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Note

Use of nuclear Overhauser enhancements for determination of the glycosylation site in nucleosides of *s*-triazole

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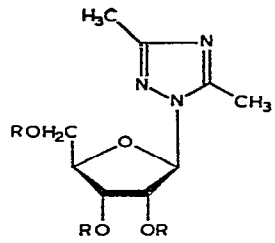
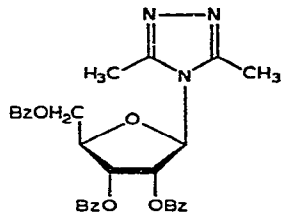
The synthesis of nucleosides by glycosylation of nitrogenous bases, using the three basic approaches[1], does not yield a single predictable product, and definitive proof of the site of glycosylation is required. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (virazole)[2,3] shows significant antiviral activity[4], because the molecule apparently mimics inosine or guanosine. 5-Substituted 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamides lose their antiviral activity[5]. We have been interested in the effect of methyl substituents on rotation about the glycosylic C–N bond and have prepared 3,5-dimethyl-1-β-D-ribofuranosyl-1,2,4-triazole as a model compound. We now report that whereas the site of glycosylation cannot be definitely assigned on the basis of ^1H or ^{13}C chemical-shift data on a single product, the nuclear Overhauser effect, which yields data that directly depend on molecular geometry[6,7], can be utilised for this purpose.

The acid-catalysed fusion procedure proved to be the most suitable route to the desired nucleoside. Fusion of 3,5-dimethyl-1,2,4-triazole[8] with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, in the presence of iodine, gave a mixture of two isomeric protected nucleosides (**1** and **2**, in the ratio 10:1) that were separable by chromatography on silica gel. The main product **1** was debenzoylated to give the nucleoside **3**, but, because of the low yield of the minor isomer **2**, the corresponding debenzoylated product was not obtained. The benzoyl groups in **2** complicated the ^1H-n.m.r. spectrum, and hence the structural assignment is tentative. It could be argued that **2** is the α anomer of **1** ($J_{1',2'}$ 4 Hz), as no 4-β-D-ribofuranosyl-1,2,4-triazoles have been obtained to our knowledge by the application of the acid-catalysed fusion procedure[5]. The β configuration of **1** and **3** was readily assigned on the basis of the singlet for H-1′ present in the ^1H-n.m.r. spectrum of **1**.

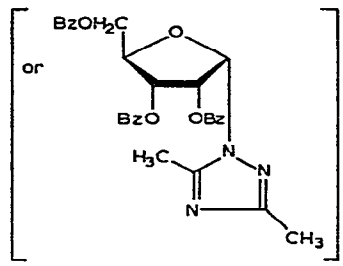
Since the 1,2,4-triazole is symmetrically substituted, only two β-D-ribofuranosyl derivatives are possible (**1** and **2**). Unambiguous assignment of structure to these derivatives on the basis of u.v. spectra was not possible because of weak absorption. Therefore, ^1H- and ^{13}C-n.m.r. data were examined.



1 R = Bz
3 R = H



2



The signals of the sugar protons were assigned on the basis of decoupling experiments. Signals for two Me groups were observed for **1–3**; the magnetic equivalence due to the rapid exchange of the NH protons between three nitrogen atoms should be removed upon ribosylation at N-1 or N-4 if there is no free rotation about the C–N bond. The downfield shift (18 Hz) of the signal for one Me group in **3** compared to those for 3,5-dimethyl-1,2,4-triazole in D_2O is consistent with, but does not prove, the structure assigned. The ^{13}C-resonances (see Experimental) of the β-D-ribofuranosyl carbon atoms in **3** conform to the established pattern[10]. In the off-resonance proton-decoupled spectrum, from low field to high field, the signals follow the sequence C-1′(d), C-4′(d), C-2′(d), C-3′(d), and C-5′(t). The chemical shifts of C-3, C-5, Me-3, and Me-5 were assigned by comparison with those of the 3,5-dimethyl-1,2,4-triazole anion, formed[2] by neutralization with LiOH in D_2O. The agreement with the α- and β-substitution shifts observed in other heterocyclic systems, and in monosubstituted *s*-triazoles, allows only a tentative assignment of structure. $\Delta C\alpha$ (+3.26 p.p.m.) for the δ −158.5 carbon atom and $\Delta C\beta$ (−1.4 p.p.m.) for the δ −163.2 carbon atom are consistent with the direction of the shift changes, but the magnitude (4–9 p.p.m.) in *s*-triazole systems[10] cannot be compared directly, because it depends on the nature of the substituent attached to carbon[11–13]. The signals for the methyl groups are slightly perturbed. That for hindered Me-5 (determined by selective, off-resonance proton decoupling) at 15.1 p.p.m. was moved downfield, and the unperturbed signal for Me-3 upfield by 1.1 p.p.m. Thus, the ^{13}C-n.m.r. data do not permit an unequivocal assignment of structure to **3**.

Molecular models show that the interproton distances (r_{ij}) H-1′/Me-5 and H-1′/Me-3 are significantly different in **3**, but similar in **2**. Spin–lattice relaxation times (T_1) and/or nuclear Overhauser effects (NOE) are dependent on r_{ij}^{-6}, and hence should allow these structures to be distinguished. The NOE were determined by using the continuous-wave mode, in which a proton resonance is saturated and the resulting change in the magnetisation of another proton is observed[6]. For dipole–dipole relaxation, the Overhauser enhancements are related inversely to the sixth power of the internuclear distance[14], $1/\mathrm{NOE} = Ar_{ij}^6$, and have been successfully applied for investigating internuclear distances, including Me/H interactions.

Under experimental conditions where it can be assumed that there are only intramolecular dipole–dipole interactions, the NOE enhancements must reflect the geometry of the nucleoside 3. The experimental NOE enhancements are presented in Table I. Enhancements of <5% were not taken into account, and only the distances from 0.2–0.3 nm gave a well-defined though qualitative picture suitable for assignment of structure. Considerable enhancements were observed when irradiating H-1′ and observing the downfield methyl group (7.5%), and a larger value (21%) by a reverse operation. These results unequivocally establish structure 3 only, because both methyl groups in 2 showed positive enhancements after saturation of H-1′ and H-2′, and even saturation of H-5′a and H-5′b must influence the nearby methyl groups.

TABLE I

NUCLEAR OVERHAUSER ENHANCEMENTS FOR 3,5-DIMETHYL-1-β-D-RIBOFURANOSYL-1,2,4-TRIAZOLE (3)

Protons irradiated	*Protons observed*	*NOE (±1.5%)*
H-1′	Me-5	7.6
H-2′	Me-5	1.5
H-3′	Me-5	0.0
Me-5	H-1′	21.0
Me-5	H-2′	2.5
Me-5	H-3′	2.1
H-1′	Me-3	2.2
H-2′	Me-3	2.2
H-3′	Me-3	3.0
Me-3	H-1′	0.0
Me-3	H-2′	2.0

EXPERIMENTAL

Melting points were determined on a Kofler microscope and are uncorrected. Concentrations were accomplished with a Büchi rotary evaporator under reduced pressure. P.m.r. spectra (100 MHz) were recorded on a JEOL PS 100 spectrometer. The purity of compounds was checked by t.l.c., with detection by u.v. light (254 nm) or by charring with sulphuric acid.

NOE samples (0.2M nucleoside in D_2O or $CDCl_3$) were placed in coaxial, precision n.m.r. tubes (5-mm diameter), with 1% Me_4Si in CCl_4 as external reference. The nucleoside 3 was dissolved in D_2O, to effect exchange of OH protons, and lyophilized twice before the n.m.r. solution was made. The solution was degassed by three freeze–thaw cycles at $<10^{-3}$ Torr before being sealed under argon. ^{1}H-NOE measurements were made at 100 MHz using the "frequency sweep" internal lock method, and the enhancements were measured by an integrator, and more precisely by measuring peak areas with a special computer programme made on a Lab PDP 8

computer. The NOE enhancement was defined as the difference of peak areas on saturation of a particular proton and when a decoupling frequency was set off-resonance, usually at 7300 or 6900 Hz. At least ten consecutive measurements were performed and the areas calculated and averaged to obtain an improved S/N ratio. The standard deviation (σ) of the measurements of peak areas was always <0.015, but varied depending on the magnitude of the enhancement.

^{13}C-N.m.r. spectra were recorded with a JEOL-FX-100 instrument operating in the FT-mode. Chemical-shift measurements were made with full proton-noise decoupling for solutions (50 mg/ml) in D_2O, and assignments were made by off-resonance decoupling.

3,5-Dimethyl-1- (**1**) *and -4-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-1,2,4-triazole* (**2**). — A mixture of 3,5-dimethyl-1,2,4-triazole[8] (500 mg, 5 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.5 g) was kept at 180–208° until a melt was achieved, and a catalytic amount of iodine was then added. Heating *in vacuo* at the above temperature was continued for 15 min. A solution of the dark residue in chloroform was added to a column (3 × 40 cm) of silica gel (100 g) prepacked in chloroform. The fractionation was monitored by t.l.c. (methanol–chloroform, 1:9). Elution with chloroform (300 ml) gave 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (0.7 g). Chloroform–ethyl acetate (5:1, 300 ml) then gave syrupy **1** (1 g, 30%), a mixture of **1** and **2**, and syrupy **2** (100 mg, 3%).

Compound **1** had $[\alpha]_D^{20}$ −2.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.28 (s, 3 H, Me-3), 2.52 (s, 3 H, Me-5), 4.6–4.9 (m, H-3,4′,5′,5′), 6.10 (s, 1 H, H-1′, from superimposed spectrum), 6.15 (d, 1 H, H-2′, from superimposed spectrum), 6.33 (dd, 1 H, H-3′), 7.5 and 8.1 (2 m, 15 H, 3 Ph).

Anal. Calc. for $C_{30}H_{27}N_3O_7$: C, 66.51; H, 5.02; N, 7.76. Found: C, 66.23; H, 5.56; N, 7.69.

Compound **2** had $[\alpha]_D^{20}$ −3° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): δ 2.28 [s, 3 H, Me-3(5)], 2.43 [s, 3 H, Me-5(3)], 4.7 (m, 2 H, H-5′,5′), 5.40 (m, 1 H, H-4′), 5.88 (m, 2 H, H-2′,3′), 6.44 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1′), 7.5 and 8.1 (2 m, 15 H, 3 Ph).

Anal. Calc. for $C_{30}H_{27}N_3O_7$: C, 66.51; H, 5.02; N, 7.76. Found: C, 65.95; H, 5.60; N, 7.53.

A solution of **1** (1 g, 1.8 mmol) in methanol (100 ml) saturated with ammonia was stored for 48 h at room temperature, and then concentrated. The residue was dissolved in ethanol–ethyl acetate and adsorbed onto silica gel (1 g), which was added to a column of silica gel (10 g) prepacked in ethyl acetate. Elution with ethyl acetate–methanol (19:1 followed by 1:1) gave **3** (350 mg, 85%), m.p. 125–126°, $[\alpha]_D^{20}$ −58° (*c* 1, water). ^{1}H-N.m.r. data (D_2O): δ 2.23 (s, 3 H, Me-3), 2.43 (s, 3 H, Me-5), 3.71 (m, 2 H, H-5′,5′), 4.12 (m, 1 H, H-4′), 4.35 (dd, 1 H, $J_{3',4'}$ 4 Hz, H-3′), 4.56 (dd, 1 H, $J_{2',3'}$ 5 Hz, H-2′), and 5.78 (d, 1 H, $J_{1',2'}$ 5 Hz, H-1′). ^{13}C-N.m.r. data (D_2O): δ 163.2 (s, C-3), 158.5 (s, C-5), 91.2 (d, C-1′), 87.6 (d, C-4′), 76.4 (d, C-2′), 72.9 (d, C-3′), 64.4 (t, C-5′), 15.1 (q, Me-5), and 13.3 (q, Me-3).

Anal. Calc. for $C_9H_{15}N_3O_4$: C, 47.13; H, 6.59; N, 18.33. Found: C, 47.16; H, 6.78; N, 18.02.

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REFERENCES

1 K. A. Watanabe, D. H. Hollemberg, and J. J. Fox, *J. Carbohydr. Nucleos. Nucleot.*, 1 (1974) 1–37.
2 G. P. Kreishman, J. T. Witkowski, R. K. Robins, and M. P. Schweizer, *J. Am. Chem. Soc.*, 94 (1972) 5894–5896.
3 P. Prusiner and M. Sundaralingam, *Nature (London), New Biol.*, 244 (1973) 116–118.
4 P. Dea, M. P. Schweizer, and G. P. Kreishman, *Biochemistry*, 13 (1974) 1862–1867.
5 S. R. Naik, J. T. Witkowski, and R. K. Robins, *J. Heterocycl. Chem.*, 11 (1974) 57–61.
6 J. H. Noggle and R. E. Schirmer, *The Nuclear Overhauser Effect*, Academic Press, New York, 1971.
7 A. Abragam, *Principles of Nuclear Magnetism*, Oxford University Press, 1961.
8 K. Brunner, *Monatsh. Chem.*, 36 (1905) 509–534.
9 K. Imai, H. Nohara, and M. Honjo, *Chem. Pharm. Bull.*, 14 (1966) 1377–1381.
10 M. T. Chenon, R. P. Panzica, J. C. Smith, R. J. Pugmire, D. M. Grant, and L. B. Townsend, *J. Am. Chem. Soc.*, 98 (1976) 4736–4745.
11 P. Dea, G. R. Revankar, R. L. Tolman, R. K. Robins, and M. P. Schweizer, *J. Org. Chem.*, 39 (1974) 3226–3231.
12 F. J. Weigert and J. D. Roberts, *J. Am. Chem. Soc.*, 90 (1968) 3543–3549.
13 R. J. Pugmire and D. M. Grant, *J. Am. Chem. Soc.*, 90 (1968) 4232–4238.
14 R. A. Bell and J. K. Saunders, *Can. J. Chem.*, 48 (1970) 1114–1122.