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DIHEDRAL-ANGLE DEPENDENCE OF GEMINAL, SCALAR COUPLING-CONSTANTS IN [1-^{13}C]-AMINO SUGARS*

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ABSTRACT

2-Amino-2-deoxy-D-[1-^{13}C]-glucose and -mannose hydrochlorides have been studied by ^{13}C and ^{1}H n.m.r. spectroscopy. The carbon–carbon and carbon–hydrogen coupling-patterns observed are qualitatively similar to results obtained with the [1-^{13}C]hexoses. In both of the amino sugars studied, resolvable C-1–C-3 coupling was observed only for the β anomers, whereas C-1–C-5 coupling was observed only for the α anomers. In addition, the C-1–H-2 couplings exhibit the same dihedral-angle dependence found for other [1-^{13}C]hexoses. Specific, homonuclear decoupling of H-1 indicates that the C-1–H-2 coupling constants are negative for the α and β anomers of both amino sugars. The dihedral-angle dependence of the geminal C-1–H-2 coupling is thus qualitatively invariant to the presence of the amino group and appears to be a useful conformational probe.

INTRODUCTION

Nuclear magnetic resonance studies of molecules in solution have frequently been used to obtain conformational information. Such applications have been based on dipolar-relaxation interactions, for instance the nuclear Overhauser enhancement effect[1–4], on rare-earth ion "shift reagents"[5–8], and on the interpretation of vicinal proton coupling-constants[9–11]. The latter may be related to molecular conformation by the dihedral-angle dependence originally deduced by Karplus[12]. Recently, it has been found that $^{3}J_{CCCH}$ (refs. 13–15) and $^{3}J_{CCCC}$ (refs. 16 and 17) couplings also conform to a Karplus type of relation. In addition, geminal $^{2}J_{CCH}$ (refs. 18–20), $^{2}J_{CCC}$ (ref. 21), and $^{1}J_{CH}$ (refs. 22 and 23) couplings have been reported that also appear to show a significant conformational dependence. In particular, the $^{2}J_{CCH}$

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coupling can be related to the dihedral angle defined by the bonds XCCH, where X is an electronegative substituent.

Perlin and coworkers have found that the $^2J_{CCH}$ coupling observed between C-1 and H-2 in several hexopyranoses obeys the general rule that an oxygen substituent at C-1 in a gauche orientation relative to H-2 makes a negative contribution to the sign of the C-1–H-2 coupling constant, whereas a C-1 oxygen atom anti to H-2 makes a positive contribution[20]. Furthermore, they noted that this effect on the sign of the coupling constant is potentially useful in conformational studies, especially as the $^2J_{CCH}$ coupling is a single-valued function of the O-C-C-H dihedral angle in the range: $0° < \alpha < 180°$, rather than a double-valued one, as is the case with vicinal coupling-constants.

In the present article, we extend these observations to some amino sugars. The ^{13}C n.m.r. spectra of 1-^{13}C-labeled amino sugars are useful for obtaining both the proton couplings as well as the carbon–carbon couplings. Furthermore, the fact that the H-2 resonance is shifted upfield in 2-amino-2-deoxy sugars facilitates measurements of the sign of the C-1–H-2 coupling-constants.

MATERIALS AND METHODS

The [1-^{13}C]amino sugars were synthesized by adaptations of standard procedures[24]. Unlabeled 2-amino-2-deoxy-D-glucose hydrochloride was purchased from Eastman Organic Chemicals, and unlabeled 2-amino-2-deoxy-D-mannose hydrochloride was purchased from Pfanstiehl Chemicals.

N.m.r. spectra were obtained with a Varian XL-100-15 spectrometer operating in the continuous-wave mode (proton) or the Fourier-transform mode (carbon) and locked to the resonance of solvent D_2O (15.4 MHz). Proton n.m.r. spectra of the H-2 region of the amino sugars were recorded with a sweep width of 100 Hz and a sweep rate of 0.2 Hz sec^{-1}. Proton-noise-decoupled ^{13}C n.m.r. spectra were obtained with 2K spectral points, a spectral width of 1000 Hz, and a filter setting of 500 Hz. By setting the filter smaller than the spectral width, it was possible to minimize the intensity of the ^{13}C-1 resonance in the enriched compounds without allowing the residual ^{13}C-1 resonance to fold over into the spectral region of interest. Although this method introduces phase distortions into the spectrum, it avoids the dynamic-range problem inherent in observing weak resonances in the presence of strong ones. For obtaining a proton-coupled ^{13}C n.m.r. spectrum, a spectral width of 500 Hz with a filter setting of 650 Hz and 2K spectral points was used. All chemical shifts are given relative to external tetramethylsilane.

RESULTS AND DISCUSSION

The proton-coupled ^{13}C-1 resonances observed for 2-amino-2-deoxy-D-glucose · DCl and 2-amino-2-deoxy-D-mannose · DCl are shown in Fig. 1. Most readily apparent are the large $^1J_{CH}$ couplings (Table I), which have been observed in other hexo-

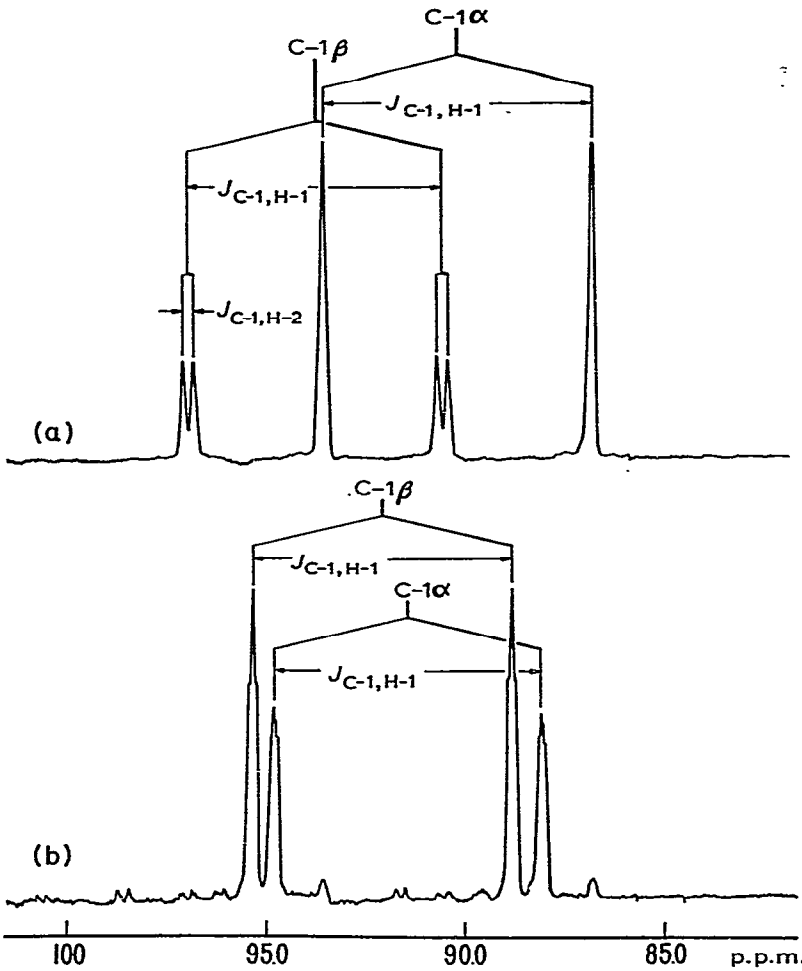

Fig. 1. Proton-coupled, ^{13}C Fourier-transform n.m.r. spectra of the C-1 region of 1-^{13}C-labeled 2-amino-2-deoxy-D-glucose · DCl (a), and 2-amino-2-deoxy-D-mannose · DCl (b). The large couplings with H-1 are indicated. The smaller, long-range C-1–H-2 coupling is resolved only for 2-amino-2-deoxy-β-D-glucose DCl.

pyranoses[14,21–23]. As expected, these couplings show the anomeric dependence described by Bock and Pedersen[22,23], and a smaller coupling is observed for the β anomer, where H-1 is axial. Also apparent are smaller, remote couplings of which only one, the $^{2}J_{CCH}$ coupling between C-1 and H-2 in 2-amino-2-deoxy-β-D-glucose, is readily resolved. This pattern is similar to that observed for the ^{13}C-1 proton-coupled resonances of D-glucose and D-mannose[21]. 2-Amino-2-deoxy-β-D-glucose is unique among the sugars studied here in that both oxygen atoms attached to C-1 are gauche relative to H-2 (Fig. 2a). In 2-amino-2-deoxy-α-D-glucose as well as 2-amino-2-deoxy-α and β-D-mannose, one of the oxygen atoms attached to C-1 is gauche and the other anti, relative to H-2. Thus, based on the dihedral-angle effects

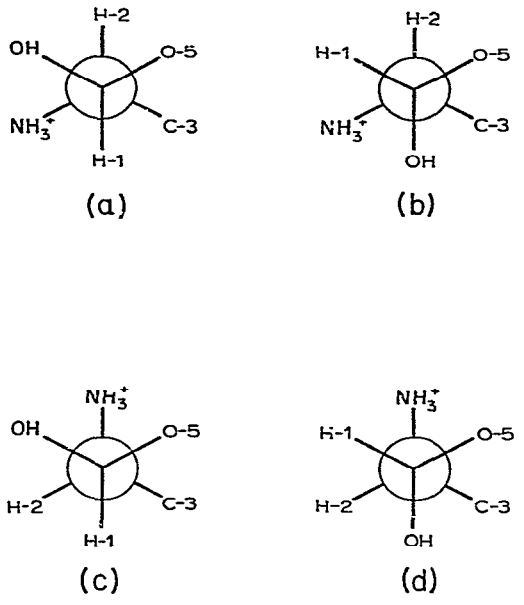

Fig. 2. The rotamer projections along the C-1–C-2 bond for the $^4C_1$ conformation of (a) 2-amino-2-deoxy-β-D-glucose · DCl, (b) 2-amino-2-deoxy-α-D-glucose · DCl, (c) 2-amino-2-deoxy-β-D-mannose · DCl, and (d) 2-amino-2-deoxy-α-D-mannose DCl.

deduced by Perlin and coworkers, a large, negative coupling is expected for 2-amino-2-deoxy-β-D-glucose. Correspondingly, the opposing contributions of the anti and gauche oxygen atoms in the other sugars should result in the smaller couplings observed for these sugars. Thus, in contrast to the spectrum of 2-amino-2-deoxy-β-D-glucose, the proton-coupled spectra of the ^{13}C-1 resonances of 2-amino-2-deoxy-α-D-glucose (Fig. 1a) and 2-amino-2-deoxy-α and β-D-mannose (Fig. 1b) show no resolved coupling, but only substantial broadening, which may reflect coupling to H-2, as well as to the vicinal protons (H-3 and H-5). The question of whether or not coupling to H-2 is significant can be resolved by examining the *proton spectra* of the ^{13}C-1 labeled sugars. In all instances, small C-1–H-2 couplings could be resolved (Figs. 3 and 4).

TABLE I

PROTON AND CARBON COUPLING CONSTANTS FOR [1-^{13}C]AMINO SUGARS[a]

Sugar	*C-1,H-1*	*C-1,H-2*	*H-1,H-2*	*H-2,H-3*
2-Amino-2-deoxy-α-D-[1-^{13}C]glucose · DCl	170	2.6	3.6	10.9
2-Amino-2-deoxy-β-D-[1-^{13}C]glucose · DCl	161	6.9	8.2	10.0
2-Amino-2-deoxy-α-D-[1-^{13}C]mannose · DCl	170	3.1	1.6	4.6
2-Amino-2-deoxy-β-D-[1-^{13}C]mannose · DCl	165	3.0	1.6	4.6

[a]Spectra were obtained in the continuous-wave mode at 25°, $J = \pm 20\%$.

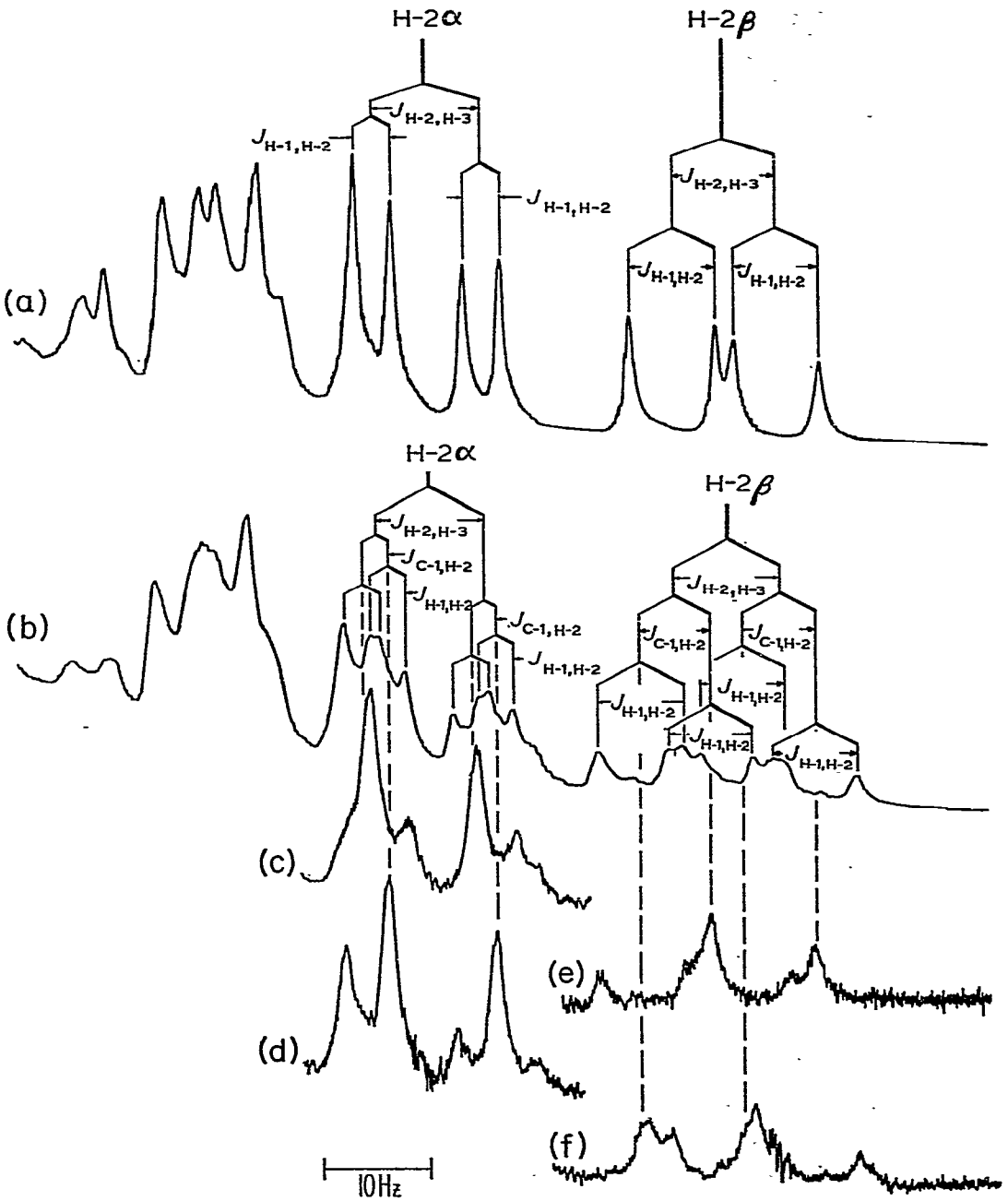

Fig. 3. Continuous-wave proton spectra of the upfield region of 2-amino-2-deoxy-D-glucose · DCl. (a) Natural abundance 2-amino-2-deoxy-D-glucose · DCl, (b) 90% 2-amino-2-deoxy-D-[1-^{13}C]glucose · DCl, (c) and (d) illustrate the effect on the H-2α resonance of decoupling the H-1α resonances at δ 4.62 and 6.32, respectively; (e) and (f) illustrate the effect on H-2β of decoupling the H-1β resonances at δ 5.78 and 4.17, respectively. Thus, in both the α and β sugars, irradiation of the low-(high-) field transitions of H-1 leads to decoupling of the high-(low-) field transitions of H-2 such that $^1J_{C-1,H-1}$ and $^2J_{C-1,H-2}$ are opposite in sign.

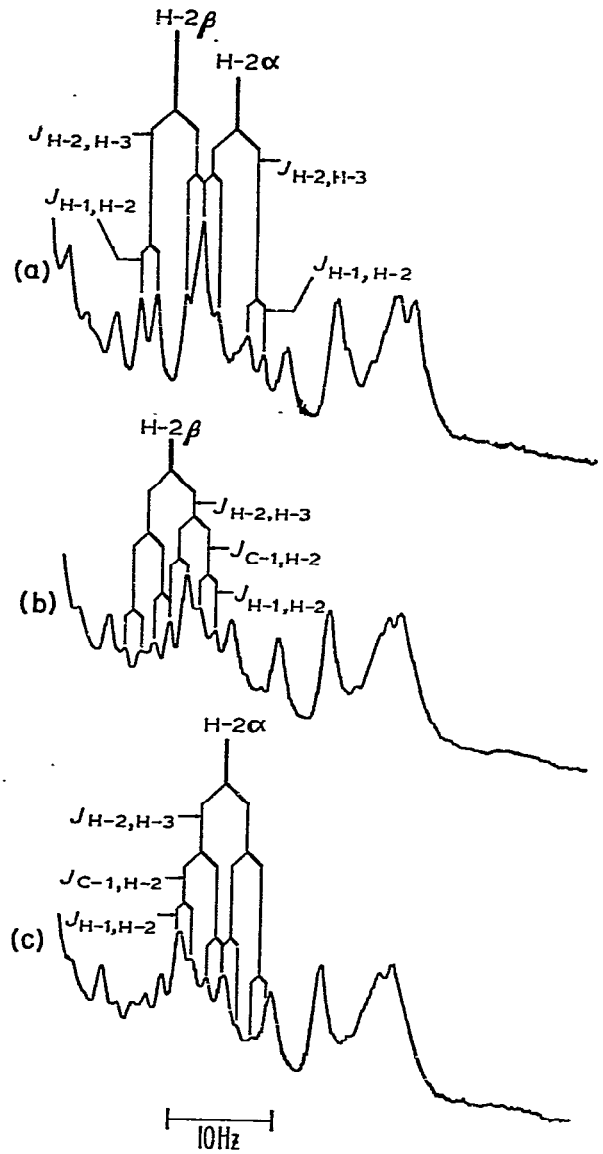

Fig. 4. Continuous-wave spectra of the upfield region of 2-amino-2-deoxy-D-mannose: (a) natural-abundance spectrum; (b) and (c) 90% 1-^{13}C-labeled 2-amino-2-deoxy-D-mannose. The coupling constants were deduced by comparison of the labeled and unlabeled sugar resonances and by the specific H-1 decoupling experiments which also gave the sign of the C-1–H-2 coupling constants. As is clear from the spectrum, there is considerable overlap of the H-2α and H-2β resonances.

The signs of the coupling constants were measured by double-resonance techniques[25]. In these experiments, H-1 is decoupled from H-2 corresponding to a given spin-state of C-1. The relative sign of $J_{C-1,H-1}$ and $J_{C-1,H-2}$ may then be determined. As all directly bonded C-H coupling-constants are assumed to be

positive[26], this allows a determination of the signs of $^2J_{C-1,H-2}$. Ideally, for an AMX system, four experiments can be performed: the decoupling of H-1 from H-2 for each of the two C-1 spin-states, and decoupling of H-2 from H-1 for each of the C-1 spin-states. However, these systems are complicated by the additional coupling of H-2 to H-3. As, in all cases, $^3J_{H-2,H-3} > {}^3J_{H-1,H-2}$, it was not possible to decouple H-2 from H-1 with a single decoupling-frequency corresponding to a given C-1 spin-state. Instead, only the H-1 resonances were decoupled and the H-2 resonances observed. This experiment was sufficient for determining the signs of $^2J_{C-1,H-2}$.

Spectra from the decoupling experiments on 2-amino-2-deoxy-D-glucose are shown in Fig. 3. The H-2 resonances are readily resolved because they are furthest upfield, reflecting the fact that aminomethine protons (>CH-ND_2) resonate at higher fields than hydroxylmethine protons (>CH-OD)[27]. In natural-abundance 2-amino-2-deoxy-D-glucose (Fig. 3a), H-2α and H-2β are quartets because of non-equivalent coupling with H-1 and H-3 (Table I). In the 1-^{13}C-labeled sugar (Fig. 3b) *both* α and β resonances exhibit coupling to C-1. For example, in the β sugar, where $^2J_{C-1,H-2} \simeq {}^3J_{H-1,H-2} \simeq {}^3J_{H-2,H-3}$, the H-2 resonance is approximately a 1:3:3:1 quartet, as illustrated in Fig. 3b. For the α sugar, $^3J_{H-2,H-3} > {}^3J_{H-2,H-1} \simeq {}^2J_{H-2,C-1}$, so that the H-2 resonance appears as two quartets (Fig. 3b). Despite the complexity of the resonance patterns, the H-1 decoupling experiments give unequivocal results, indicating negative values for both $^2J_{C-1,H-2\alpha}$ and $^2J_{C-1,H-2\beta}$. Thus, in both the α and β sugars, irradiation of the low (high) field transitions of H-1 leads to a decoupling of the high (low) field transitions of H-2, such that the $^1J_{C-1,H-1}$ and $^2J_{C-1,H-2}$ are opposite in sign.

The spectra of 2-amino-2-deoxy-D-mannose are difficult to analyze. The chemical shifts of H-2 have not been assigned previously. They were determined by double resonance to be $\delta_{H-2} = 3.67$ p.p.m. for the α anomer, and $\delta_{H-2} = 3.73$ p.p.m. for the β anomer, relative to external tetramethylsilane. As in the case of 2-amino-2-deoxy-α-D-glucose, $J_{H-2,H-3} > J_{H-2,H-1}$ (Table I), so that in the natural-abundance sugar, the H-2 resonances of both the α and β anomers appear as doublets of doublets (Fig. 4a). The spectra of 1-^{13}C-labeled 2-amino-2-deoxy-D-mannose are quite complex (Fig. 4b,c), but coupling of C-1 to H-2 in both anomeric forms is readily apparent, and careful analysis of the decoupled spectrum shows that the coupling constants are negative in both cases.

The conformational dependence of the carbon–carbon coupling constants in a number of hexopyranoses has been noted recently[21]. The ^{13}C n.m.r. spectrum of the natural-abundance, carbon positions of the 1-^{13}C-labeled amino sugars can be used to study this coupling. The results (Table II) are completely consistent with the generalizations noted for the hexopyranoses, namely:

(*1*) The two vicinal couplings between C-1 and C-4, and C-1 and C-6, obey a Karplus type of rule, so that observable coupling occurs in the latter case with the two carbon atoms anti, and not in the former instance with the two carbon atoms gauche.

(*2*) The geminal carbon–carbon coupling-constants follow the empirical

TABLE II

CARBON–CARBON COUPLING CONSTANTS FOR [1-^{13}C]AMINO SUGARS[a]

Sugar	*C-1,C-2*	*C-1,C-3*	*C-1,C-5*	*C-1,C-6*	*C-1,C-4*
2-Amino-2-deoxy-α-D-[1-^{13}C]glucose · DCl	44.3	[b]	1.5	[c]	[b]
2-Amino-2-deoxy-β-D-[1-^{13}C]glucose · DCl	43.8	2.4	[b]	[c]	[b]
2-Amino-2-deoxy-α-D-[1-^{13}C]mannose · DCl	44.7	[b]	1.7	[c]	[b]
2-Amino-2-deoxy-β-D-[1-^{13}C]mannose · DCl	41.3	1.8	[b]	[c]	[b]

[a]Fourier-transform spectra were obtained with a spectral resolution of 1 Hz at 50°. [b]No observable coupling. [c]Coupling observed, but not measured.

observation of a C-1–C-3 resolvable coupling only in the β anomer and a C-1–C-5 resolvable coupling only in the α anomer. The C-C coupling data (Table II) confirm the ^{13}C resonance-assignments of the non-acetylated amino sugars made by Bock and Pedersen[22] and by Yamaoka, *et al.*[28] except for C-5β of 2-amino-2-deoxy-D-glucose, which is at 77.5 p.p.m. rather than 71.0 p.p.m. as reported by Yamaoka *et al.* Although only the amino sugar hydrochlorides have been examined, our results are in general agreement with the assignments of Bundle, *et al.*[29] for the *N*-acetyl derivatives.

The trends established for coupling between protons tend to be observed for couplings involving carbon as well. Thus, a Karplus or modified Karplus type of dependence on the dihedral angle predicted for $^3J_{HH}$ is also observed for $^3J_{CH}$ (refs. 13 and 14) and for $^3J_{CC}$ (refs. 16, 17, and 21). It is, therefore, reasonable to expect qualitative similarities in the conformational dependence of the $^2J_{CCH}$ and $^2J_{CCC}$ couplings. However, several differences are apparent. Thus, for the coupling between C-1 and C-3, a larger magnitude is observed in the β sugars, corresponding to O-5 gauche and O-1 anti relative to C-3. Alternatively, a larger $^2J_{CCH}$ coupling is observed if both of the oxygen substituents at C-1 are gauche relative to the coupled proton, that is, in β-D-glucose, than if one oxygen atom is gauche and one anti. Clearly, a determination of the signs of the $^2J_{CCC}$ couplings would be useful for resolving the problem. Preliminary molecular-orbital (MO) calculations, based on the finite-perturbation theory formulation in the INDO approximation of self-consistent-field MO theory, appear to predict the correct signs and the observed trends in the $^2J_{CCH}$ values but not in the $^2J_{CCC}$ values. Additional theoretical investigations into the conformational nature of the $^2J_{CCC}$ values are currently in progress.

Finally, we note that the $^3J_{HH}$ gauche couplings obtained for the amino sugars present a pattern consistent with an empirical relationship recently deduced for other carbohydrates[30]. In particular, it has been reported that the magnitude of the $^3J_{HH}$ gauche coupling-constants is decreased if one or more of the coupled protons is antiperiplanar relative to an oxygen substituent. In the present case, the $^3J_{H-2,H-3}$ value for 2-amino-2-deoxy-α and β-D-mannose is larger than any of the $^3J_{H-1,H-2}$ gauche couplings. The actual couplings are slightly outside the ranges suggested in

ref. 30, and indicate that the effect of a nitrogen atom anti relative to one of the coupled protons is qualitatively more similar to that of an anti carbon atom than an anti oxygen atom.

CONCLUSIONS

Numerous empirical observations have been made regarding the scalar spin-couplings observed in carbohydrates. The structural constraints present in these systems make them ideal for studying the dependence of scalar coupling on conformation. In all cases, the $^3J_{HH}$ and $^3J_{CC}$ couplings exhibit a Karplus type of dependence on dihedral angle. The observed two-bond couplings $^2J_{CCH}$, $^2J_{CCC}$ and $^2J_{COC}$ involving C-1 similarly appear to depend on the dihedral angle between the coupled proton or carbon atom and the oxygen substituents at C-1. However, the relationships are not so readily generalized, as similar conformational arrangements lead to apparent increases in one case and apparent decreases in another. The present studies indicate that the $^2J_{CCH}$ values are negative in all instances, so that the decrease in magnitude observed when the coupled proton is anti relative to one of the C-1 oxygen substituents actually corresponds to a more-positive coupling. These results are consistent with the sign determinations of Schwarcz *et al.* for allose[20] and with the general dihedral-angle dependence deduced by Schwarcz *et al.*[14,20]. Comparison of the results obtained with the amino sugars to results obtained for other hexopyranoses[21] indicates no qualitative differences. Thus, from the standpoint of couplings, the replacement of a hydroxyl group by an amino group at C-2 is not a major perturbation.

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