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2′-O-ACYL RIBONUCLEOSIDE DERIVATIVES

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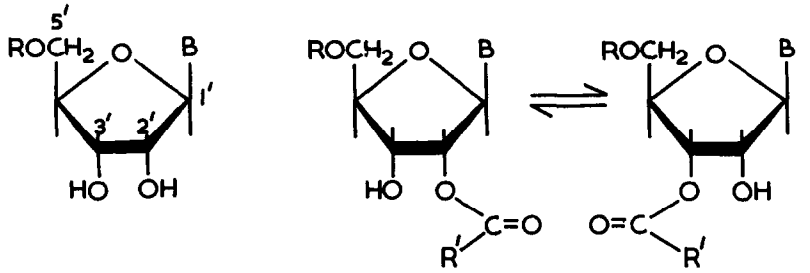
In connection with our interest in oligoribonucleotide synthesis (1), it became essential to be able to distinguish between pairs of isomeric 2′- and 3′-acyl derivatives of ribonucleosides (such as II and III respectively), and to determine whether such isomers were readily interconvertible. These considerations are also directly relevant to the orientation (2 - 5) of amino-acyl-s-RNA.



(I) (II) (III)

The earlier literature on this subject suggested that 2′-acyl derivatives (II) were unstable with respect to their 3′-isomers (III). Thus (6) when 5′-O-acetyluridine (I; B=uracil-1, R=Ac) was treated with ca. 1 mol. of acetic anhydride in pyridine solution and the diacetate fraction isolated, 3′,5′-di-O-acetyluridine (III; B=uracil-1, R=Ac, R′=Me) only, was obtained in a pure crystalline form. In the same way (7), 3′,5′-di-O-acetyladenosine

was prepared from 5'-O-acetyladenosine. These results were particularly interesting in that both nucleoside 5'-acetates were reported (6, 8) to yield predominantly 2'-O-tosyl derivatives when treated with limited amounts of tosyl chloride. More recently, however, the separation of two pure isomeric tribenzoylcytidines (respectively II and III; B=N^4-benzoylcytosine-1, R=Bz, R'=Ph) has been claimed by Rammler and Khorana (9). These workers phosphorylated the 3'-benzoate in pyridine solution and, after the removal of protecting groups, obtained cytidine 2'-phosphate as the sole nucleotidic product. However, phosphorylation of a specimen of the 2'-benzoate, and work-up under identical conditions, gave a mixture of cytidine 3'-phosphate (82%) and cytidine 2'-phosphate (18%).

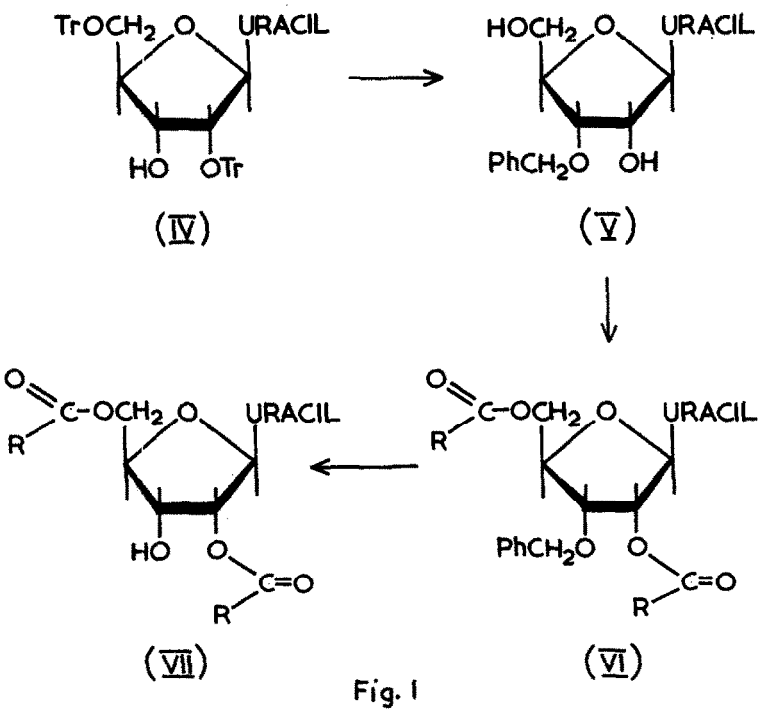


Fig. 1

In order to examine the stability of 2'-O-acyl nucleosides, we devised the general and unambiguous synthesis of 2',5'-di-O-acyluridines (VII), outlined in Fig. 1. Yung and Fox (10) characterized 2',5'-di-O-trityluridine (IV) as one of the products of the reaction between uridine and excess trityl chloride.† Alkylation of (IV) with benzyl chloride and potassium hydroxide in hot benzene/dioxane (11) gave its 3'-benzyl ether, which was subsequently de-tritylated by treatment with aqueous acid. The product, 3'-O-benzyluridine (V)‡ crystallized from water as colourless rods, m.p. 204-206°, in 50% yield based on 2',5'-di-O-trityluridine. When (V) was allowed to react with acetic anhydride in pyridine solution, 2',5'-di-O-acetyl-3'-O-benzyluridine (VI; R=Me) was isolated from the products as fine colourless needles, m.p. 134.5-135.5°, in 91% yield. A solution of this material (R_F*0.92) in anhydrous dioxane was shaken with hydrogen in the presence of palladium catalyst at 20° and atmospheric pressure. The hydrogenolysis product, which had the same paper chromatographic properties as 3',5'-di-O-acetyluridine (R_F0.77), was isolated as a colourless glass. In the same way, reaction of (V) with benzoyl chloride in pyridine gave an 88% yield of a dibenzoate (VI; R=Ph), which on hydrogenolysis gave dibenzoyluridine as a colourless oil. Clearly, an analytical method was required to determine whether the hydrogenolysis products were pure 2',5'-di-O-acetyl and 2',5'-di-O-benzoyluridines (VII; R=Me and Ph respectively), uncontaminated with their corresponding 3',5'-isomers.

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† From uridine (20 g), we obtained 2',5'-di-O-trityluridine (IV) in 44% yield (28 g) and the 3',5'-isomer, m.p. 137-140° in 20% yield (13 g). Žemlička reported (12) the isolation of the latter isomer in 27% yield (m.p. 135-145°), but gave no evidence to support its orientation. We have prepared distinguishable O-mesyl as well as O-benzyl derivatives of both isomers.

‡ Satisfactory elemental analyses were obtained for all new crystalline compounds described. By a similar route, 2'-O-benzyluridine was obtained as colourless needles, m.p. 181-182° in 59% yield from 3',5'-di-O-trityluridine. Both 2'- and 3'-O-benzyluridines showed the expected ultraviolet absorption; neither consumed periodate. The potential uses of 2'-O-benzyluridine in oligoribonucleotide synthesis are currently under investigation.

*Chromatographic solvent system: butan-1-ol - acetic acid - water (5, 2, 3; v./v.).

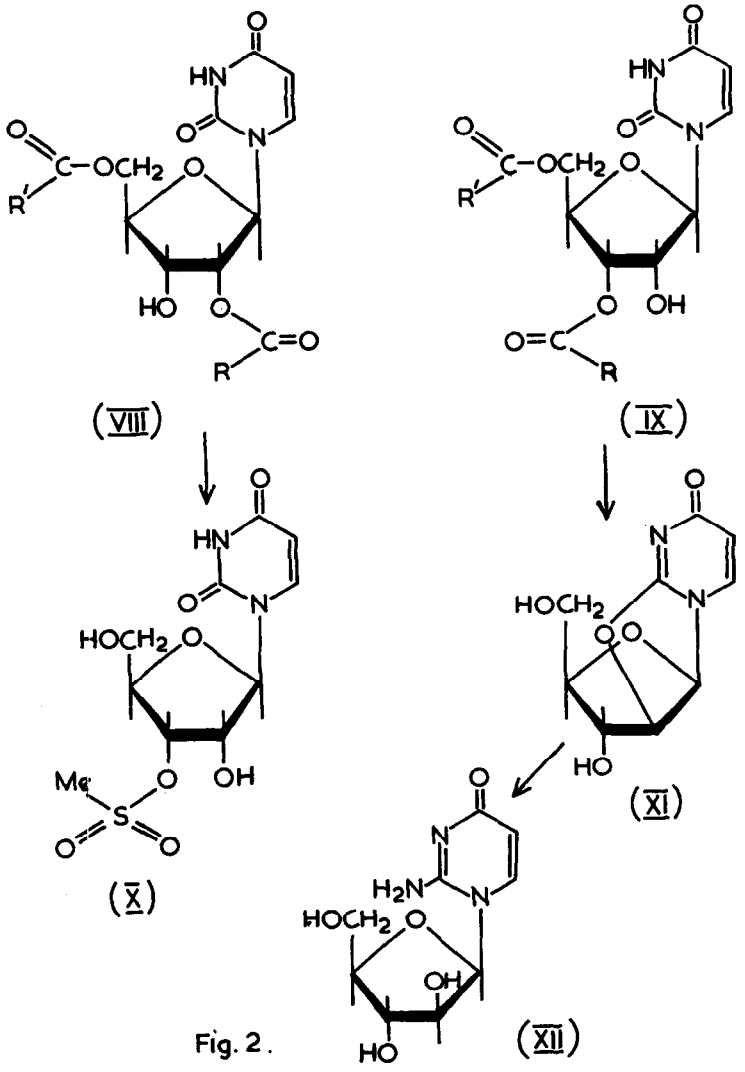

Fig. 2.

A suitable analytical procedure, which depends on the greater ease of anhydronucleoside formation from a 2′-sulphonate (6) than from a 3′-sulphonate (10, 13, 14) ester of uridine, is outlined in Fig. 2. When a mixture (15) of diacyl compounds (VIII and IX) is treated with an excess of methanesulphonyl chloride in pyridine solution at or below 0°, and the resulting mesyl derivatives treated with methanolic ammonia, the 2′,5′-diacyl compound (VIII) is converted into 3′-O-mesyluridine (X) and the 3′,5′-isomer (IX) is converted (13

into a mixture of 2,2'-anhydro-1-β-D-arabinofuranosyluracil (XI) and 1-β-D-arabinofuranosylisocytosine (XII). If the resultant mixture of (X), (XI) and (XII) is then submitted to paper electrophoresis in 0.02 M sodium borate buffer at pH 9, 3'-O-mesyluridine (X) has virtually zero mobility and is separated from the anhydronucleoside (XI) and the isocytosine derivative (XII), which both migrate towards the cathode with similar mobilities. The neutral and cationic fractions are then independently eluted with 0.1 N hydrochloric acid and estimated spectrophotometrically at 256 mμ[†]. As virtually no acyl migration occurs during mesylation (see below), this allows a direct measurement of the proportions of the diacyl compounds (VIII and IX) in the original mixture.

The diacetyluridine, prepared by the hydrogenolysis of 2',5'-di-O-acetyl-3'-O-benzyluridine (VI; R=Me), was treated with an excess of methanesulphonyl chloride in pyridine solution at -30° for 16 hr. When the product, isolated as a chromatographically homogeneous (R_F 0.81) oil[‡], was treated with methanolic ammonia, a sole product which had the same paper electrophoretic and paper chromatographic (R_F 0.61) properties as 3'-O-mesyluridine (X) (10) was obtained*. No anhydronucleoside (XI) or its ammonolysis product (XII) (both have R_F 0.51) was observed although the limit of detection was estimated to be ca. 1%. Thus the hydrogenolysis product of (VI; R=Me) was shown to be 2',5'-di-O-acetyluridine (VII; R=Me), free from its 3',5'-isomer (IX; R=R'=Me). In the same way the hydrogenolysis product of (VI; R=Ph) was established to be 2',5'-di-O-benzoyluridine (VII; R=Ph).

When 3',5'-di-O-acetyluridine (IX; R=R'=Me) was treated with methanesulphonyl chloride and then with methanolic ammonia, as above, no 3'-O-mesyluridine (X) was detected. The orientations of several other crystalline

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[†]At 256 mμ, both (XI) and (XII) have ε=7920, and it is thus unimportant that they have similar electrophoretic mobilities. At 256 mμ, (X) has ε=9940.

[‡]The analytical procedure requires that pyridinium salts should be removed at this stage.

*Crystalline 3'-O-mesyluridine was subsequently isolated from the products in 49% yield, based on (VI; R=Me).

di-O-acyluridines, prepared by the orthoester exchange method (16), were established in the same way: 3′,5′-di-O-formyluridine (IX; R=R′=H), 3′-O-acetyl-5′-O-trimethylacetyluridine (IX; R=Me, R′=t-Bu), and 3′-O-benzoyl-5′-O-formyluridine (IX; R=Ph, R′=H). In none of these cases was the presence of 2′,5′-isomer detected.

2′,5′-Di-O-acetyl- and 2′,5′-di-O-benzoyluridine are the first reported 2′-O-acyl ribonucleoside derivatives, with unprotected neighbouring hydroxyl groups, which have been established conclusively to be free from their 3′-isomers†.

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† A solution of 2′,5′-di-O-benzoyluridine, 2′,3′-di-O-acetyluridine 5′-phosphate and an excess of dicyclohexylcarbodiimide in anhydrous pyridine solution was allowed to stand for 58 hr. at 20°. After the products had been worked up and the protecting groups removed, the uridylyl (3′→5′) uridine so obtained was ca. 98% degraded to uridine 3′-phosphate and uridine in the presence of pancreatic ribonuclease. The reported studies (9) on the phosphorylation of tribenzoylcytidines are surprising in the light of this result.

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