tion of methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-*arabino*-hexopyranosid-2-ulose were 301 mg (52.9%) of **9** and 192 mg (33.7%) of **11** (the gluco:manno ratio being thus 1.57:1). The analytical sample of **11**, an amorphous solid showed an $[\alpha]^{27}D$ +76° (*c* 1.0, $CHCl_3$); ir ($CHCl_3$) 3560 (broad peak) and 3470 (shoulder) (OH) nmr ($CDCl_3$) δ 7.6–7.3 (m, 5, phenyl), 5.58 (s, 1, methine H from benzylidene group), 4.77 (d, $J_{1,2}$ = 1.4 Hz, 1, H-1), 4.4–3.6 (m, 6, H-2, H-3, H-4, H-5, H-6 and H′-6), 3.53 and 3.37 (two s, 6, C-1 and C-3 methoxy groups), 2.65 (d, *J* = 1.4 Hz, 1, OH).

Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 61.05; H, 7.00.

Methyl 4,6-*O*-Benzylidene-3-*O*-methyl-2-*O*-methylsulfonyl-α-D-mannopyranoside (3). To a pyridine solution of **11** (87 mg; 0.29 mmol) methanesulfonyl chloride (0.100 ml, 0.59 mmol) was added and the reaction mixture was kept at room temperature for 2 hr. The excess of methanesulfonyl chloride was destroyed by methanol and the solvents were evaporated *in vacuo*. The residue was chromatographed on silica gel (20 g). Elution with 97:3 benzene–2-propanol afforded pure crystalline **3** (105 mg, 95%). An analytical sample was obtained by recrystallizing **3** from acetone: mp 185–186°; $[\alpha]^{27}D$ +22° (*c* 0.7, $CHCl_3$); ir ($CHCl_3$) 1363 and 1170 cm^{-1} (asymmetric and symmetric SO_2 stretch); nmr ($CDCl_3$) δ 7.6–7.2 (m, 5 phenyl), 5.62 (s, 1, methine H from benzylidene group), 5.0 (m, 1, H-2), 4.90 (d, $J_{1,2} \leq 1$ Hz, 1, H-1), 4.4–3.8 (m, 5, H-3, H-4, H-5, H-6, and H′-6), 3.56 and 3.40 (two s, 6, C-1 and C-3 methoxy groups), 3.13 (s, 3, methyl from C-2 methylsulfonyl group).

Anal. Calcd for $C_{16}H_{22}O_8S$: C, 51.33; H, 5.92; S, 8.57. Found: C, 51.39; H, 5.90; S, 8.79.

Reaction of Methyl 4,6-*O*-Benzylidene-3-*O*-methyl-2-*O*-methylsulfonyl-α-D-mannopyranoside 3 with Potassium Benzoate in Refluxing *N,N*-Dimethylformamide. An *N,N*-dimethylformamide solution (10 ml) containing **3** (202 mg, 0.54 mmol) and potassium benzoate (202 mg, 1.26 mmol) was heated at reflux for 120 hr. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (30 g). Elution with 2:1 benzene–ethyl acetate afforded four fractions. The third fraction was pure starting material **3** (59 mg, 29%), whereas the other three fractions were unidentified products of decomposition of **3** under the given experimental conditions.

Methyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (15). To a pyridine solution of **11** (81 mg, 0.27 mmol) benzoyl chloride (0.100 ml, 0.59 mmol) was added and the reaction mixture was kept at room temperature for 2 hr. The pyridine was removed *in vacuo* and the residue was chromatographed on silica gel (20 g). Elution with 98:2 benzene–2-propanol gave slightly impure **15** (111 mg). The rechromatography on slica gel (16 g) and elution with 98:2 benzene–2-propanol afforded pure **15** (110 mg; 100%) as an amorphous solid: $[\alpha]^{27}D$ −48° (*c* 1.0, $CHCl_3$); ir ($CHCl_3$) 1720 and 1265 cm^{-1} (C=O and C—O stretch, benzoate); nmr ($CDCl_3$) δ 8.2–7.2 (m, 10, phenyl), 5.68 (s, 1, methine H from benzylidene group), 5.61 (m, 1, H-2), 4.85 (d, $J_{1,2} \leq 1$ Hz, 1, H-1), 4.5–3.7 (m, 5, H-3, H-4, H-5, H-6, and H′-6), 3.46 and 3.43 (two s, 6, C-1 and C-3 methoxy groups).

Anal. Calcd for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04. Found: C, 66.18; H, 6.20.

Registry No.—**1**, 51016-19-4; **2**, 52260-45-4; **3**, 52260-46-5; **4**, 52260-47-6; **10**, 35775-68-9; **11**, 52260-48-7; **12**, 51364-57-9; **13**, 52260-49-8; **14**, 52260-50-1; **15**, 52260-51-2; methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-*arabino*-hexopyranosid-2-ulose, 29774-59-2.

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Use of Carbon-13 and Proton Magnetic Resonance Studies for the Determination of Glycosylation Site in Nucleosides of Fused Nitrogen Heterocycles

Phoebe Dea,*[1] Ganapathi R. Revankar, Richard L. Tolman, Roland K. Robins, and Martin P. Schweizer

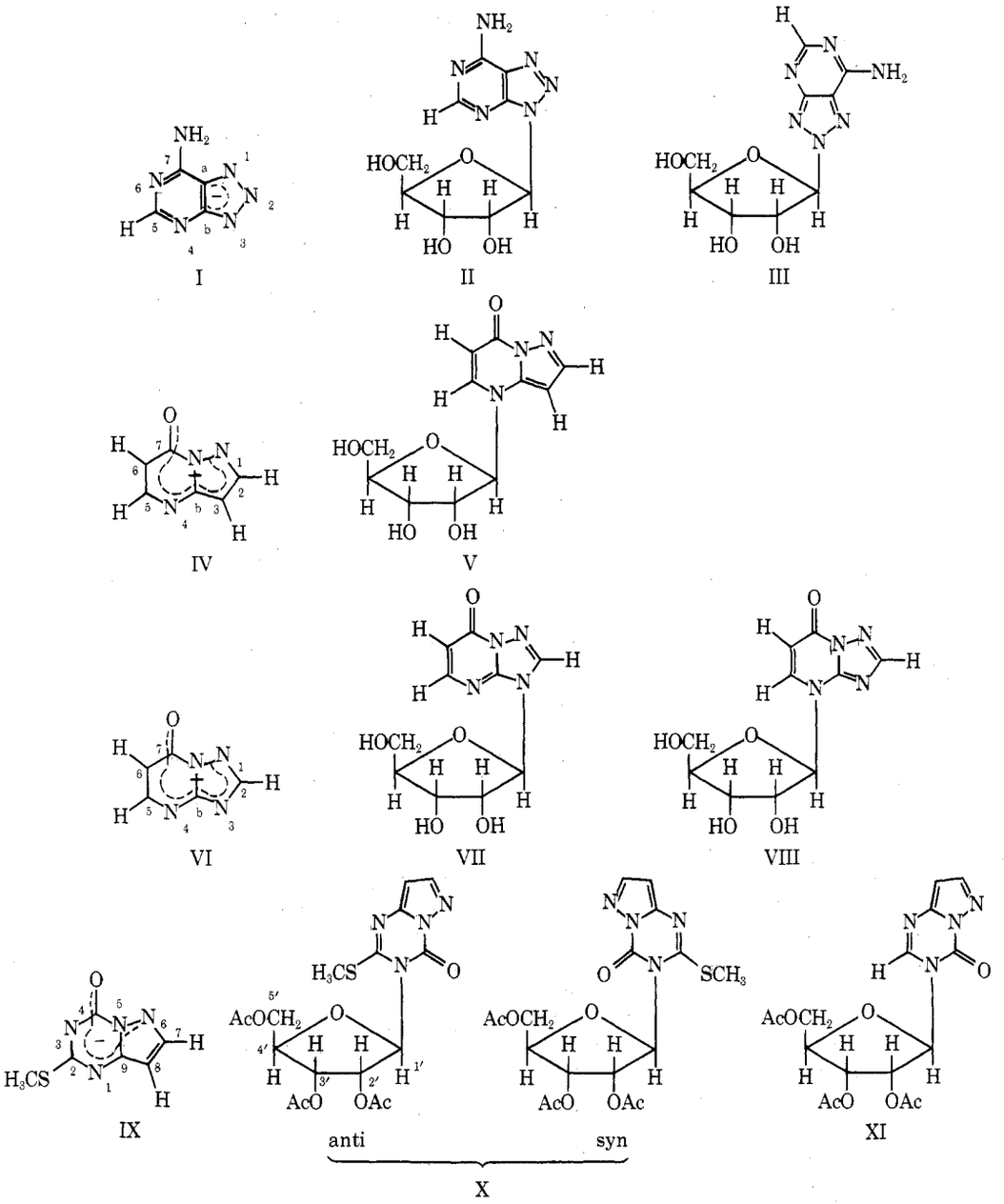
ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92664

Received July 1, 1974

Several selected fused nitrogen heterocyclic systems, 7-amino-*v*-triazolo[4,5-*d*]pyrimidine (I), pyrazolo[1,5-*a*]pyrimidin-7-one (IV), *s*-triazolo[1,5-*a*]pyrimidin-7-one (VI), and 2-methylthiopyrazolo[1,5-*a*]-*s*-triazin-4-one (IX), and their *N*-ribofuranosides have been studied with respect to the effect of N-ribosylation on the carbon-13 chemical shifts of the neighboring carbons. Large upfield α shifts and small downfield β shifts were observed in the nucleoside when compared to the base anion, thereby providing a convenient general method for the assignment of the glycosylation site in complex fused nitrogen heterocyclic systems.

Recently, several nmr studies in this laboratory have demonstrated the potential use of carbon-13 nuclear magnetic resonance spectroscopy as a general unequivocal method for the assignment of glycosylation site in both five- and six-membered nitrogen heterocycles.[2,3] The assignments were based upon the use of previously reported α- and β-substitution shifts observed in other heterocyclic systems when the neutral species is compared with the anionic form.[4-6] These shift parameters were first described by Pugmire and Grant from studies on the azines and their charged species,[4,5] where nitrogen protonation resulted in an upfield shift for the α carbon and downfield shifts were

Chart I



observed for the β and γ carbon atoms when compared to the free base. From the results of their theoretical calculations on these systems, the observed α-substitution shifts have been explained on the basis of a decrease in bonding between N–C_α while β and γ shifts are the result of charge polarization effects.

Our carbon-13 nmr results on several five- and six-membered nitrogen heterocycles have indicated that N-protonation and N-ribosylation result in similar α and β shifts. In view of the success using these substitution parameters in determinations of the preferred positions of the labile protons in the simple azines as well as benzimidazole, various purines and other fused-ring heteroaromatic systems,[6,7] we have extended our carbon-13 nmr studies to examination of the effect of ribosylation on the chemical shifts of neighboring carbon atoms in several selected fused nitrogen heterocycles. In these systems, the existence of the second ring provides an opportunity to examine long-range cross-ring effects as well as the unusual characteristics of the bridgehead carbons.

Results and Discussion

A. 7-Amino-*v*-triazolo[4,5-*d*]pyrimidine (I). To determine whether the effect of N-ribosylation in the fused-ring heterocycles is similar to the previously studied five- and six-membered rings, we have chosen to examine the carbon-13 chemical shifts of 7-amino-3-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]pyrimidine (II, Chart I) and 7-amino-2-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]pyrimidine (III) and have compared their chemical shifts to those of the anion of 7-amino-*v*-triazolo[4,5-*d*]pyrimidine (I). The glycosylation site in these two nucleosides had previously been assigned from uv spectra.[8] The carbon-13 chemical shifts are summarized in Table I. The C_5 and C_7 carbons are assigned based on the splitting patterns observed in the proton coupled spectra. The chemical shifts of the C_a and C_b carbons are found to be similar to those reported for adenosine.[9]

In this series of compounds the C_b carbon is α to the glycosylation site in II but is β to the glycosylated nitrogen in III. A corresponding upfield shift of 6.8 ppm was observed

Table I
Comparison of Carbon-13 Chemical Shifts for the Various Fused Nitrogen Heterocyclic Systems (See Chart I)

Compd	Chemical shift, δ ppm[a]						
	C_2	C_3	C_5	C_6	C_7	C_a	C_b
I			155.8		158.9	124.0	152.4
II			153.0		154.0	121.1	145.6
III			153.7		154.4	122.9	153.5
$\Delta\delta_{I-II}$			+2.8		+4.9	+2.9	+6.8
$\Delta\delta_{I-III}$			+2.1		+4.5	+1.1	−1.1
IV	151.3	92.9	141.2	91.7	159.4		151.7
V	142.8	96.6	138.1	93.8	156.0		141.4
$\Delta\delta_{IV-V}$	+8.5	−3.7	+3.1	−2.1	+3.4		+10.3
VI	152.5		154.1	97.2	160.1		158.3
VII	140.8		153.3	104.0	156.8		149.6
VIII	151.6		138.9	100.2	155.4		143.1
$\Delta\delta_{VI-VII}$	+11.7		+0.8	−6.8	+3.3		+8.7
$\Delta\delta_{VI-VIII}$	+0.9		+15.2	−3.0	+4.7		+15.2

	C_7	C_8	C_2	C_4	C_9	$C_{1'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	$C_{5'}$
IX	143.0	92.8	150.6	167.1	151.0					
X	147.0	98.1	146.0	156.6	142.9	90.6	72.2	68.7	78.8	62.3
XI	148.8[b]	102.0	148.5[b]	153.3[b]	145.8[b]	91.4	74.6	70.9	80.9	63.8
$\Delta\delta_{IX-X}$	−4.0	−5.3	+4.6	+10.5	+8.1					
$\Delta\delta_{IX-XI}$	−5.8	−9.2	+2.1	+12.8	+5.2					
$\Delta\delta_{X-XI}$						−0.8	−2.1	−2.4	−2.2	−1.5

[a] Chemical shifts measured from DMSO-d_6, converted to TMS scale using $\delta_{TMS} = \delta_{DMSO} + 39.5$ ppm. [b] Assignments tentative owing to low signal to noise ratio in the proton-coupled spectrum.

for the C_b resonance in II whereas a downfield shift of 1.1 ppm was observed for III when compared to the C_b chemical shift in I (Table I). The substitution parameters observed here are very similar to the previously reported α- and β-protonation parameters of +9.04 and −1.59 ppm, respectively, for the five-membered azines[5] and the corresponding values of +7.8 and −4.4 ppm reported for the six-membered azines.[4] It must be noted in the case of III that the change in chemical shift of C_b is actually an average of β and γ positional effects. Furthermore, the C_a carbon of II and III which is β and γ to the respective glycosylation site shows upfield shifts of 2.9 and 1.1 ppm. This is similar to the reversal in trend noted by Pugmire, *et al.*,[6] at the bridgehead positions in some methylpurines.

We also note that the long-range cross-ring effects are not preserved for the γ carbons. Upfield shifts of 2.1 and 2.8 ppm for C_5 and 4.5 and 4.9 ppm for C_7 were observed rather than the downfield γ shifts reported in other simpler systems.

B. Pyrazolo[1,5-*a*]pyrimidin-7-one (IV). Evidence that glycosylation in pyrazolo[1,5-*a*]pyrimidin-7-one (IV) occurs at the N_4 position was first obtained by comparing the pmr spectrum of this nucleoside (V) with those of several related heterocycles. It has been observed that N-methylation results in an increase in the coupling constant between protons on neighboring carbons. Some of our results are summarized in Table II. We note that in cases where methylation occurs at the N_1 position, the H_{2-3} coupling constants are of the order of 3.5 Hz, whereas methylation at N_4 results in smaller couplings of 2.0 Hz.[10] Since ribosylation or methylation is expected to produce very similar inductive effects, the fact that the observed $J_{H_{2-3}}$ in *N*-β-D-ribofuranosylpyrazolo[1,5-*a*]pyrimidin-7-one is only 2.0 Hz leads to the conclusion that N_4 is the glycosylation site.

Confirmation of this assignment was derived from the following carbon-13 nmr studies. The proton splitting pattern in the carbon-13 spectrum enables one to distinguish the C_7 and C_b carbons where the large carbon-13–proton coupling is absent, since there are no protons directly attached. The carbonyl carbon is assigned to the resonance furthest downfield in the spectrum. The specific assignment of the remaining C_2, C_3, C_5, and C_6 carbons in the heterocycle or the nucleoside is not possible from the proton-coupled spectra alone, since they all exhibit identical proton splitting patterns. From chemical shift considerations, it is expected that the C_2 and C_5 carbons should occur considerably more downfield compared to the C_3 and C_6 carbons. The specific assignments of these carbons were achieved using off-resonance proton decoupling techniques. The results are summarized in Table I.

The two carbons adjacent to the glycosylation site N_4 are C_b and C_5. When the carbon-13 shifts of the nucleoside are compared with those of the anion of the heterocycle, a large upfield shift of 10.3 ppm was observed for C_b and a smaller but also upfield shift of 3.1 ppm was observed for C_5. The two β carbons C_3 and C_6 also exhibit the usual downfield shifts of 3.7 and 2.1 ppm, respectively. The γ shifts for C_2

Table II
Some Typical Changes in Proton Coupling Constants as a Result of N-Methylation

Compd	J_H
1,5-Dimethylpyrazolo[1,5-*a*]pyrimidin-7-one	3.55
1,2-Dimethylpyrazol-3-one	3.50
4,5-Dimethylpyrazolo[1,5-*a*]pyrimidin-7-one	2.05
5-Methyl-4-β-D-ribofuranosylpyrazolo-[1,5-*a*]-pyrimidin-7-one	2.00
4-β-D-Ribofuranosylpyrazolo[1,5-*a*]pyrimidin-7-one	2.00

and C_7, however, are upfield, being 8.5 and 3.4 ppm, again showing a reversal in trend in the long-range inductive effects.

C. *s*-Triazolo[1,5-*a*]pyrimidin-7-one (VI). There are three possible glycosylation sites in *s*-triazolo[1,5-*a*]pyrimidin-7-one, namely, N_1, N_3, and N_4. Although the attachment of the β-D-ribofuranosyl moiety in position 1 could not be ruled out absolutely from uv data, tentative assignments of the 3- and 4-β-D-ribofuranosyl isomers had been reported based on comparisons of the uv spectra[11] with those of the corresponding N_3 and N_4 methyl derivatives.[10,11]

The carbon-13 chemical shifts obtained for the two nucleosides of this series are presented in Table I, along with the carbon-13 chemical shifts of the heterocycle anion for comparison. The carbonyl resonance was assigned to the most downfield signal from chemical shift considerations and C_b can be assigned from the proton-coupled spectrum, since it is the only other carbon with no directly bonded protons. The C_5 and C_6 resonances in both nucleosides can be distinguished from the C_2 resonance by a small geminal coupling arising from the adjacent proton. The upfield resonance is assigned to the C_6 carbon by comparison with the chemical shifts of related compounds.

The possibility that glycosylation had occurred at N_1 can be eliminated from the positive β shift of 3.3 and 4.7 ppm observed for C_7 for nucleosides VII and VIII, respectively, when compared to the carbon shifts of the base anion. The nucleoside VII exhibits large upfield shifts of 11.7 and 8.7 ppm for the C_2 and C_b carbon resonances, whereas the C_5 resonance remains essentially unchanged. These α shifts confirm that this nucleoside is 3-β-D-ribofuranosyl-*s*-triazolo[1,5-*a*]pyrimidin-7-one (VII). In the case of the 4 isomer VIII, there is little change in the chemical shift for the C_2 carbon when compared to the base anion, but both C_b and C_5 resonances move upfield by 15.2 ppm and both of these carbons are adjacent to the glycosylation site. The C_6 resonance exhibits a downfield β shift of 3.0 ppm. The γ shifts in both nucleosides are again inconsistent with the downfield shifts reported for other simpler ring systems, being +0.8 ppm for C_5 and +3.3 ppm for C_7 in the 3 isomer, and +0.9 and +4.7 ppm for the C_2 and the carbonyl carbon in the 4 isomer, respectively.

D. 2-Methylthiopyrazolo[1,5-*a*]-*s*-triazin-4-one (IX). The pyrazolotriazine heterocycle (IX) also provides three possible sites for glycosylation, *i.e.*, N_1, N_3, and N_6. We have examined the carbon-13 spectra of the base anion (IX), the corresponding nucleoside (X), and the dethiated derivative of the nucleoside (XI). All pertinent carbon-13 nmr data are summarized in Table I. The assignment of the various carbon resonances in the heterocycle is based on comparison of the proton coupled and decoupled spectra. The C_7 and C_8 carbon resonances can be identified from large carbon-13 proton couplings. Since carbons bonded to nitrogen atoms are generally known to be appreciably deshielded relative to benzene while β carbons are shielded,[12] the 50-ppm difference between these two resonances strongly supports the assignment of the lower field signal to the C_7 carbon α to the nitrogen. The most downfield resonance in the spectrum is assigned to the carbonyl, C_4. The remaining C_2 and C_9 resonances can be unequivocally assigned by examining their proton splitting patterns in the undecoupled spectra. The C_2 resonance appears as a singlet while C_9 appears as a doublet, split by the vicinal H_7 proton.

In the nucleoside spectrum, C_7, C_8, and C_4 carbons are assigned in a similar manner. The C_9 resonance is again identified as a closely spaced doublet in the proton coupled spectrum, but the splitting pattern for the C_2 resonance cannot be distinguished owing to the overlap with one leg of the C_7 doublet. However, the C_2 carbon can be assigned to the only remaining downfield resonance. The chemical shifts of the ribose carbons are assigned by comparison with previously reported spectra.[13]

When the chemical shifts of the base carbons of the nucleoside X were compared to those of the triazine anion, large upfield shifts were noted for three carbons, namely, C_2, C_4, and C_9, the shifts being 4.6, 10.5, and 8.1 ppm, respectively. Using the previously reported large positive shifts observed in other heterocyclic systems upon comparison of a neutral species with an anionic form, we can eliminate N_6 as the site of glycosylation because of the negative α shift (−4.0 ppm) at C_7 but it is not possible to establish whether N_1 or N_3 is the glycosylation site from these chemical shift data alone.

Let us first consider the case where the ribose is attached at the N_3 position. Examination of molecular models reveals that the anti conformation [for purposes of discussion here, the anti glycosidic conformation refers to the range of torsional angles about the glycoside bond such that the 4-keto is directed away from the furanose ring (see Chart I)] is impossible, since the SCH_3 substituent is too bulky to go over the ribose ring. In the syn conformation, the 4-keto group of the base would be located over the ribose ring. The presence of the keto group over the ring as occurs in 6-methylcytidine and other 2,6-dioxopyrimidine nucleosides has been shown[14] to result in C–H bond polarization whereby the $C_{2'}$ is observed to shift upfield by 2–2.5 ppm while the $H_{2'}$ and $H_{3'}$ protons shift downfield by 0.2 and 0.1 ppm, respectively. We have therefore examined the carbon-13 ribose chemical shifts and have compared them to those of the dethiated nucleoside XI where the bulky SCH_3 group is absent and the ribose is expected to be free to rotate round the glycosyl bond. Our results (Table I) indicate that all the ribose carbon resonances in X are upfield compared to the dethiated analog (XI). In particular, the $C_{2'}$ and $C_{3'}$ carbons in X were shifted upfield by 2.1 and 2.4 ppm, respectively. In the ^1H nmr spectra, the $H_{2'}$ and $H_{3'}$ in X occur at −5.90 and −5.66 ppm, respectively, whereas those for XI appear at −5.58 and −5.52 ppm. Therefore, the carbon shifts were observed to change in opposite directions to the proton shifts of the directly bonded hydrogens, indicating the presence of a carbonyl group over the ring in X. Both the carbon-13 and proton chemical shift data indicate that the nucleoside X exists predominantly in the syn conformation whereas the nucleoside XI exists predominantly in the anti conformation. In order to account for the presence of the keto group over the ring, the ribose must be attached to the N_3 position, since the keto group would be too far away to exert any effect on the ribose shifts if the ribosylation had occurred at N_1. The large positive α shifts observed in C_2 and C_4 are also consistent with this structure. As has been observed in all of the nucleosides examined in this study, a positive γ shift is noted at C_9 (8.1 ppm).

In summary, carbon-13 nmr study of the four heterocyclic series have shown that large upfield α shifts and the small downfield β shifts are preserved in these complex fused-ring systems. The only exception observed in the β shift is in the case of 7-amino-2-β-D-ribofuranosyl-υ-triazolo[4,5-*d*]pyrimidine (III), where a bridgehead carbon is involved. The γ shifts in these systems, however, are entirely inconsistent with past observations in other fused nitrogen heterocycles, namely, the *N*-methylpurines.[6] The γ carbons in the nucleosides all exhibit upfield rather than downfield shifts and a wide range of magnitudes are observed. Nonetheless, in most heterocyclic systems, measurement of the α and β shifts alone are sufficient to estab-

lish the glycosylation site. This study shows that carbon-13 nmr can readily be applied for structural assignments of the nitrogen at which alkylation or glycosylation has occurred in complex fused heterocyclic systems.

Experimental Section

Proton magnetic resonance spectra were obtained at 60 MHz on a Hitachi Perkin-Elmer R-20A spectrometer in DMSO-d_6 using DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) as an internal reference. A Bruker HX-90 nmr spectrometer operating at 22.62 MHz in the Fourier transform mode was used to obtain the carbon-13 nmr spectra. A Fabri-Tek 1074 signal averager with 4096 word memory was used for data accumulation and a PDP-8/e computer (Digital Equipment Corp.) for data processing. Saturated solutions were prepared in DMSO-d_6 and were studied in 10-mm tubes. Chemical shifts were reported in parts per million relative to TMS using the relationship $\delta_{TMS} = \delta_{DMSO\text{-}d_6} + 39.5$ ppm.

Ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer and infrared spectra on a Perkin-Elmer 257 spectrophotometer (KBr pellets). Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Elemental analyses were performed by MHW Laboratories, Garden City, Mich.

Evaporations were carried out under reduced pressure with bath temperature below 30°. Detection of components on silica gel F-254 (EM Reagents) was by ultraviolet light and by a 10% sulfuric acid in methanol spray followed by heating. Chromatography solvent mixtures were by volume.

The anions of various heterocycles were formed by neutralization with LiOH in DMSO-d_6. Trimethylsilyl derivatives of heterocycles were prepared using the general procedure of Wittenburg.[15] The heterocycle of interest was heated under reflux in an excess of freshly distilled hexamethyldisilazane with a catalytic amount of ammonium sulfate under anhydrous conditions until complete solution was achieved and evolution of ammonia ceased (~15 hr). The excess hexamethyldisilazane was removed by distillation under reduced pressure and the residue (syrup or crystalline solid) was used directly without further purification. 7-Amino-*v*-triazolo[4,5-*d*]pyrimidine, 7-amino-3-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]pyrimidine, and 7-amino-2-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]pyrimidine used in this study were prepared as previously reported.[8] The synthesis of 3- and 4-(β-D-ribofuranosyl)-*s*-triazolo[1,5-*a*]pyrimidin-7-one had been reported by Winkley, *et al.*,[11] and Revankar, *et al.*[16]

4-β-D-Ribofuranosylpyrazolo[1,5-*a*]pyrimidin-7-one (V). To tetra-*O*-acetyl-β-D-ribofuranose (10.5 g, 0.033 mol) in dry dichloromethane (50 ml) at −20° was added a solution of dry dichloromethane (originally 50 ml) which had been saturated at −20° with dry hydrogen bromide gas. The mixture was protected from moisture with a drying tube and allowed to warm to 0°. The solvent was evaporated and the resulting syrup was coevaporated twice with dry toluene (50 ml). The residual syrup was dissolved in "Nanograde" acetonitrile (100 ml) and was added to the syrupy trimethylsilyl derivative of 7-hydroxyryrazolo[1,5-*a*]pyridine [prepared from 4.05 g (0.030 mol) of base[10]] in dry acetonitrile (50 ml). The reaction vessel was sealed and the mixture was stirred at room temperature. After 3 days the reaction mixture was filtered to remove some heterocyclic starting material (0.7 g) and the dark filtrate was evaporated to a syrup. Sodium bicarbonate (5.0 g), water (15 ml), and ethanol (50 ml) were added. The mixture was evaporated to dryness. Coevaporation with absolute ethanol several times afforded a dry residue which was extracted with chloroform (3 × 100 ml). The combined extracts were washed with cold saturated aqueous sodium bicarbonate solution (2 × 10 ml) followed by water (3 × 10 ml) and dried over anhydrous sodium sulfate. The chloroform was evaporated to dryness and the residual syrup was dissolved in a minimum volume of chloroform and applied to a silica gel column (4.5 × 35 cm) prepacked in chloroform. The column was eluted with chloroform–acetone (8:2) and each of the 25-ml fractions was collected. The fractionation was monitored by tlc and appropriate fractions were pooled and solvent evaporated to yield colorless foam, 7.40 g (75.8%).

The above blocked nucleoside (7.0 g) was dissolved in methanolic ammonia (200 ml, methanol presaturated with ammonia at 0°) and the solution was allowed to stand at room temperature overnight. The crystalline solid that separated was collected and washed with a little methanol. The combined filtrate and washings were evaporated to dryness. The resulting foam was triturated with cold methanol, and the solid that separated was collected and crystallized from aqueous ethanol to provide pure V, 4.4 g (92.5%) (70% for two steps): mp 265°; $[\alpha]^{25}D$ −23.0° (*c* 1.0, DMSO); uv λ_{max} (pH 1) 255 nm (ε 7200), 303 (8300); λ_{max} (pH 7) 255 nm (ε 7500), 303 (8800); λ_{max} (pH 11) 255 nm (ε 7200), 303 (8550); ir λ_{max} (KBr) 1690 cm^{-1} (C=O of heterocycle); pmr (DMSO-d_6) δ 8.30 and 5.95 (doublets, for C-5 H and C-6 H, respectively, $J_{5,6}$ = 7.5 Hz) and 8.00 and 6.60 (doublets, for C-2 H and C-3 H, respectively, $J_{2,3}$ = 2 Hz).

Anal. Calcd for $C_{11}H_{13}N_3O_5$: C, 49.44; H, 4.90; N, 15.72. Found: C, 49.49; H, 4.89; N, 15.71.

5-Methyl-4-β-D-ribofuranosylpyrazolo[1,5-*a*]pyrimidin-7-one. A solution of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide from 5.25 g (0.0165 mol) of tetra-*O*-acetyl-β-D-ribofuranose in dry acetonitrile (80 ml) was added to the trimethylsilyl derivative of 5-methylpyrazolo[1,5-*a*]pyrimidin-7-one [prepared from 2.24 g (0.015 mol) of base[17]] and the resulting solution was stirred at room temperature for 4 days in a sealed reaction vessel. The dark reaction mixture was filtered to remove some heterocyclic starting material (0.9 g) and the filtrate was evaporated to dryness. The residue was extracted with chloroform (150 ml), and the chloroform phase was washed with saturated aqueous sodium bicarbonate solution followed by water (3 × 75 ml) and dried over anhydrous sodium sulfate. The chloroform was evaporated to dryness and the residual syrup was dissolved in a minimum volume of ethyl acetate and applied to a silica gel column (4.5 × 35 cm) prepacked in ethyl acetate–water–1-propanol (4:2:1, upper phase). The column was eluted with the same solvent system and 15-ml fractions were collected. The fractionation was monitored by tlc on silica gel using the eluting solvent as the developer. Fractions 31–80 were pooled and the solvent was evaporated to yield cream-colored foam, 2.25 g (61.5%): uv λ_{max} (pH 1) 253 nm (sh) (ε 6500), 298 (10,600); λ_{max} (pH 7) 253 nm (sh) (ε 6500), 300 (10,200); λ_{max} (pH 11) 253 nm (sh) (ε 6500), 300 (10,200).

The above blocked nucleoside (2.0 g) was dissolved in methanolic ammonia (80 ml, methanol presaturated with ammonia at 0°) and the solution was allowed to stand at room temperature overnight. The solution was evaporated to dryness and the residue was triturated with anhydrous ether (5 × 30 ml). The ether-insoluble material was dissolved in ethyl acetate containing a few drops of water and chromatographed on a silica gel column (2.5 × 35 cm) eluting with ethyl acetate–water–1-propanol (4:2:1, upper phase). The appropriate fractions were pooled and the solvent was evaporated. The residue was dissolved in water (50 ml), frozen, and lyophilized to obtain hygroscopic solid, 0.9 g (65.2%), no definite melting point: uv λ_{max} (pH 1) 250 nm (ε 5900), 298 (8150); λ_{max} (pH 7) 250 nm (ε 5050), 298 (8150); λ_{max} (pH 11) 250 nm (ε 5600), 298 (8400); ir λ_{max} (KBr) 1680 cm^{-1} (C=O of heterocycle); pmr (DMSO-d_6) δ 7.90 and 6.87 (doublets, for C-2 H and C-3 H, respectively, $J_{2,3}$ = 2 Hz), 5.85 (singlet, C-6 H).

Anal. Calcd for $C_{12}H_{15}N_3O_5 \cdot H_2O$: C, 48.16; H, 5.72; N. 14.04. Found: C, 48.50; H, 5.51; N, 13.81.

2-Methylthio-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[1,5-*a*]-*s*-triazin-4-one (X). A solution of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide from 7.0 g (0.022 mol) of tetra-*O*-acetyl-β-D-ribofuranose in dry acetonitrile (100 ml) was added to the trimethylsilyl derivative of 2-methylthiopyrazolo[1,5-*a*]-*s*-triazin-4-one [prepared from 3.64 g (0.020 mol) of base[18]] and the resulting solution was stirred at room temperature for 2 days in a sealed reaction vessel. The brown reaction mixture was filtered to remove 0.6 g of heterocyclic starting material. The filtrate was evaporated to dryness and dissolved in chloroform (150 ml). The chloroform solution was washed successively with saturated aqueous sodium bicarbonate solution (3 × 75 ml) and water (3 × 100 ml) and dried over anhydrous sodium sulfate. The residual foam, after removal of chloroform, was dissolved in a minimum volume of chloroform and chromatographed on a silica gel column (3.5 × 50 cm); the eluting solvent was chloroform–acetone (8:2). The appropriate fractions were pooled and the solvent was evaporated, which afforded orange-colored foam, 6.10 g (83.0%): $[\alpha]^{25}D$ +13.7° (*c* 1.0, DMSO); uv λ_{max} (pH 1) 226 nm (ε 8600), 287 (9900); λ_{max} (pH 7) 226 nm (ε 9050), 286 (10,300); λ_{max} (pH 11) 230 nm (ε 5500), 286 (9900); ir λ_{max} (KBr) 1340 (SCH_3), 1750 cm^{-1} (OAc); pmr (DMSO-d_6) δ 8.08 and 6.45 (doublets, for C-2 H and C-3 H, respectively, $J_{2,3}$ = 2 Hz), 2.65 (singlet, for CH_3).

Anal. Calcd for $C_{17}H_{20}N_4O_8S$: C, 46.36; H, 4.58; N, 12.72. Found: C, 46.56; H, 4.43; N, 12.94.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[1,5-*a*]-*s*-triazin-4-one (XI). Freshly prepared Raney nickel (30 g) was added to a solution of 2-methythio-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[1,5-*a*]-*s*-triazin-4-one (X, 3.0 g, 0.0068 mol) in

50 ml of absolute ethanol. The suspension was heated at reflux on a steam bath for 1 hr. The catalyst was removed by filtration on a Celite pad and washed with hot ethanol (3 × 15 ml). The combined filtrate and washings were evaporated to dryness, dissolved in the minimum volume of chloroform, and chromatographed on a silica gel column (3.5 × 50 cm) eluting with chloroform–acetone (8.5:1.5). The appropriate fractions were pooled and the solvent was evaporated to yield a colorless foam, 1.80 g (67.0%): $[\alpha]^{25}D$ +2.7° (c 1.0, DMSO); uv λ_{max} (pH 1) 262 nm (ε 10,300); λ_{max} (pH 7) 262 nm (ε 9900); λ_{max} (pH 11) 262 nm (ε 9900); ir λ_{max} (KBr) 1750 cm^{-1} (OAc); pmr (DMSO-d_6) δ 8.05 and 6.50 (doublets for C-7 H and C-8 H, respectively, $J_{7,8}$ = 2 Hz), 8.20 (singlet for C-2 H).

Anal. Calcd for $C_{16}H_{18}N_4O_8 \cdot H_2O$: C, 46.60; H, 4.89; N, 13.59. Found: C, 46.80; H, 4.80; N, 13.49.

Acknowledgment. The authors wish to thank Drs. Mason G. Stout and Rich B. Meyer, Jr., for helpful discussions of this work.

Registry No.—I, 52259-78-6; II, 10299-44-2; III, 28279-62-1; IV, 52259-79-7; V, 52217-05-7; V 5-methyl derivative, 52217-08-0; VI, 52259-80-0; VII, 32817-07-5; VIII, 33037-54-6; IX, 52259-81-1; X, 52217-06-8; XI, 52217-07-9; tetra-O-acetyl-β-D-ribofuranose, 13035-61-5; 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide, 39110-68-4.

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"Octakis-O-(3-aminopropyl)sucrose" as a Bifunctional Catalyst for the Dedeuteration of Isobutyraldehyde-2-d[1]

Jack Hine* and Stephen S. Ulrey

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received January 15, 1974

"Octakis-O-(3-aminopropyl)sucrose" (OAPS) containing about seven aminopropyl side chains per sucrose moiety has been prepared by reduction of octakis-O-(2-cyanoethyl)sucrose. Measurements of its basicity and equilibrium constant for forming imines with isobutyraldehyde have been made. OAPS is an effective catalyst for the dedeuteration of isobutyraldehyde-2-d in aqueous solution; a pH–rate plot shows a maximum around pH 8.4 at 35°. Under these conditions the catalytic activity is about 14 times that which would be expected if the catalyst were acting only monofunctionally. Hence it is concluded that one amino group on the catalyst transforms the aldehyde to an iminium ion, and then the activated α-deuteron in this isobutyraldiminium ion is removed by another amino group from the same molecule of catalyst. Reasons why this bifunctional catalytic activity is more efficient than that due to polyethylenimines, but less efficient than that due to 8-amino-1-dimethylamino-2-octyne, are discussed.

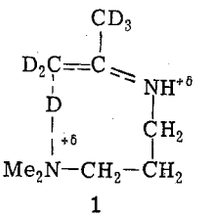
Primary amine salts catalyze α-hydrogen exchange reactions of isobutyraldehyde-2-d, acetone-d_6, and other aldehydes and ketones by transforming them to iminium ions, whose α-hydrogen atoms are much more rapidly removed by bases than are the α-hydrogen atoms of the original carbonyl compounds.[2,3] The monoprotonated forms of

$$Me_2CDCHO + RNH_3^+ \rightleftharpoons Me_2CDCH{=}NHR^+ + H_2O$$

$$Me_2CDCH{=}NHR^+ + B \rightleftharpoons Me_2C{=}CHNHR + BD$$

$$Me_2C{=}CHNHR \xrightarrow{BH} \xrightarrow{H_2O} Me_2CHCHO + RNH_3^+$$

amines of the type $Me_2N(CH_2)_nNH_2$, where n is 2, 3, 4, and 5, show a catalytic activity toward isobutyraldehyde-2-d that increases monotonically with increasing basicity (increasing n),[4,5] but the monoprotonated form of 3-dimethylaminopropylamine is by far the best catalyst toward acetone-d_6.[4,6] These results indicate bifunctional catalysis of the dedeuteration of acetone-d_6 *via* a transition state like **1**. The absence of such catalysis in the case of isobutyraldehyde-2-d was explained in terms of the destabilizing steric interactions between a methyl group from the aldehyde and the NH-bound methylene group from the catalyst that would be present in the analogous transition state for isobutyraldehyde. Such strain may be avoided if the basic group that removes the α-deuteron and the primary amino group that forms the iminium ion are separated by a long enough chain for internal deuteron removal



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