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Note

The structure of the "uridine dialdehyde"–benzoylhydrazine reaction product

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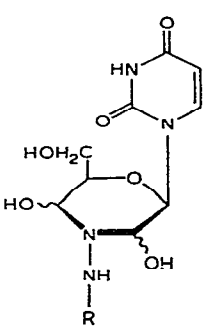
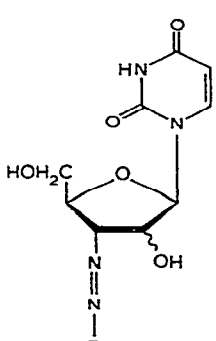
We have been interested for some time in the synthesis of polynucleotide analogues that do not contain phosphorus[1–6], some of which interact with polynucleotides. One type of analogue used was obtained by the reaction of a "ribonucleoside dialdehyde" (formed by periodate oxidation of a ribonucleoside) with polyacrylic acid hydrazide[5,6], but the structures of these polymers have not been established. Similar compounds are obtained during one method of purifying specific tRNA. The purified tRNA species required from a mixture is aminoacylated, and the mixture is then subjected to periodate oxidation. The "tRNA dialdehydes" so formed can then be removed by reaction with a suitable hydrazide to leave the purified aminoacylated tRNA[7,8]. It is known that only one "ribonucleoside dialdehyde" molecule reacts per molecule of hydrazide[9], and two cyclic structures for compounds of a similar type, namely **1**[7] and **2**[10], have been suggested, although the proposed intermediate **3** in the formation of **2** should not be excluded. The work described here was designed to determine which, if any, of these structures are produced when a "nucleoside dialdehyde" reacts with a monomeric hydrazide (benzoylhydrazine), such that the product could be isolated and characterised.



1



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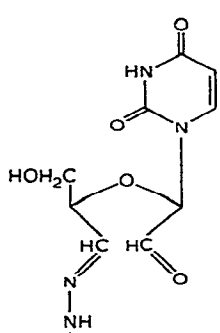

3

The product from the reaction between benzoylhydrazine and "uridine dialdehyde", which were allowed to react under conditions identical to those previously used for the preparation of the polynucleotide analogues, was isolated in good yield, and no other u.v.-absorbing compounds could be detected in the reaction mixture. The compound had an analysis compatible with structure **1** (R = Bz), or with a monohydrate of structures **2** and **3**. N.m.r. spectroscopy indicated that the product was actually a mixture of diastereoisomers of general structure **1** (R = Bz).

Most of the peaks in the 100-MHz spectrum [$(CD_3)_2SO$] of **1** could be assigned. The presence of two NH protons indicated that compounds of structure **1**, or possibly structure **3**, were present and excluded structure **2**. The absence of an aldehydic proton signal excluded structure **3**; furthermore, all signals in the region where vinylic proton resonances might be expected can be assigned and only signals below δ 4.82 remain. The hydrazide NH signal appeared as two peaks which were not coupled to each other and were present in the ratio 1:1. This could be due to restricted rotation about the amide bond, to the existence of two molecules with different stereochemistry at the ring nitrogen atom, or to a mixture of compounds of the types **1** and **3**. Attempts to distinguish between the first two of these alternatives by high temperature n.m.r. spectroscopy failed because the compound decomposed in solution at temperatures above 40°, but it is thought that the second explanation is more likely. Thus, despite the complex n.m.r. spectra, the possibility of a mixture of compounds of widely differing structures (such as **1** and **3**) can be excluded because the n.m.r. signals are in the positions expected for compounds of structure **1** and the integration is correct.

Determination of the stereochemistry at C-2′ and C-4′ is more complicated as no individual signals can be assigned to the H or OH of C-1′, C-2′, or C-4′. The n.m.r. spectrum is somewhat simplified when D_2O is added, in that the two NH and three OH protons exchange. However, in the region δ 6–5 p.p.m., there were at least 11 lines that included a doublet from the heterocyclic H-5, leaving the remaining signals to be assigned to H-1′. This means that the anomeric proton must be influenced by the protons at C-2′ and at C-4′ being present in at least three of the four possible diastereomeric forms (4 lines from each). Spin-decoupling experiments also demonstrated the presence of several isomers, and attempts to separate these failed because their chromatographic mobility is mainly influenced by the aromatic and heterocyclic ring-systems.

It is of interest to note that reaction of the dialdehyde prepared from methyl 4,6-*O*-benzylidene-α-D-glucoside with phenylhydrazine, under conditions similar to those used here[10], gave a compound of the type **2**, whereas "uridine dialdehyde" has here given compounds of structure **1**. For the D-glucose derivative to give compounds of structure **1**, the formation of a seven-membered ring fused to the six-membered benzylidene ring would be involved, and this is probably less favourable than the ring expansion from a five- to a six-membered ring in the case of uridine.

However, it has been shown that the reaction of a hydrazine, containing a fairly bulky group, with a "nucleoside dialdehyde" gives a series of isomeric compounds of general structure **1**. It seems likely that the polynucleotide analogues prepared under

similar conditions have the same structure, but with several stereoisomers present on one chain of polyacrylic acid hydrazide.

EXPERIMENTAL

Benzoylhydrazine was prepared as described by Curtius[11]. "Uridine dialdehyde" was prepared by the periodate oxidation of uridine, as previously described[5]; it was not isolated and was treated in solution with benzoylhydrazine as follows: uridine (488 mg, 2 mmoles) was dissolved in water (200 ml), sodium metaperiodate (2.2 mmoles) was added, and the solution was left at room temperature for 1 h. T.l.c. showed that no nucleoside was present after this time, and only one u.v.-absorbing compound, corresponding in mobility with uridine dialdehyde, was present. Benzoylhydrazine (300 mg, 2.2 mmoles) in water (100 ml) was then added, and the solution was left at room temperature overnight, concentrated to 100 ml under reduced pressure, and left at 4°. The product, which was homogeneous by t.l.c., was filtered off and dried to give **1** (R = Bz) as a white, crystalline solid (0.37 g, 1.0 mmole, 50% yield), m.p. 160–164° (dec.) (Found: C, 50.5; H, 5.1; N, 14.5. $C_{16}H_{18}N_4O_7$ calc.: C, 50.8; H, 4.8; N, 14.8%); u.v. data: λ_{max} (0.1M HCl and H_2O pH 7) 261 nm (ε 9,600), λ_{min} 230 nm; λ_{max} (0.1M NaOH) 264 nm (ε 7,200), λ_{min} 243 nm. N.m.r. data [$(CD_3)_2SO$]: δ 11.25 (1-proton singlet, 3N-H), 9.44 and 9.23 (singlets, 1 proton, hydrazide N-H), 7.89–7.54 (6-proton multiplet, C_6H_5 and H-6), 6.30–5.38 (4-proton multiplet, HO-2′, HO-4′, H-5, and H-1′), 4.82–4.56 (3-proton multiplet, HO-6, H-2′, and H-4′), 4.00 (1-proton singlet, H-5′), 3.65 (2-proton singlet, H-6′); [$(CD_3)_2SO+D_2O$]: δ 7.87–7.50 (6-proton multiplet, C_6H_5 and H-6), 5.80–5.47 (2-proton multiplet, H-5 and H-1′), 4.82–4.44 (2-proton multiplet, H-2′ and H-4′), 4.00 (1-proton singlet, H-5′), 3.65 (2-proton singlet, H-6′).

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