530, 3470 (ν_{OH}), 3300–2700 cm^{-1} (ν_{NH}), NMR (DMSO-d_6): δ 11·9 (NH), 8·65 (s, H-6), 6·15 (d, H-1'), 4·70 ppm (t, H-2'), $J_{1',2'} = J_{2',3'} =$ 4 Hz (Found: C, 28·83; H, 2·83; Br, 41·11; N, 7·19. $C_9H_8O_5Br_2N_2$ requires: C, 28·16; H, 2·10; Br, 41·73; N, 7·30%).

(b) A slurry of **7** (0·6 g) in trifluoro acetic acid (20 ml) saturated with HBr at 0°, was stirred at room temp. for 5 days. Unreacted **7** was removed by filtration, the filtrate was evaporated and the yellow syrup was purified by column chromatography (solvent C). Recrystallization of the evaporated residue from EtOAc afforded **6** (0·5 g; 65%), identical with that, obtained according to route a.

(c) 2' - Bromo - 2' - deoxy - uridine[9] (0·3 g) was treated with Br_2 water, until the yellow colour of the mixture remained unchanged. The excess of Br_2 was removed by air and the soln was evaporated. The residue gave on purification by column chromatography (solvent D) and recrystallization from EtOAc pure **6** (0·2 g, 53%), identical with that, mentioned above.

Hydrogenation of compound **6**. A soln of **6** (0·1 g) in water (5 ml) was hydrogenated at room temp in the presence of 2 N NaOH (0·2 ml) using $Pd(OH)_2/C$ as catalyst. The residue, obtained after filtration and evaporation was hydrolysed with 0·1 N HCl (5 ml) on a stream bath for 8 hr. The mixture was chromatographed on Schleicher-Schüll 2043/6 mg paper, using n-BuOH saturated with water for elution. On detection by aniline-phthalate the spot of 2 - deoxy - ribose appeared at R_f 0·30.

2,2' - *Anhydro* - [1 - (*β* - *D* - *arabofuranosyl*)] - 5 - *bromo* - *uracil* (**7**)

A soln of **6** (4·8 g) in dry MeOH (60 ml) was treated with 0·4 N methanolic NaOMe (30 ml). The slurry was stirred at room temp. overnight. The residue obtained after evaporation was purified by column chromatography (solvent B). Recrystallization from EtOH gave **7** as colorless platelets (3·6 g; 86%), m.p. 214°, R_f 0·58 (D), $[\alpha]_D^{20} = -120°$ (*c* 1, DMF), lit.[8] m.p. 195°, $[\alpha]_D^{20} = +20·7$ IR: no pyrimidine-dione band, 1640 (amide-I), 1530 ($\nu_{C=C}$), 1480 cm^{-1} (ν_{NH}), NMR (DMSO-d_6): δ 8·50 (s, H-6), 6·37 (d, H-1'), 5·29 (d, 3'-OH; $J_{H-OH} = 4·5$ Hz), 5·28 (d, H-2'), 5·00 (t, 5'-OH; $J_{H-OH} =$ 5 Hz), 4·43 (2xd, H-3'), 4·17 (2xt, H-4'), 3·30 ppm (d, 2xH-5'), $J_{1',2'} = 6$, $J_{2',3'} = 0$, $J_{3',4'} = 1$, $J_{4',5'} = 4$ Hz. (Found: C, 35·45; H, 3·19; Br, 26·31; N, 8·99. $C_9H_9O_5BrN_2$ requires: C, 35·43; H, 2·97; Br, 26·20; N, 9·18%).

1 - *β* - *D* - (3',5' - *di* - *O* - *acetyl* - 2' - *bromo* - 2' - *deoxy*) - *ribofuranosyl* - 5 - *bromo* - *uracil* (**8**)

A soln of **6** (0·8 g) in pyridine (9 ml) and Ac_2O (9 ml) was kept at room temp. for 24 hr. After usual work up the $CHCl_3$ soln was evaporated and the residue was purified by column chromatography (solvent D). Compound **8** was obtained as a solid foam (0·6 g; 64%), m.p. 170–195° (dec), R_f 0·60 (D), $[\alpha]_D^{20} = -39·7°$ (*c* 1, $CHCl_3$), IR: 3300–2700 (ν_{NH}), 1740 ($\nu_{C=O}$), 1690 (amide-I), 1620 ($\nu_{C=C}$), 1230 and 1050 cm^{-1} (ν_{C-O}), NMR ($CDCl_3$): δ 8·8 (NH), 7·93 (s, H-6), 6·25 (d, H-1'), 5·10 (t, H-3'), 4·65 (t, H-2'), 2·20 and 2·23 ppm (2xO-Ac), $J_{1',2'} = J_{2',3'} = J_{3',4'} = 4$ Hz. (Found: C, 35·68; H, 3·19; Br, 33·44; N, 4·02. $C_{15}H_{12}O_7Br_2N_2$ requires: C, 33·36; H 2·64; Br, 34·15; N, 5·99%).

Oxidation of 2,2' - *anhydro* - 3',5' - *di* - *O* - *acetyl* - *uridine with* $KMnO_4$

A soln 2,2' - anhydro - 3',5' - di - O - acetyl - uridine[13] (0·6 g) in water (80 ml) and BuOH (80 ml) was treated with $KMnO_4$ (1 g). The brown slurry was stirred overnight. The filtered soln was evaporated, the residue was treated with EtOH, filtered and reevaporated. The oily residue (0·8 g) was acetylated in pyridine (8 ml) with Ac_2O (4 ml). After usual work up the crude mixture was separated by column chromatography (solvent E) yielding **4** (0·15 g) and **5** (0·2 g), both identical with those described above.

REFERENCES

[1]T. Kanai, M. Ichino, A. Hoshi, F. Kanzawa and K. Kuretani, *J. Med. Chem.* **15**, 1218 (1972); A. Hoshi, F. Kanzawa, K. Kuretani, T. Kanai, K. Kikugawa and M. Ichino, *Gann* **64**, 519 (1973); J. H. Hunter, *U. S.* 3,155,646; *Chem. Abstr.* **62**, 2821a; G. E.

Underwood, C. A. Wisner and S. D. Weed, *Arch. Ophtamol.* **72**, 505 (1964); P. Lepine, A. M. Michelson and M. P. Garilhe, *Fr.* M3783; *Chem. Abstr.* **67**, 54403k; J. Rudder and M. P. Garilhe, *Antimicrobial Agents Chemotherapy* 578 (1965); *Chem. Abstr.* **65**, 9362 g.

[2]A. Hoshi, F. Kanzawa, K. Kuretani, M. Saneyoshi and Y. Arai, *Gann* **62**, 145 (1971); Kohjin Co Ltd. 02-08-71 J4 8023-788, Takeda Chemical Inds Ltd. TAKE 03-06-71 JA 4844267-Q; F. Hoffmann-La Roche Co NL 7312-201; Kohjin KK DT-2159181-Q.

[3]Kohjin Co Ltd. JA-4864079-Q; Syntex Corp. DT-2112724-Q.

[4]N. C. Yung, J. H. Burchenal, R. Fecher, R. Duschinsky and J. J. Fox, *J. Am. Chem. Soc.* **83**, 4060 (1961); J. J. Fox, N. C. Miller and R. C. Cushley, *Tetrahedron Letters* 4927 (1966).

[5]R. A. Sanchez and L. E. Orgel, *J. Mol. Biol.* **47**, 531 (1970); D. H. Shannahoff and R. A. Sanchez, *J. Org. Chem.* **38**, 593 (1973).

[6]R. S. Goody and R. T. Walker, *Tetrahedron* **27**, 65 (1971).

[7]C.f.: T. Radford and D. C. Dejongh, *Biochemical Applications of Mass Spectrometry*, (Edited by G. R. Waller) p. 339. Wiley Interscience, New York (1972).

[8]M. M. Ponpipom and S. Hanessian, *Can. J. Chem.* **50**, 246 (1972).

[9]J. F. Codington, I. L. Doerr and J. J. Fox, *J. Org. Chem.* **29**, 558 (1964).

[10]C.f.: C. Hignite, *Biochemical Applications of Mass Spectrometry* (Edited by G. R. Waller) Wiley-Interscience, New York (1972).

[11]J. Ulrich, R. Teoule, R. Massot and A. Cornu, *Org. Mass Spectrom.* **2**, 1183 (1969).

[12]J. B. Westmore, D. C. K. Lin, K. K. Ogilvie, H. Wayborn and J. Berestiansky, *Org. Mass Spectrom.* **6**, 1243 (1972).

[13]D. M. Brown, D. B. Parihar and A. Todd, *J. Chem. Soc.* 4242 (1958).