Isomeric 2′- and 3′-*O*-Acyl Ribonucleoside Derivatives

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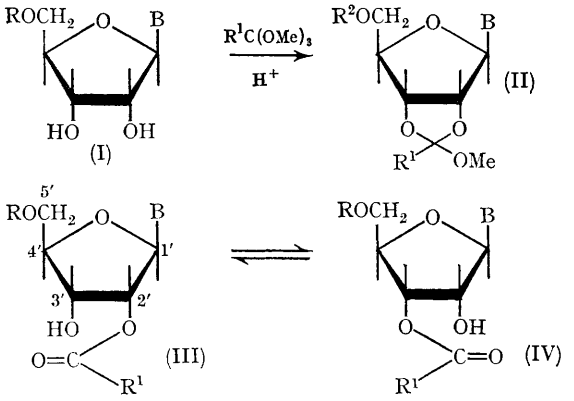
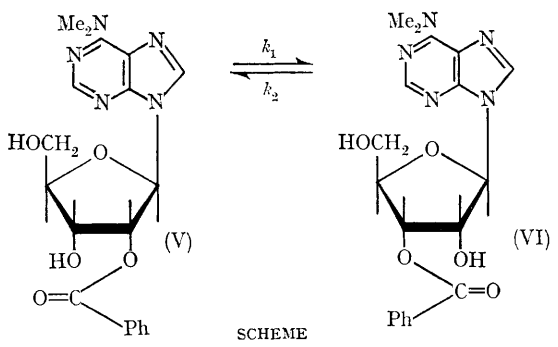
THE comparative ease of interconversion of isomeric 2′- and 3′-*O*-acyl ribonucleoside derivatives (III and IV) is important in the determination of the structures of aminoacyl-transfer-ribonucleic acids,[1] and therefore in the elucidation of the detailed mechanism of *in vivo* protein synthesis; and also in the chemical synthesis of oligoribonucleotides.[2] We have developed a procedure for the monoacylation of the ribonucleoside 2′,3′-diol system:[3] acid-catalyzed exchange between a ribonucleoside (I; R=H) or its 5′-derivative and a trimethyl orthoester gives a 2′,3′-*O*-methoxyalkylidene derivative (II) which, on mild acidic hydrolysis, is converted into a mixture of the corresponding 2′- and 3′-esters (III and IV) in



good overall yield. Usually, an acid-free solution of this mixture in a suitable solvent deposits one or other isomer (III or IV) in a pure crystalline state; its orientation is then determined by n.m.r. spectroscopy.[4] Generally, if the mother-liquors are allowed to stand, equilibration occurs and further crops of the same crystalline isomer are obtained.

We had not previously[3] isolated both the 2′- and 3′-isomers (III and IV) as pure crystalline compounds.

When 2′,3′-*O*-methoxybenzylidene-N^6,N^6-dimethyladenosine had been treated with 80% acetic acid at 20°, t.l.c. [$CHCl_3$–MeOH (37: 3; v/v)] revealed the presence of two products (R_F 0·63 and 0·75) in the proportion *ca.* 1:10,† which were readily separated by chromatography on silicic acid, and were both obtained crystalline. The major component (R_F 0·75), (75%),‡ m.p. 176—177°, was identified as 3′-*O*-benzoyl-N^6,N^6-dimethyladenosine (VI); the minor component (R_F 0·63), (7%), m.p. 149—151°, was identified as 2′-*O*-benzoyl-N^6,N^6-dimethyladenosine§ (V).



SCHEME

Structures (V) and (VI) have been assigned to the above products on the basis of their n.m.r. spectra (Figure), the most significant features of which are listed in the Table. The orientations of these isomers, which follow[4] from the relative chemical shifts and splittings of their H(1′) resonances,¶ have been confirmed by a more detailed analysis of their n.m.r. spectra. Thus, in the spectrum of

† The acidic hydrolysis of a 2′,3′-*O*-methoxyalkylidene ribonucleoside gives a mixture of 2′- and 3′-esters, which is apparently always richer in the 3′-isomer (D. P. L. Green and C. B. Reese, unpublished results). However, this ratio is not generally as high as 10:1 in favour of the latter.

‡ Based on N^6,N^6-dimethyladenosine as starting material. Satisfactory analytical data were obtained for all new compounds described.

§ Since the completion of this work, two other pairs of isomeric 2′- and 3′-*O*-acyl ribonucleoside derivatives have been obtained crystalline (R. Saffhill and J. C. M. Stewart, unpublished results). However, in neither case was the chromatographic separation on silicic acid as satisfactory, and neither pair of isomers could be clearly resolved by t.l.c. on Merck Kieselgel GF_{254}.

¶ As H(1′) of the 2-isomer (III) is always more deshielded[4] than H(1′) of the 3′-isomer (IV), H(4′) of (III) would be expected to be more shielded than H(4′) of (IV). It can be seen from the Table that this effect is indeed observed with (V) and (VI).

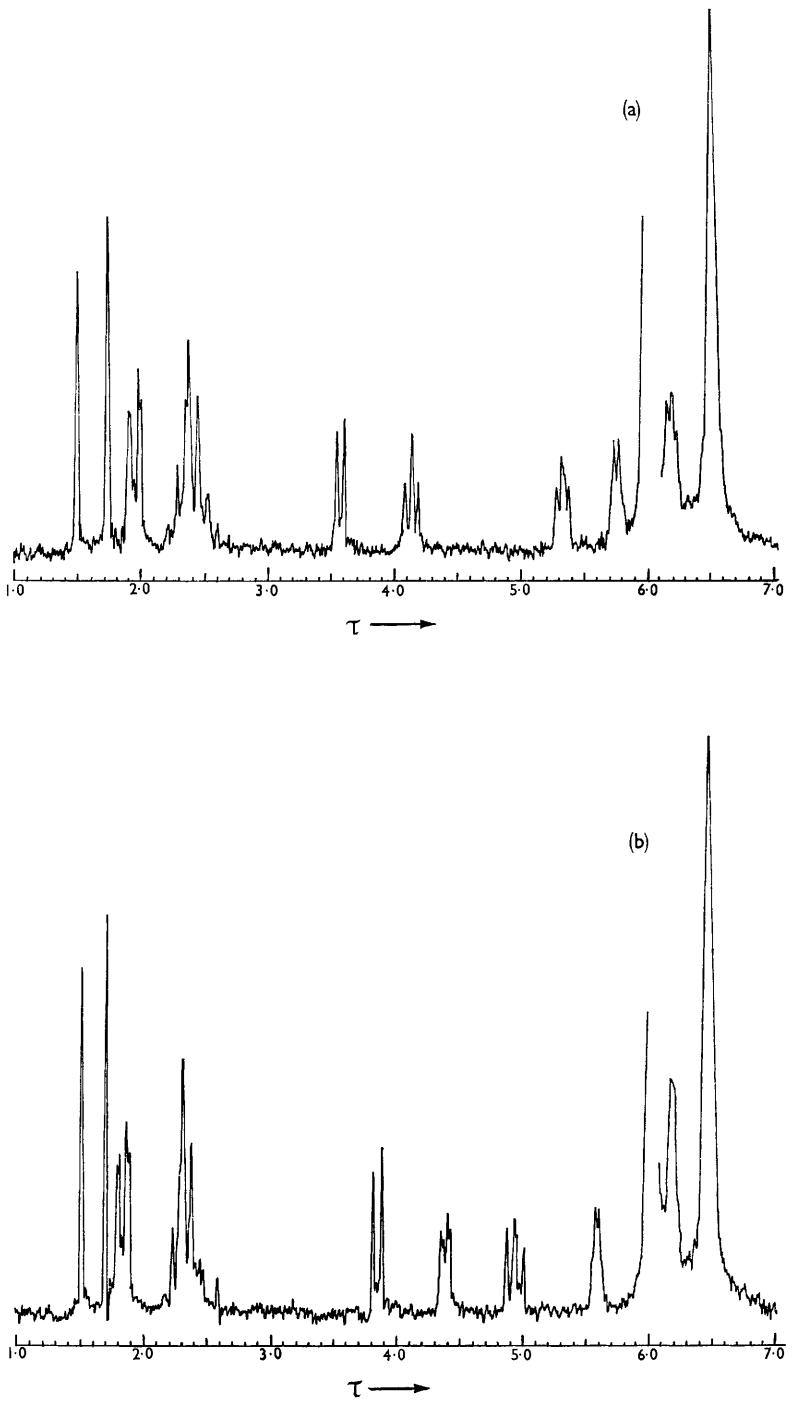


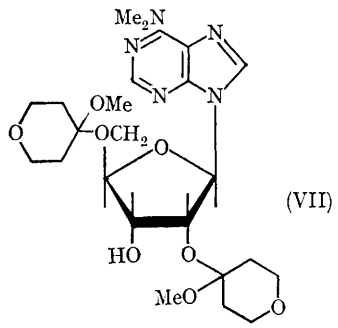
FIGURE. 100 *Mc./sec. n.m.r. spectra in* $(D_3C)_2SO$–D_2O [0·1 N *with respect to* HCl] (20: 3; v/v) of (a) 2′-*O*-benzoyl-N^6,N^6-dimethyladenosine (V), and (b) 3′-*O*-benzoyl-N^6,N^6-dimethyladenosine (VI).

Features of 100 *Mc./sec. n.m.r. spectra of* 2′- *and* 3′-O-*benzoyl*-N^6,N^6-*dimethyladenosines in acidified* $(D_3C)_2SO$–D_2O *solution.*

Compound	Chemical shifts (τ, p.p.m.)				Coupling constants (c./sec.)		
	H(1′)	H(2′)	H(3′)	H(4′)	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$
(V)	3·58	4·14	5·34	5·76	6	5	~4
(VI)	3·86	4·95	4·40	5·59	7	~5·5	~2

the 2′-isomer (V) (Figure a), the signal at τ 4·14 may be assigned to the H(2′) resonance as the splitting of H(1′) [$J_{1',2'}$ 6 c./sec., see Table] corresponds to one of its coupling constants (5 and 6 c./sec.) and not to either of those (4 and 5 c./sec.) of the signal at τ 5·34, assigned to the H(3′) resonance. These assignments are in accordance with the expectation that H(2′) would be considerably less shielded than H(3′) in a 2′-*O*-acyl ribonucleoside, and thus the orientation of (V) is confirmed. Further confirmation was obtained by spin decoupling: double irradiation at τ 5·34 caused the signal at τ 4·14 to collapse to a doublet (J 6 c./sec.), but had no effect on the signal at τ 3·58. By similar considerations (see Figure b and Table), the orientation of the 3′-isomer (VI) may be confirmed. In the latter case double irradiation at τ 4·95 caused the signal at τ 3·86 to collapse to a singlet.

Both 2′- and 3′-*O*-benzoyl-*N*^{6},*N*^{6}-dimethyladenosines were shown by t.l.c. to undergo extremely rapid equilibration in 50% aqueous pyridine (v/v) solution at room temperature; when a solution of the 3′-benzoate (VI) in this solvent was allowed to stand at 20° overnight and the products then chromatographed on silicic acid, starting material (VI) and 2′-isomer (V) were isolated in the proportion of 3·3 : 1, and in combined quantitative yield. Equilibration occurred only very slowly in anhydrous pyridine solution, but was markedly catalyzed by the addition of water.[5] Thus the isomers (V and VI) were found** to undergo equilibration in C_5D_5N/D_2O (95 : 5; v/v) solution with the same first-order rate constant ($k_1 + k_2$, see Scheme) of $1{\cdot}72 \times 10^{-2}$ min.$^{-1}$ ($t_{\frac{1}{2}}$, 40 min.) at 37°. The equilibrium constant (k_1/k_2) in this medium, 5·53, was greater than that observed in 50% aqueous pyridine solution.



(VII)

When 3′-*O*-benzoyl-*N*^{6},*N*^{6}-dimethyladenosine (VI) was allowed to undergo acid-catalyzed reaction with 4-methoxy-5,6-dihydro-2H-pyran[6] and the product treated with methanolic ammonia, the bis-ketal (VII) was obtained as a crystalline solid (75%), m.p. 133—133·5°. (VII) is a valuable intermediate in oligoribonucleotide synthesis.

One of us (D.P.L.G.) thanks the S.R.C. for a research studentship.

(*Received, April* 29*th,* 1968; *Com.* 519.)

** The equilibration rates of both isomers (V and VI) and the equilibrium constant were determined by n.m.r. spectroscopy with a Perkin Elmer 60 Mc./sec. spectrometer. Proportions of 2′- and 3′-isomers were estimated by measuring the areas under the corresponding H(1′) resonance signals.

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