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^{13}C NMR IN CONFIGURATIONAL ASSIGNMENT OF RIBOFURANOSYL PURINE NUCLEOSIDES

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Reaction of *N*(7,9)-trimethylsilylated chloropurines with either α- or β-1-halo-2',3'-*O*-isopropylidene riburonic esters yields three isomeric nucleosides;[1] they are listed, together with some model compounds, in Table I, with the glycosidic linkage already designated correctly. The *elemental composition* of the individual products is confirmed by elemental analyses and mass spectra;[1] for one derivative each (**3B, 4B, 5B**), β-anomeric *configuration* is established unequivocally by the $^{3}J_{(H^{1'}H^{2'})}$ coupling (< 0.8 Hz).[2,3] Since the ^{1}H carbohydrate partial spectra[3] hold no promise, though, for distinguishing between possible 7- and 9-purine substitution, we turned to ^{13}C NMR in quest of definitive structural criteria.[5]

Of the compounds in Table I, two are established 9-purine derivatives: 2,6-dichloro-9-methylpurine (**6A**)[6] and 2',3'-*O*-isopropylidene-nebularine (**1**) which retains the 9-β glycosidic linkage of its precursor nebularine. Accordingly, as demonstrated by a schematical ^{13}C shift diagram (Fig. 1), the purine carbon shift pattern of **1** and **6A** is practically identical (the straightforward assignment of the five resonances each is based on the fully coupled spectra[5]). Six of our isopropylidene nucleosides, with an aglycone shift pattern[7] closely similar to that of **1** and **6A**, thence are likewise assigned the 9-purinyl structure, including **5A** for which 9-α configuration has been established by X-ray analysis.[4] The anomer pair **5A/B**, by the way, does not obey Imbach's criterion for anomeric configuration assignment:[9] $\Delta\delta^{H}_{CH_3\text{-}exo/\,CH_3\text{-}endo}$ for the α-compound **5A** is 0.22 ppm, far larger than Imbach's limiting value[9] and larger also than $\Delta\delta_{CH_3}$ for the β-nucleoside **5B**.[3])

For 2,6-dichloro-7-methylpurine (**6a**), on the other hand, the ^{13}C spectrum is completely different (Fig. 1). With but small individual shifts, three of our nucleosides (**3a, 4a, 5a**) display this same, distinctly disparate pattern, and so must be 7-purine derivatives, too; for **5a**, a 7-α configuration is proven by crystal structure analysis.[4] Due to the wide separation of the five signals, they may be correlated directly with those of **6a**; no rationalization of these - except for C-2 - dramatic shifts from the 9-purine compounds shall be attempted here.[5]

The ^{13}C shift schematic in Fig. 1 clearly demonstrates that the heterocyclic carbon shift pattern allows an immediate decision on whether the glycosidic

linkage is in 7- or 9-position of the purine nucleus. No differentiation is possible, though, between α- and β-nucleosides (**4A/B, 5A/B**) or - for the chloro derivatives - with respect to the place of substitution. These *configurational* changes result in shifts of comparable magnitude (1 - 2 ppm) as those induced by a change in glycoside *conformation* (**2** → **3B**).[3] Solvent shifts are even more pronounced; C-2,6,8 in the conformationally rigid methylpurines **6a** and **6A**, for instance, are shifted up to ± 2.5 ppm between $CDCl_3$ and d_6-DMSO (30° C).[5]

Figure 1. *^{13}C chemical shift diagram ($CDCl_3$, 30°C) for the heterocyclic carbons of 7- and 9-purine nucleosides (see Table I; asterisks indicate definitive assignment from fully coupled spectra[5]).*

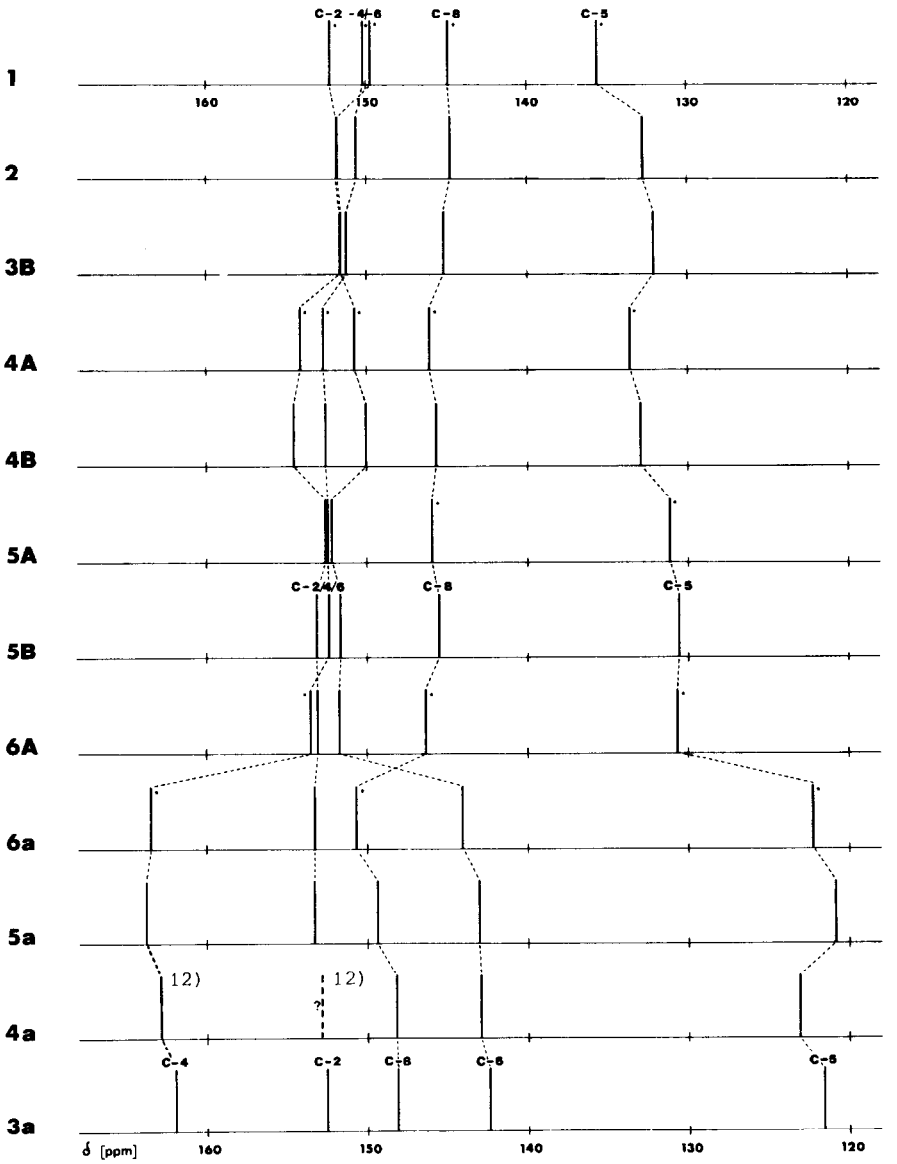
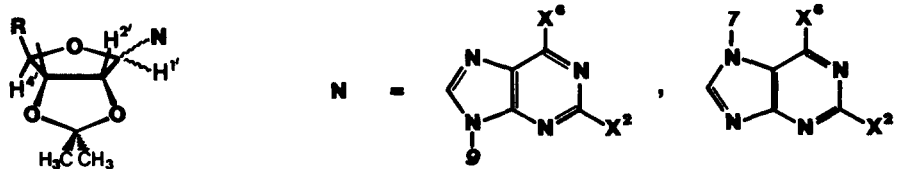
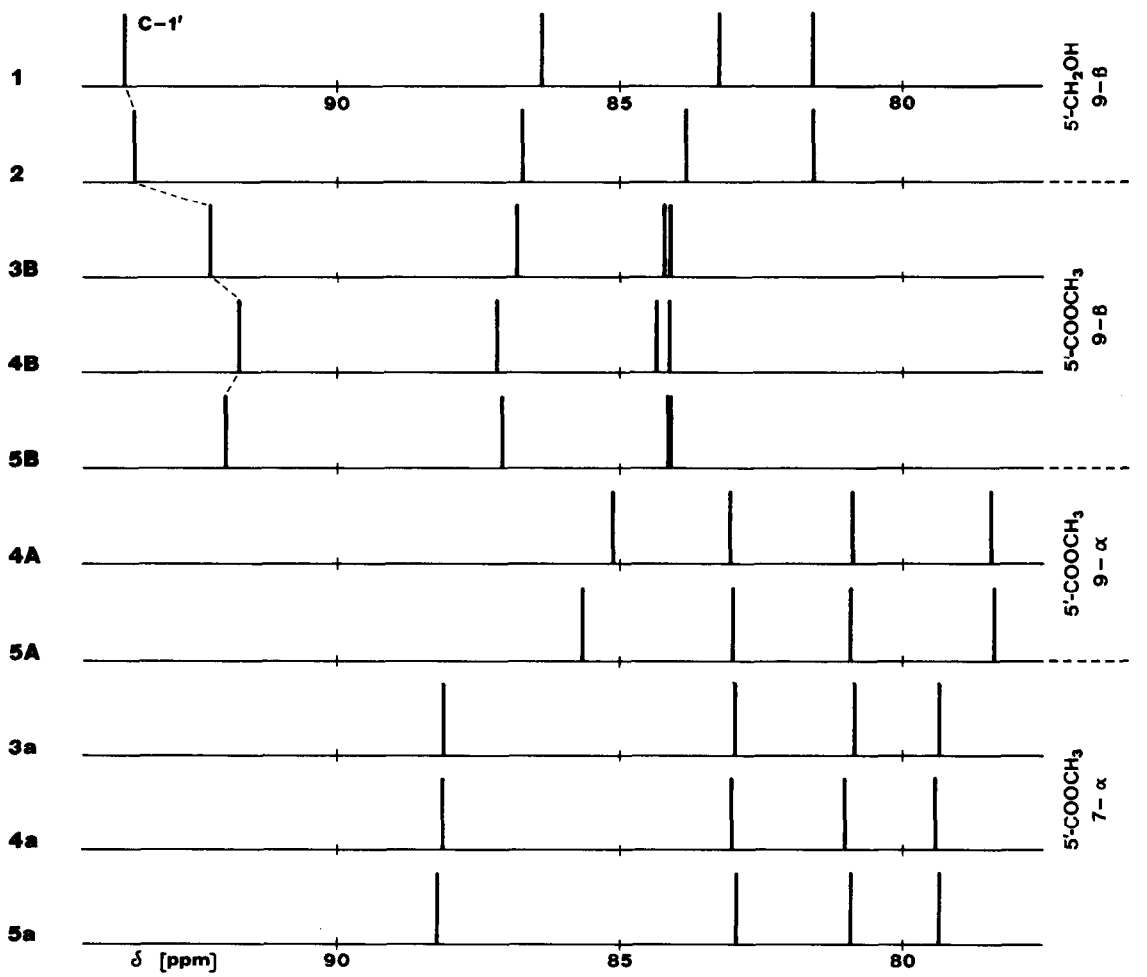


Table I. *1'-Deoxy-1'-(chloropurinyl)-2',3'-O-isopropylidene-D-ribofuranuronic acid methyl esters with different glycosidic linkages (7-α, 9-α, 9-β) and some model compounds.*



	1	2	3a	3B	4a	4A	4B	5a	5A	5B	6a	6A
R	CH_2OH		$COOCH_3$								—	
glycosidic linkage	9-β	9-β	7-α	9-β	7-α	9-α	9-β	7-α	9-α	9-β	$7\text{-}CH_3$	$9\text{-}CH_3$
X^2	H	H	H	H	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl
X^6	H	Cl	Cl	Cl	H	H	H	Cl	Cl	Cl	Cl	Cl

Figure 2. *Furanosyl ^{13}C shifts (in $CDCl_3$) for the 7- and 9-purine nucleosides* **1 - 5B** *(see Table I)*



As illustrated by Fig. 2, the furanosyl ring carbons (C-1' to C-4') of the 2',3'-*o*-isopropylidene purine nucleosides exhibit distinctly different shift patterns, depending on whether the carbohydrate moiety is incorporated as 7-α, 9-α or 9-β methyl riburonate, or in the form of the 9-β-ribofuranoside. Due to the tightly coupled proton resonance sets,[3] the ^{1}H-undecoupled ^{13}C spectra deviate so far from first order that a straightforward shift assignment from C,H long range couplings is not possible;[10] of the four lines, only C-1' can thus be assigned with certainty.[11] It is immediately apparent, though, that in the α-anomers all resonances appear substantially better shielded (probably because of the steric interaction between the glycosidic $C^{1'}$-N and the $C^{2'},^{3'}$-O bonds). In going from the 9-α to the 7-α nucleosides, C-1' moves downfield again (Fig. 2); the same *peri*-effect on δ_{CH_3} is observed between **6A** and **6a**.

Discussion of the individual shifts must await definite assignment of each resonance.[5] Even without that, however, an unequivocal differentiation between the various possible glycosidic linkages of purine ribofuranosides seems feasible, based on the carbohydrate ^{13}C NMR spectral pattern and independent of substitution or modification of the aglycone. In conjunction with the spectral behaviour of the heterocyclic carbons, ^{13}C NMR thus holds promise for structural assignment of nucleosides in general. Work is in progress on the extension of the basic set of representative model compounds which at the same time should establish the limits of this classification procedure.

REFERENCES and FOOTNOTES

1. A fourth one is isolated only in trace quantities; G.Lösch, *Ph.D.Thesis*, Stuttgart 1978.
2. M.J.Robins and M.MacCoss, *J.Am.Chem.Soc.* 99, 4654 (1977); J.A.May Jr. and L.B.Townsend, *J.Org.Chem.* 41, 1449 (1976); R.L.Rinehardt, W.S.Chilton, M.Hickens and W.von Phillipsborn, *J.Am.Chem.Soc.* 84, 3216 (1962).
3. A detailed discussion, based on the numerical evaluation (δ,*J*), of the ^{1}H NMR spectra will be reported in conjunction with the crystal structure investigations.[4]
4. R.Prewo and J.J.Stezowski, to be published.
5. A full account of the ^{13}C NMR investigation (P.Fischer and G.Lösch) will be published separately: The ^{13}C spectra were measured in $CDCl_3$ solution (0.2 - 1.0 M; 30°C; 22.63 MHz; 16k interferograms; 0.02 ppm/address) to allow a direct (structural and configurational) correlation with the respective ^{1}H NMR data[3] and to also exclude specific solvation.
6. A.G.Beaman and R.K.Robins, *J.Org.Chem.* 28, 2301 (1963).
7. C-5 appears especially well shielded (δ < 135 ppm, a fact noted already for purine[8]). The imidazole carbon C-8 is likewise set off to higher field; C-2,4,6, however, absorb within such a narrow shift range that they can be assigned only from fully coupled spectra or by careful incremental comparison within a series of model compounds.[5]
8. R.J.Pugmire, D.M.Grant, R.K.Robins and G.W.Rhodes, *J.Am.Chem.Soc.* 87, 2225 (1965).
9. J.-L.Imbach, *Ann.N.Y.Acad.Sci.* 255, 177 (1975).
10. For 2',3'-*O*-isopropylidene-nebularine (**1**), the carbohydrate resonance at highest field can be assigned to C-4' from the $^3J(C^5H_2)$ triplet splitting (see Fig. 2).
11. On the basis of the larger $^1J_{C-H}$ value (two α-heteroatoms).
12. Since of **4a** only 20 mg were available, the extremely weak signals for the non-protonated carbons C-2,4 cannot be assigned with absolute certainty.