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Acetylation and cleavage of purine nucleosides. Synthesis of 6-azauridine, 5-fluorouridine, and 5-methyluridine.

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ABSTRACT

Inosine (I) when acetylated with acetic anhydride in the presence of acetyl chloride in acetic acid solution (the so called "acid acetylation"), affords an acetylated nucleoside III (75%) along with cleavage products of the nucleoside (hypoxanthine, 19%). The reaction of I with acetyl chloride (7 days) results in the formation of hypoxanthine (95%) and triacetylribofuranosyl chloride (IV) isolated in the form of tetraacetylribofuranose (47%). The acetylated purine nucleoside affords a similar result by reaction with acetyl chloride or acetyl bromide. 2'-Deoxyuridine gives a diacetyl derivative (80%) by reaction with acetyl bromide. On treatment with acetyl bromide, the nucleoside bond of purine nucleosides is quantitatively cleavaged (4 h, 20°C) with the formation of tri-O-acetyl-D-ribofuranosyl bromide (X). The halogenose X affords pure β-anomers, namely, 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (75%), the triacetyl derivatives of 5-methyluridine (XVIIa; 75%, referred to guanosine), 6-azauridine (XVIII; 71%), and 5-fluorouridine (XIXa; 75%).

INTRODUCTION

Some time ago, a report has been published on acetylation[1] of pyrimidine nucleosides by the action of acetic anhydride in the presence of acetyl chloride; this reaction may be conducted in the solution of acetic acid as a suitable solvent of nucleosides. This so called "acid acetylation" may also be performed on treatment with acetyl chloride alone. A simple work-up of the reaction mixture by evaporation and crystallisation of the residue makes the method particularly advantageous for the preparation of water-soluble acetyl derivatives. The method has been utilised in the preparation of acetylated ribo[1,2], arabino[2], and deoxy[3-6] derivatives of pyrimidine nucleosides. Another

advantageous feature of the method consists in the selective O-acetylation of the nucleoside amino derivatives as it has been demonstrated in the case of pyrimidine nucleosides bearing an amino group in the aglycon[7] or sugar[8] moiety.

In the literature, a report has appeared on acetolysis of the acetylated 2′-deoxyadenosine (by the action of acetic acid and acetic anhydride at 100°C) with the formation of 1,3,5-tri-O-acetyl-2-deoxy-D-ribose[9] and analogous acetolysis of purine nucleosides with the formation of 1,2,3,5--tetra-O-acetyl-β-D-ribofuranose[10,11]. The reaction of uridine with acetyl bromide has been recently effected to afford 3′,5′-di-O-acetyl-2′-bromo-2′-deoxyuridine[12].

RESULTS AND DISCUSSION

The "acid acetylation" of purine nucleosides with acetic anhydride in the presence of a small amount of acetyl chloride also results in acetylation of the nucleoside (70%), but the acetylation is accompanied by a partial cleavage of the nucleoside bond. This cleavage reaction has been now examined in detail.

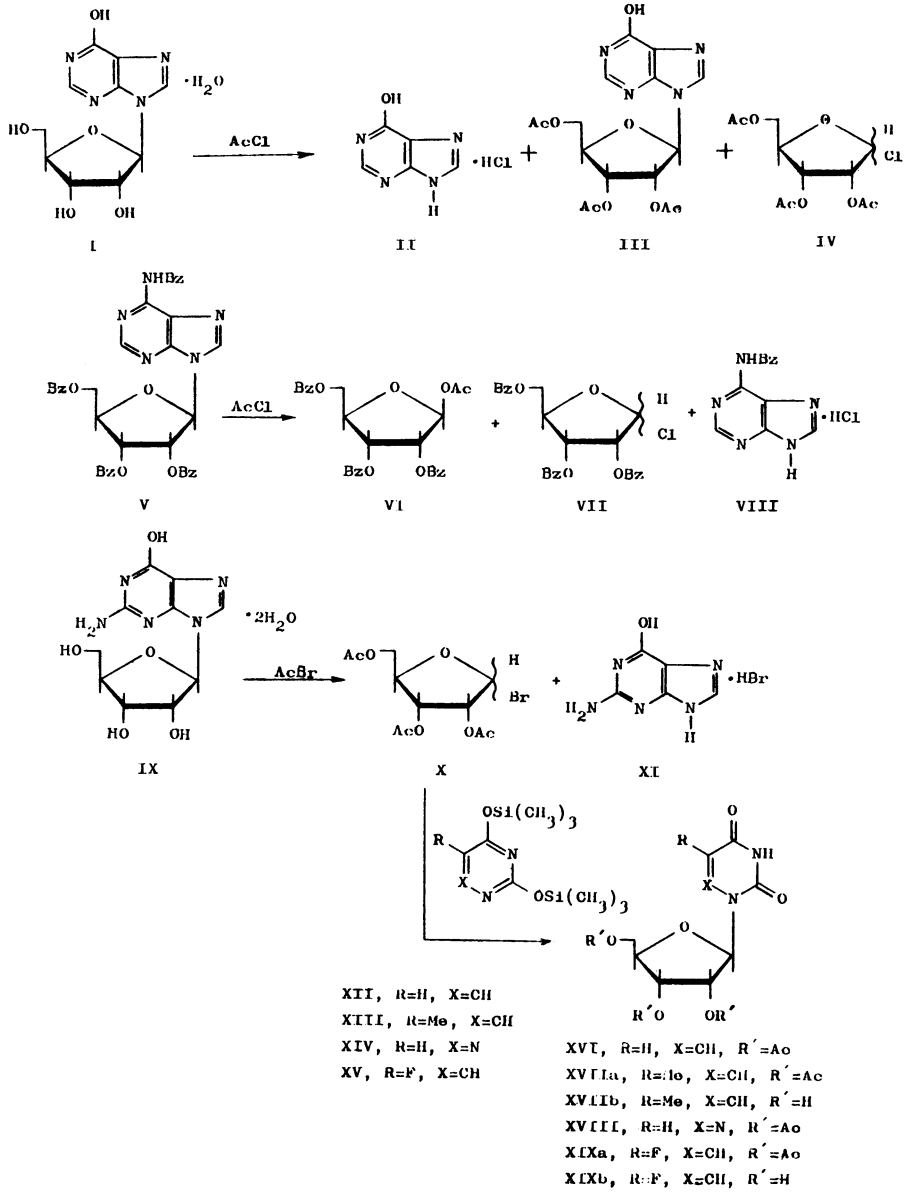
The reaction of acetyl chloride with purine nucleosides in acetic acid afforded a mixture of products of the acetylation and cleavage reaction. Thus, from the reaction of inosine (I) with acetyl chloride, the following substances were isolated after 4 h at room temperature: triacetylinosine (III; 46%), hypoxanthine hydrochloride (II; 43%), and triacetylribofuranosyl chloride (IV) which was transformed into tetraacetylribofuranose (37%) by reaction with silver acetate. The content of products and their ratio remained almost the same when the reaction was conducted for a prolonged time (24 h) or at an elevated temperature (50°C). Addition of hydrogen chloride (20%) to the reaction mixture did not accelerate the cleavage reaction. In the reaction of acetyl chloride with a purine nucleoside proceeding for a considerably longer reaction period of time (7-14 days), a quantitative cleavage of the nucleoside bond was observed with the formation of the purine base hydrochloride (95%) and the acetylated halogenose (44%). In the case of the free nucleoside, a simultaneous acetylation and cleavage of the

nucleoside bond occurs in the initial stage of the reaction. The acetylated nucleoside is then slowly cleaved in the further stage.

The cleavage of the nucleoside bond in acetylated nucleosides proceeds with similar results. Thus, analogous products are obtained in the reaction with acetyl chloride or acetyl bromide. The use of a longer reaction time results in a partial decomposition of the halogenose. The reaction of triacetylinosine with acetyl chloride (acetyl bromide) affords hypoxanthine (95%) and a sugar derivative which was isolated in the form of a tetraacetylribofuranose (40-50%); the cleavage with acetyl bromide was by an order of magnitude faster than in the case of acetyl chloride. When treated with acetyl chloride, tetrabenzoyladenosine (V) affords N-benzoyladenine (after neutralisation of compound VIII; 93%), 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (VI; 25%), and tribenzoylribofuranosyl chloride (VII) which was isolated in the form of ethyl tri-O-benzoyl-β-D-ribofuranoside (35%). The reaction of 2′-deoxyuridine with acetyl bromide affords diacetyl-2′-deoxyuridine in 80% yield.

The best yields of the acetylated halogenose were obtained by cleavage of purine nucleosides with acetyl bromide. As it may be seen from Table I, all the naturally occuring purine nucleosides afforded a high yield of the acetylated halogenose under very mild conditions, namely, at room temperature and in the course of 4 hours. The extent of the cleavage reaction was checked by yields of the nucleobase and the halogenose. The thus-formed halogenose retains the ribo configuration of the sugar moiety of the starting nucleoside and affords unequivocally either the β-anomer of tetraacetylribofuranose (75%, referred to the starting nucleoside) when treated with silver acetate or the β-anomer of a nucleoside when treated with a silylated nucleobase. This "transnucleosidation method" was examplified by the preparation of some biologically active substances such as 5-fluorouridine[13] (XIXb) and tri-O-acetyl-6-azauridine[14] (XVIII).

Triacetyl-D-ribofuranosyl bromide (X) was prepared by reaction of acetyl bromide with guanosine (IX) because of



the most readily reproducible results and yields of the "transnucleosidation reaction" and owing to the best commercial availability of guanosine from all the naturally occurring nucleosides. Condensation of the halogenose with one equivalent of the silylated nucleobase afforded the β-ano-

mers of acetylated nucleosides in a high yield. The thus--obtained acetyl derivatives of 5-methyluridine (XVIIa; 75%), 6-azauridine (XVIII; 71%), and 5-fluorouridine (XIXa; 75.5%) were converted to the free nucleosides by the subsequent deacetylation. Triacetyluridine (XVI) was prepared analogously from inosine in 69.5% yield.

The present method is the only procedure affording acetylated halogenoses in a single reaction step. When compared with the earlier methods of the cleavage of the nucleoside bond[9-11,15] and with nucleosidation reactions[16], the present procedure is extremely simple, the reaction conditions are mild, and the reaction products are pure from chemical as well as physical standpoint. The reaction could be of some both theoretical and practical value in the cleavage of complex naturally occurring purine nucleosides and resynthesis of the appropriate analogues.

EXPERIMENTAL

Melting points were taken on a heated microscope stage Boetius (Kofler block). Ultraviolet spectra were measured on a CF-4 Optica Milano apparatus. Infrared spectra were recorded on a UR-20 apparatus (Carl Zeiss, Jena). The ^{1}H NMR spectra were taken on a Varian HA-100 apparatus (100 MHz). Optical rotations were measured on an automatic Perkin-Elmer 141 MC polarimeter. Column chromatography was carried out on the Pitra macroporous silica gel (particle size, 30-60 micron) produced by Service Laboratories of this Institute.

2′,3′,5′-Tri-O-acetylinosine (III)

A mixture of inosine monohydrate (I; 1.43 g; 5 mmol), acetic acid (10 ml), and acetic anhydride (5 ml) was treated at 60°C with acetyl chloride (0.3 ml) and stirred (for about 2 h) until the solid dissolved. The reaction mixture was then kept at room temperature for 15 h, evaporated under diminished pressure, and the residue coevaporated with toluene (20 ml). Crystallisation of the residue from ethanol yielded 1.39 g (70.5%) of the tri-O-acetylinosine III, m.p. 239--242°C undepressed on admixture with an autentic specimen[17]. The mother liquors were evaporated and the residue kept in a mixture of pyridine (2 ml) and acetic anhydride (1 ml) for

15 h at room temperature. Methanol (2 ml) was then added, the mixture set aside for 15 min, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (50 g) in the solvent system ethyl acetate--acetone-ethanol-water (6:1:1:1) afford 100 mg (5%) of 2′,3′,5′-tri-O-acetylinosine (III) and 130 mg (19%) of hypoxanthine.

Cleavage of inosine (I) with acetyl chloride

A A mixture of inosine monohydrate (I; 286 mg; 1 mmol), acetyl chloride (2 ml), and acetic acid (2 ml) was shaken for about 30 min until the solid dissolved, the solution kept at room temperature for 4 h, and evaporated under diminished pressure. The residue was coevaporated with three 3 ml portions of toluene and finally triturated with toluene. The insoluble portion was filtered off, washed with three 2 ml portions of toluene, and distributed between water (3 ml) and chloroform (5 ml). The aqueous layer was separated and extracted with two 5 ml portions of chloroform. The chloroform layers were combined, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. Crystallisation of the residue from ethanol yielded 181 mg (46%) of 2′,3′,5′-tri-O-acetylinosine (III). The aqueous layer was neutralised with aqueous sodium hydroxide to deposit 59 mg (43%) of hypoxanthine. The filtrates after compound III and hypoxanthine were combined and treated with silver acetate (167 mg; 1 mmol). The mixture was stirred at room temperature for 12 h. The insoluble portion was filtered off and washed with toluene. The filtrate and washings were combined and evaporated under diminished pressure. Crystallisation of the residue from ethanol yielded 85 mg (27%) of 1,2,3,5--tetra-O-acetyl-β-D-ribofuranose. An additional crop (32 mg; 10%) of this furanose was obtained by chromatography of mother liquors on a column of silica gel (10 g) in the solvent system benzene-acetone (6:1).

B A mixture of inosine monohydrate (I; 286 mg; 1 mmol), acetic acid (5 ml), and acetyl chloride (5 ml) was stirred until the solid dissolved and kept at room temperature for 7 days. Toluene (10 ml) was then added and the mixture stir-

red at 3°C for 3 h to deposit hypoxanthine hydrochloride (II) which was collected with suction and washed with three 3 ml portions of toluene. The filtrate and washings were combined and evaporated under diminished pressure. The residue was co-evaporated with three 10 ml portions of toluene and finally dissolved in toluene (10 ml). The solution was treated with silver acetate (167 mg; 1 mmol) and the mixture stirred at room temperature for 15 h. The insoluble portion was filtered off and washed with three 2 ml portions of toluene. The filtrate and washings were combined and evaporated under diminished pressure. The residue was crystallised from ethanol to afford 100 mg of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose, m.p. 81-82°C undepressed on admixture with an autentic specimen. The mother liquors were chromatographed on a column of silica gel (10 g) in the solvent system benzene-acetone (6:1) to afford an additional crop (28 mg) of the same substance. Overall yield, 38% of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose. Neutralisation of the above hypoxanthine hydrochloride (II) yielded 128 mg (94%) of hypoxanthine.

Cleavage of 2',3',5'-tri-O-benzoyl-N^4-benzoyladenosine (V) with acetyl chloride

A solution of the tetrabenzoyladenosine V (684 mg; 1 mmol) in acetic acid (5 ml) and acetyl chloride (5 ml) was kept at room temperature for 5 days. Toluene (10 ml) was then added and the mixture kept at 3°C for 3 h to deposit benzoyladenine hydrochloride (VIII). The solid was collected with suction and washed with three similar portions of toluene. The filtrate and washings were combined and evaporated under diminished pressure. The residue was coevaporated with three 10 ml portions of toluene and finally crystallised from ethanol to afford 71 mg of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D--ribofuranose (VI), m.p. 131-132°C undepressed on admixture with an autentic specimen[18]. Chromatography of evaporated mother liquors on a column of silica gel (30 g) in the solvent system benzene-ethyl acetate (20:1) yielded 171 mg (35%) of ethyl 2,3,5-tri-O-benzoyl-β-D-ribofuranoside. ^{1}H NMR spectrum (deuteriochloroform; chemical shifts in p.p.m.): 1.18 (t, 3H, -O-$CH_2\underline{CH}_3$, $J_{CH_2CH_3}$ = 7.0 Hz), 4.63 (q, 2H, -O-$\underline{CH}_2CH_3$),

3.68 (m, 2H, $H_{5'}$), 4.70 (m, 1H, $H_{4'}$), 5.26 (s, 1H, $H_{1'}$, $J_{H_{1'},H_{2'}}$ = 0.7 Hz), 5.67 (d, 1H, $H_{2'}$, $J_{H_{2'},H_{3'}}$ = 5.0 Hz), 5.88 (dd, 1H, $H_{3'}$, $J_{H_{3'},H_{4'}}$ = 6.0 Hz), 7.25-7.65, 7.80-8.15 (m, 15H, arom. protons). For $C_{28}H_{26}O_8$ (490.5) calculated: 68.56% C, 5.34% H; found: 68.73% C, 5.19% H.

The subsequent fraction was evaporated and the residue crystallised from ethanol to afford 55 mg of 1-O-acetyl--2,3,5-tri-O-benzoyl-β-D-ribofuranose (VI). Overall yield, 126 mg (25%).

A suspension of the above benzoyladenine hydrochloride (VIII) in water (2 ml) was treated dropwise with 1M aqueous sodium hydroxide until neutral to deposit a solid which was collected with suction and washed successively with two 2 ml portions of water and with ethanol (1 ml). Yield, 222 mg (93%) of benzoyladenine, m.p. 242-244°C undepressed on admixture with an autentic specimen[19].

Cleavage of 2',3',5'-tri-O-acetylinosine with acetyl chloride

A solution of 2',3',5'-tri-O-acetylinosine (III; 394 mg; 1 mmol) in acetic acid (5 ml) and acetyl chloride (5 ml) was kept at room temperature for 14 days and processed analogously to the reaction of inosine with acetyl chloride. Yield, 160 mg (50%) of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose and 130 mg (95.5%) of hypoxanthine.

Cleavage of 2',3',5'-tri-O-acetylinosine with acetyl bromide

A mixture of 2',3',5'-tri-O-acetylinosine (III; 394 mg; 1 mmol), acetic acid (5 ml), and acetyl bromide (5 ml) was stirred until the solid dissolved and then kept at room temperature for 24 h. Toluene was added (10 ml), the mixture kept at 3°C for 3 h, and processed analogously to the preceding paragraph (reaction with acetyl chloride). Yield, 128 mg (94%) of hypoxanthine and 122 mg (38%) of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose.

3',5'-Di-O-acetyl-2'-deoxyuridine

A solution of 2'-deoxyuridine (46 mg; 0.2 mmol) in acetic acid (1 ml) and acetyl bromide (1 ml) was kept at room temperature for 4 h and evaporated under diminished pressure. The residue was coevaporated with three 3 ml portions of toluene

and finally dissolved in methanol. The solution was neutralised with sodium acetate and evaporated under diminished pressure. Chromatography of the residue on a column of silica gel in the solvent system benzene-acetone (3:2) yielded 50 mg (80%) of 3′,5′-di-O-acetyl-2′-deoxyuridine in the form of a chromatographically homogeneous sirup. For $C_{13}H_{16}N_2O_7$ (312.3) calculated: 50.00% C, 5.17% H, 8.97% N; found: 50.12% C, 5.33% H, 8.89% N. The infrared spectrum was identical with that of an autentic specimen[20].

Cleavage of ribofuranosylpurines with acetyl bromide

A mixture of the appropriate ribofuranosylpurine (1 mmol) acetic acid (3 ml), and acetyl bromide (3 ml) was stirred at room temperature for 4 h, diluted with toluene (6 ml), and ke t at 3°C for 3 h. The crystalline precipitate of the corresponding purine hydrobromide was collected with suction and washed with three 2 ml portions of toluene. The filtrate and washings were combined and evaporated under diminished pressure. The residue was coevaporated with three 6 ml portions of toluene and then dissolved in toluene (10 ml). Silver acetate (250 mg) was added and the mixture stirred at room temperature for 15 h. The insoluble portion was filtered off and washed with three 2 ml portions of toluene. The filtrate and washings were evaporated under diminished pressure and the residue was crystallised from ethanol to afford the main crop of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose. An additional crop was obtained by chromatography of mother liquors on a column of silica gel (15 g) in the solvent system benzene-acetone (6:1). The corresponding purine bases were liberated from their hydrobromides with 1M aqueous sodium hydroxide. For the yields of these bases and of 1,2,3,5--tetra-O-acetyl-β-D-ribofuranose see Table 1.

2,3,5-Tri-O-acetyl-D-ribofuranosyl bromide (X)

A mixture of the appropriate ribofuranosylpurine (1 mmol), acetic acid (2.5 ml), and acetyl bromide (2.5 ml) was shaken for 20 min, then kept at room temperature for 5 h, and processed under exclusion of atmospheric moisture. The final liquor was concentrated under diminished pressure to the volume of about 2 ml and the concentrate diluted with

TABLE 1

Cleavage of purine nucleosides (1 mmol) with acetyl bromide. Yields of bases and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose

Nucleoside	Base	1,2,3,5-Tetra-O-acetyl--3-D-ribofuranose
Inosine[a]	133 mg (98%)	250 mg (78.5%)
Adenosine	108 mg (80%)	238 mg (75.0%)
Guanosine[b]	130 mg (86%)[c]	240 mg (75.5%)

[a]Inosine monohydrate. [b]Guanosine dihydrate. [c]After reprecipitation from 1M aqueous sodium hydroxide with hydrochloric acid.

toluene (4 ml) to deposit the corresponding purine hydrobromide. The salt was collected with suction and washed with three 1 ml portions of toluene. The filtrate and washings were combined and evaporated under diminished pressure. After three coevaporations with toluene (5 ml each), the residual 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide was directly (without an additional purification) used in the preparation of nucleosides.

Silylation of pyrimidine bases

The procedure of Winkley and Robins was used[21]. A mixture of the appropriate pyrimidine base (1.2 mmol), hexamethyldisilazane (5 ml), and ammonium sulfate (3 mg) was heated (bath temperature, 150°C) under a reflux condenser for 4-6 h until the base dissolved. After additional one hour of heating, the mixture was evaporated under diminished pressure. The residual silylated base was coevaporated with toluene (5 ml) and then directly used in the preparation of nucleosides.

2′,3′,5′-Tri-O-acetyluridine (XVI)

A solution of 2,4-bis(trimethylsilyloxy)pyrimidine (XII; 1.2 mmol) and 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide (X; prepared from 1 mmol of inosine monohydrate I) in acetonitrile (5 ml) was treated with molecular sieves Potassit 3 (1 g) and mercuric bromide (100 mg) and the whole mixture stirred at

room temperature for 6 h. The insoluble material was filtered off and washed with three 3 ml portions of acetonitrile. The filtrate and washings were combined and evaporated under diminished pressure. The solution of the residue in chloroform (75 ml) was successively washed with three 10 ml portions of 30% aqueous potassium iodide and two 10 ml portions of water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel (30 g) in the solvent system benzene--acetone (2:1). Crystallisation from ethanol yielded 230 mg (62.5%) of 2′,3′,5′-tri-O-acetyl-uridine (XVI), m.p. 130-131°C undepressed on admixture with an autentic sample[2]. The infrared spectra of both substances were identical. Work-up of mother liquors yielded an additional crop (25 mg; 7%) of the same substance.

2′,3′,5′-Tri-O-acetyl-5-methyluridine (XVIIa)

Compound XVIIa was prepared from 5-methyl-2,4-bis(trimethyl-silyloxy)pyrimidine (XIII; 1.2 mmol) and 2,3,5-tri-O--acetyl-D-ribofuranosyl bromide (X) analogously to the preparation of compound XVI described in the preceding paragraph. The halogenose obtained from 1 mmol of guanosine dihydrate (IX) yielded 288 mg (75%) of 2′,3′,5′-tri-O-acetyl-5-methyluridine (XVIIa), $[\alpha]_D^{25}$ - 14.6° (c 0.40; ethyl acetate). For $C_{16}H_{20}N_2O_9$ (384.3) calculated: 50.00% C, 5.25% H, 7.29% N; found: 49.78% C, 5.05% H, 7.35% N.

5-Methyluridine (XVIIb)

A solution of 2′,3′,5′-tri-O-acetyl-5-methyluridine (XVIIa; 192 mg; 0.5 mmol) in 0.1M methanolic sodium methoxide (10 ml) was kept at room temperature for 1 h and neutralised with Dowex 50 (H^+ ion exchange resin previously washed with methanol. The resin was filtered off and the filtrate evaporated under diminished pressure. Crystallisation of the residue from ethanol yielded 90 mg of 5-methyluridine (XVIIb), m.p. 184-185°C; $[\alpha]_D^{25}$ - 10.3° (c 0.26; water), corresponding to the literature[22]. An additional crop (23 mg) of XVIIb was obtained from mother liquors (total yield, 87%).

2′,3′,5′-Tri-O-acetyl-6-azauridine (XVIII)

The title compound XVIII was prepared analogously to 2′,3′,5′-tri-O-acetyluridine (XVI) from 1.2 mmol of 3,5-bis(trimethylsilyloxy)-1,2,4-triazine (XIV) and 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide (X) which was obtained from 1 mmol of guanosine dihydrate (IX). Chromatography on a column of silica gel (30 g) in the solvent system benzene-acetone (3:1) and crystallisation from 2-propanol yielded 169 mg of 2′,3′,5′-tri-O-acetyl-6-azauridine (XVIII), m.p. 103-105°C undepressed on admixture with an autentic specimen[1]. An additional crop (96 mg) of XVIII was obtained from mother liquors (m.p. 101-104°C). Overall yield, 265 mg (71%). Infrared spectra of the present substance and and autentic specimen were identical.

2′,3′,5′-Tri-O-acetyl-5-fluorouridine (XIXa)

The title compound XIXa was prepared analogously to compound XVI from 1 mmol of 5-fluoro-2,4-bis(trimethylsilyloxy)pyrimidine (XV) and 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide (X) obtained from 1 mmol of guanosine dihydrate (IX). Chromatography on a column of silica gel in the solvent system benzene-acetone (5:2) yielded 293 mg (75.5%) of the chromatographically homogeneous 2′,3′,5′-tri-O-acetyl-5-fluorouridine (XIXa), $[\alpha]_D^{25}$ + 20.7° (c 0.53; ethyl acetate). Infrared spectrum (chloroform): 3384 cm^{-1} (NH), 1752 cm^{-1} (C=O, acetate), sh 1727 and 1711 cm^{-1} (C=O, uracil), 1676 cm^{-1} (C=C). For $C_{15}H_{17}FN_2O_9$ (388.3) calculated: 46.39% C, 4.41% H, 7.22% N; found: 46.60% C, 4.52% H, 6.98% N.

5-Fluorouridine (XIXb)

The deacetylation of 2′,3′,5′-tri-O-acetyl-5-fluorouridine (XIXa; 194 mg; 0.5 mmol) was effected analogously to that 2′,3′,5′-tri-O-acetyl-5-methyluridine (XVIIa). Crystallisation from ethanol y elded 80 mg of 5-fluorouridine (XIXb), m.p. 184-185°C in accordance with literature[23]. An additional crop (25 mg) of the same substance (m.p. 183-185°C) was obtained from mother liquors. Overall yield, 105 mg (80%) of 5-fluorouridine. For $C_9H_{11}FN_2O_6$ (262.2) calculated: 41.23% C, 4.23% H, 10.69% N, 7.25% F; found: 41.22% C, 4.37% H, 10.76% N, 7.22% F.

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