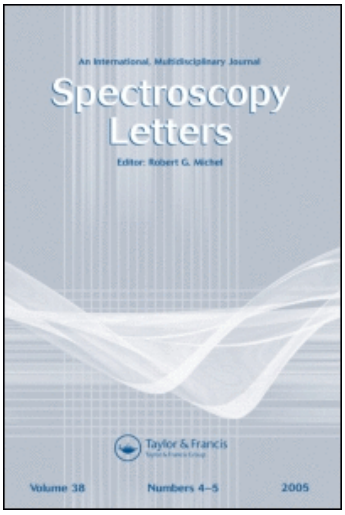

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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To cite this Article Yan, Hong , Sun, Hongzhe , Chen, Huilan and Tang, Wenxia(1993) 'Two Dimensional ^{1}HNMR Studies on 2'5'-Dideoxyadenosylcobalamin', Spectroscopy Letters, 26: 2, 319 — 329
To link to this Article: DOI: 10.1080/00387019308011534
URL: http://dx.doi.org/10.1080/00387019308011534

SPECTROSCOPY LETTERS, 26(2), 319-329 (1993)

TWO DIMENSIONAL ^{1}HNMR STUDIES ON 2′5′–DIDEOXY ADENOSYLCOBALAMIN

Key Words: Coenzyme B_{12} analogue, Cobalamin, Deoxyadenosine, Two dimensional NMR

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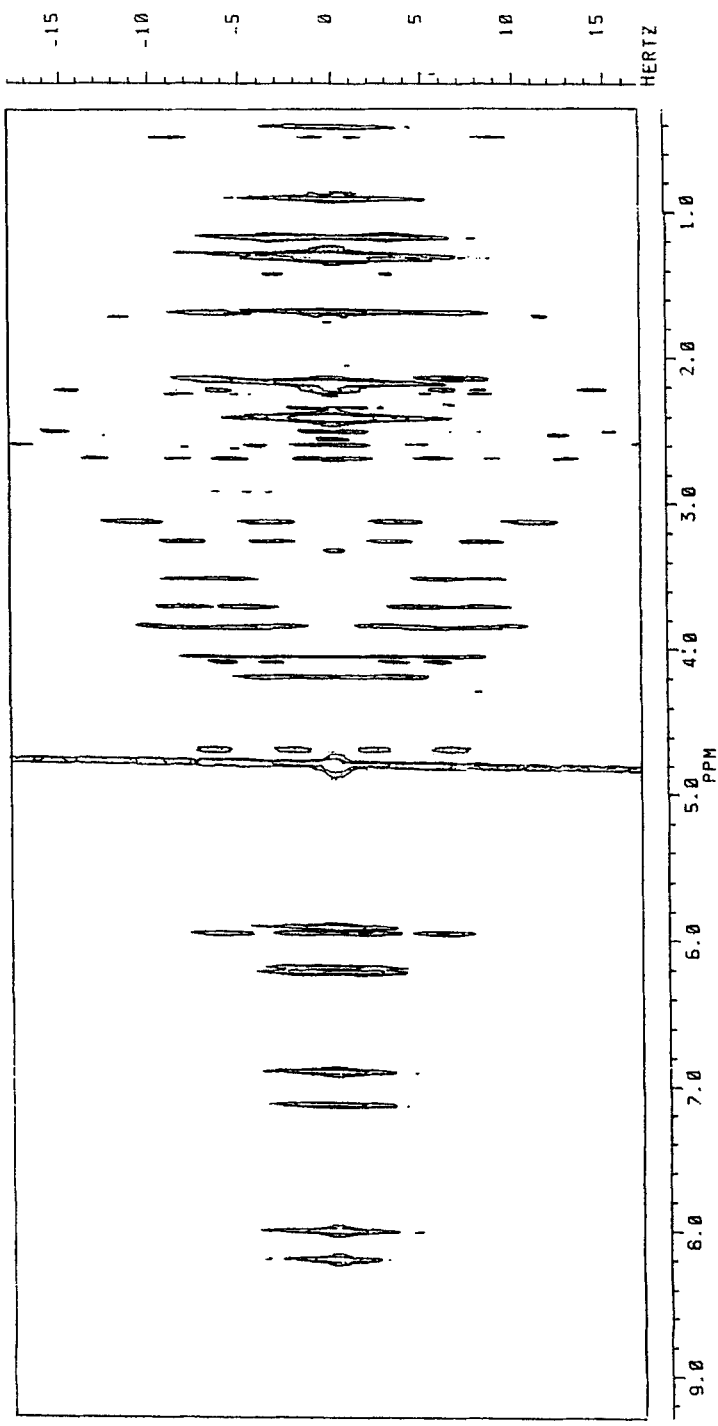
ABSTRACT

The ^{1}HNMR spectrum of 2′,5′–dideoxyadenosylcobalamin, a Coenzyme B_{12}(5′–deoxyadenosylcobalamin) analogue, has been assigned by 2D COSY . Its proton coupling constants have also been measured by J–resolved experiment. The comparison between the analogue and Coenzyme B_{12} was made.

INTRODUCTION

Since the early 1970s, Coenzyme B_{12} and related cobalamins have been subject of extensive NMR studies [1–12]. Complete ^{1}H and ^{13}C assignments of Coenzyme B_{12} through the use of new two dimensional 2D NMR techniques have been made by Ad Bax and his coworkers [9]. However, cobalamins containing axial 2′,5′–dideoxynucleosidyl ligands had only few examples and they were lack of NMR studies [13,14]. In this paper, two–dimensional ^{1}HNMR studies on 2′,5′–dideoxyadenosylcobalamin were reported. We used 2D COSY to have assigned the proton resonances and used J–resolved experiment to have determined its coupling constants. We also compared the different structure and chemical shifts between the analogue and Coenzyme B_{12} caused by their different axial deoxyadenosyl ribose rings and compared the chemical shifts of deoxyadenosyl group in uncoordinated 5′–*O*–p–tosyl–2′–deoxyadenosine with that in the analogue.



EXPERIMENTAL SECTION

2′,5′–Dideoxyadenosylcobalamin was prepared as red crystal by an improved method which consists in direct tosylation of 2′–deoxyadenosine and followed by reaction with reduced hydroxocobalamin[15]. 3.5mg of the sample (Mr = 1564) was dissolved in 0.5ml D_2O. ^{1}HNMR spectra were recorded on Bruker AM–500 NMR spectrometer (probe temperature 298K). Observation frequency of 500.13 MHz was used . All proton shifts were referenced to internal DSS . The residual HDO resonance was suppressed by presaturation method. One dimensional spectra were acquired into 32K data points. 2D COSY and J–resolved spectra were recorded with magnitude mode. For COSY, 256t_1 increments were accumulated into 1K data points with 16 scans for each and for J–resolved experiment, 128t_1 increments were accumulated into 2K data points with 16 scans for each. Prior to Fourier Transformation, the initial data matrix was zero–filled in the t_1 dimension and multiplied by a sine–bell function in both t_1 and t_2 dimensions.

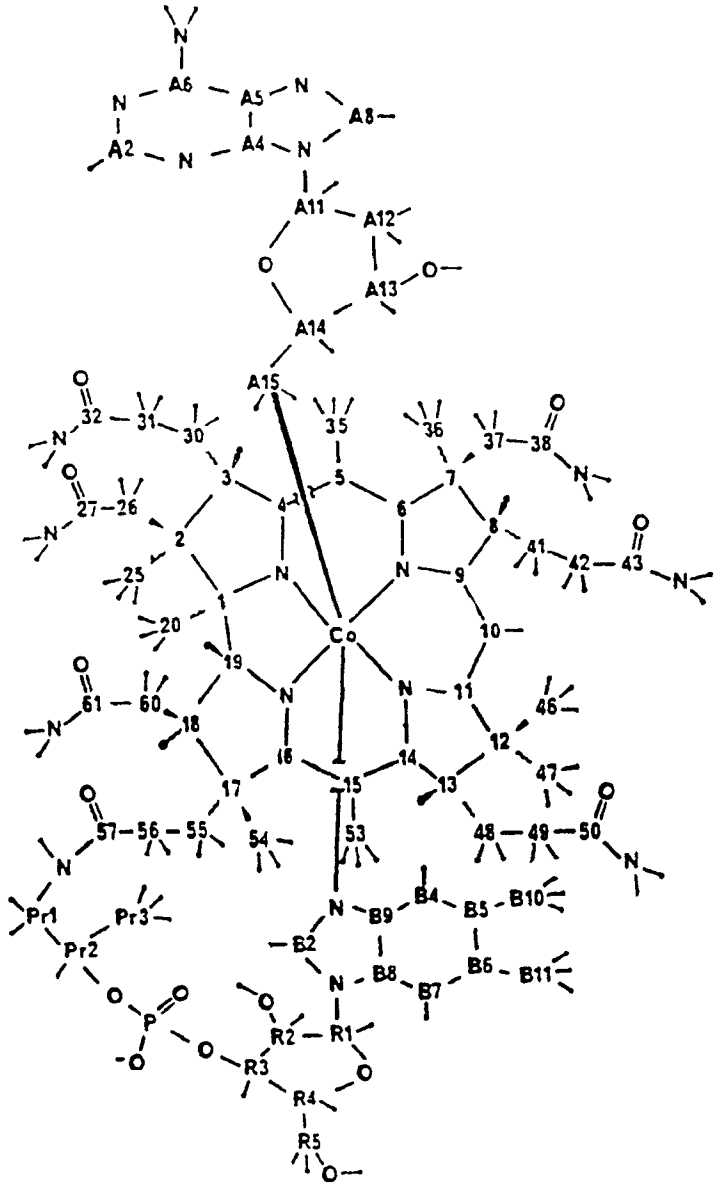
RESULT AND DISCUSSION

The structure and 2D COSY of the title compound were shown on Chart 1, Fig.1 and Fig.2 respectively. Gernerally, the downfield resonances mainly arise from the aromatic protons, immediately upfield of the suppressed residual HDO, there are some complex multiplet which arise from various methine protons. Methyl groups in the molecular are located on upfield. The analysis about ^{1}HNMR spectra of the analogue was divided into five parts.

The 2′5′–Dideoxyadenosyl Axial Ligand

According to proton chemical shifts of 5′–*O*–*p*–tosyl–2′–deoxyadenosine (Table 1) and hydroxocobalamin[4], the two singlets at very low field were assigned to A8, A2 which are lack of cross peaks on 2D COSY and the quartet at 5.979ppm with $J_{R'1-R'2A} = 7.98$Hz, $J_{R'1-R'2B} = 4.40$Hz was assigned to R′1H. The assignment of R′1 proton provided a starting point for assignments of the remaining ^{1}H resonances of 2′,5′–dideoxyadenosyl ribose ring to R′2A, R′2B, R′3, R′4, R′5A, R′5B protons(see Table 2). From Fig.2, the two protons of R′2 were separated and situated on different positions (2.265, 2.731ppm, respectively). The resonance of R′2B proton shows three cross peaks, indicating that it is coupled by three different kinds of protons, i.e. R′1, R′2A, R′3. R′4H have coupling with R′5HB, but didn't show weak coupling with R′5HA($J_{R'4-R'5A} = 2.34$Hz). Since the peaks of R′2HA and R′4H were covered by each other, it couldn't be concluded that the cross peak un-

Chart I



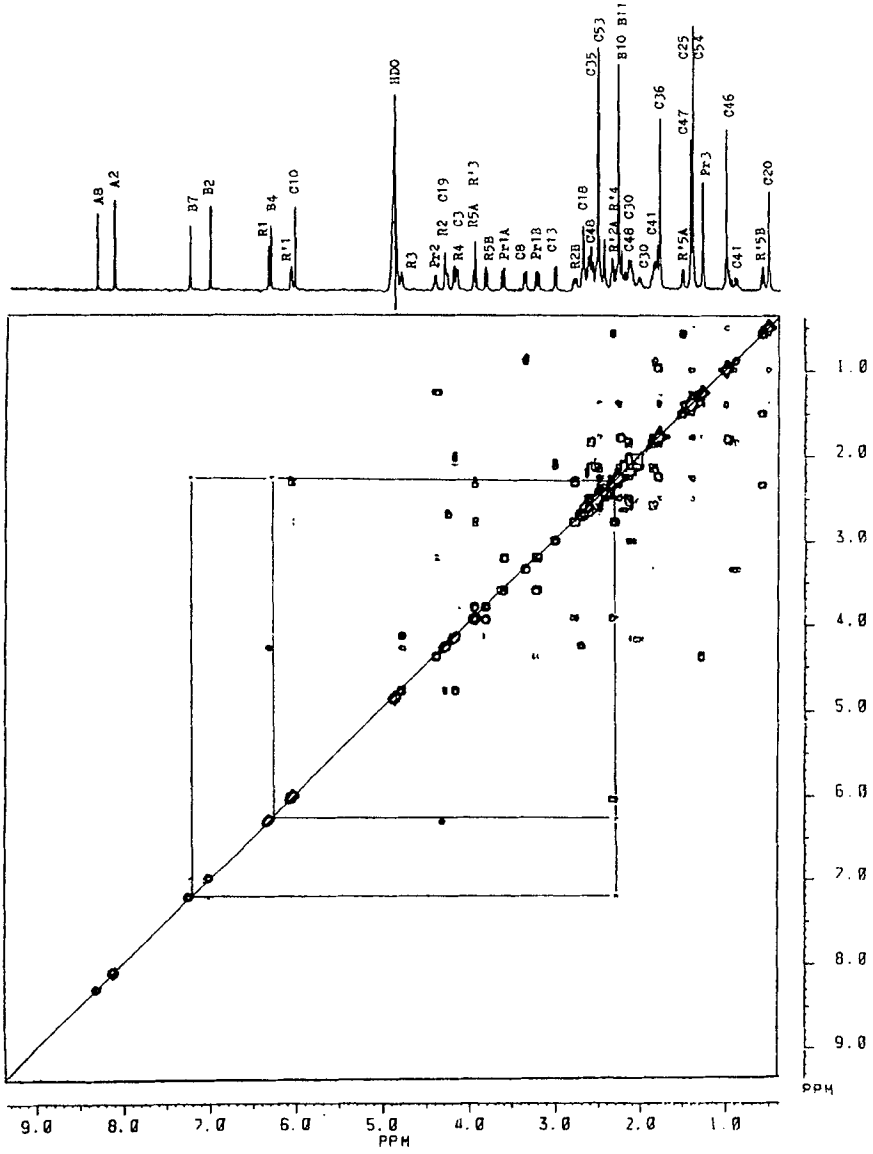


FIG.1. 2D COSY of 2',5'–dideoxyadenosylcobalamin. The coupling connectivity patterns for the 5,6–dimethylbenzimidazole protons are indicated by drawn lines.

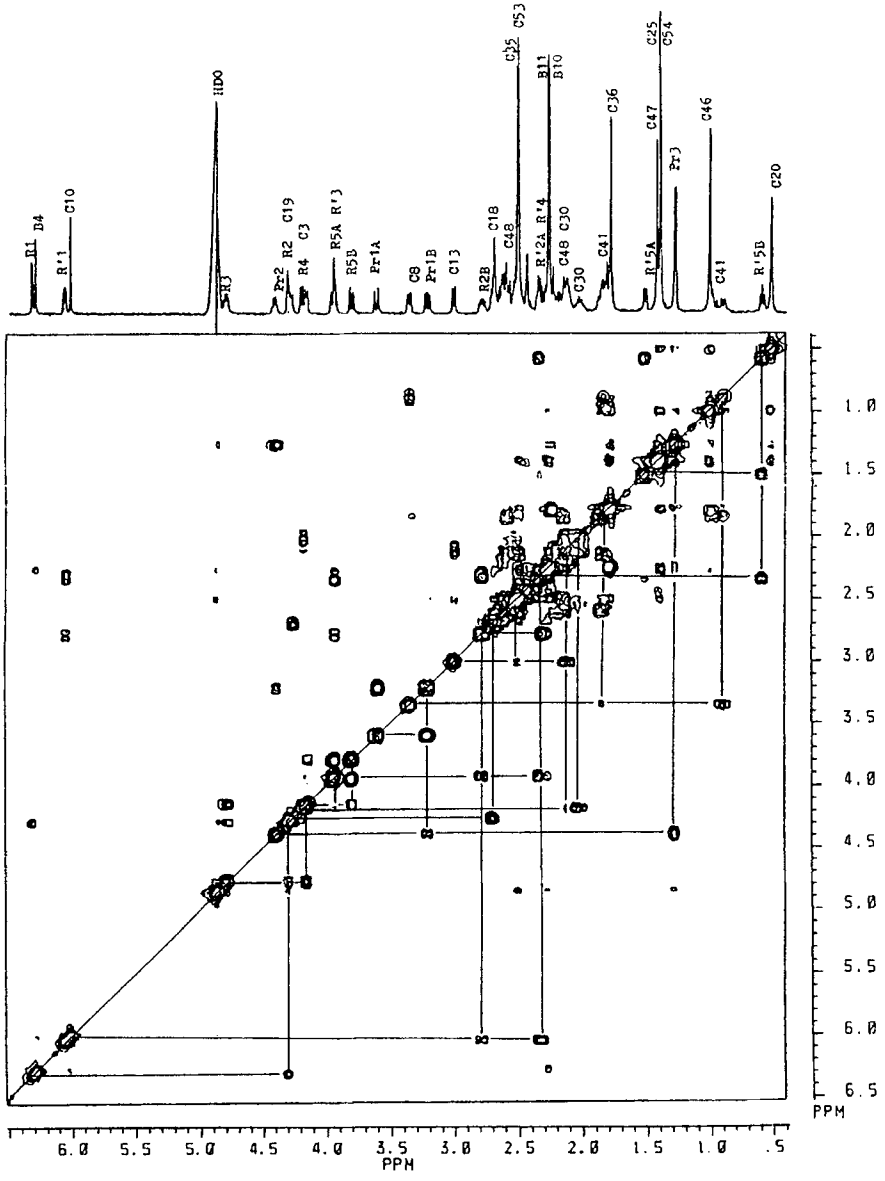


FIG.2. Expanded high–field region of the Fig.1. The coupling connectivity patterns for the protons of the two ribose rings, the propanolamine and part of the corrin and its side chains are indicated by drawn lines.

TABLE 1

The Chemical Shifts (δ / ppm) of 5′–*O*–*p*–tosyl–2′–deoxyadenosine

δ	8.431 (s,1)	8.143 (s,1)	7.505 (d,2)	7.162 (d,2)	6.623 (t,1)	2.384 (m,2)	4.558–4.881 (m,4)	2.243 (s,3)
Atom	A8	A2	Ar–a	Ar–b	R′1	R′2	R′3,R′4,R′5	Ar–CH3

s(singlet), d(doublet), t(triplet), m(multiplet)

TABLE 2

The Chemical Shifts and Coupling Constants of the Protons Associated with the 2′,5′–Dideoxyadenosyl Ligand for 2′,5′–Dideoxyadenosylcobalamin

Atom	δ(ppm)	J(Hz)
A8	8.233	
A2	8.024	
R′1	5.979	$J_{R'1-R'2A}=7.98$, $J_{R'1-R'2B}=4.40$
R′2A	2.265	$J_{R'2A-R'2B}=14.20$, $J_{R'2A-R'3}=0$
R′2B	2.731	$J_{R'2B-R'3}=7.06$
R′3	3.864	$J_{R'3-R'4}=6.60$
R′4	2.265	$J_{R'4-R'5B}=7.72$
R′5A	1.427	$J_{R'4-R'5A}=2.34$
R′5B	0.508	$J_{R'5A-R'5B}=9.19$

der R′3H was caused by coupling with R′2HA or R′4H or both of them only on the basis of 2D COSY. It was known from J–resolved spectrum that R′3H is a quartet, this is, R′3H is coupled by either of R′2HA or R′4H. The further conclusion can be drawn from J–resolved experiment in which the peaks R′2HA(quartet ,~ 2.27ppm) and R′4H (octet,~ 2.25ppm) unexactly overlap with each other that R′3H is coupled only by R′4H, not by R′2HA ($J_{R3-R2A}=0$). The final assignments about this part were listed in Table 2.

The Ribose Moiety

The assignment of R1H was quite straightforward, for it provided an easily recognizable doublet at 6.240ppm with $J_{R1-R2}=2.79$Hz, in the same

TABLE 3

The Chemical Shifts and Coupling Constants of the Protons Associated with the Ribose Moiety for 2′,5′–Dideoxyadeosylcobalamin

Atom	δ(ppm)	J(Hz)
R1	6.264	$J_{R1-R2} = 2.79$
R2	4.204	$J_{R2-R3} = 4.48$
R3	4.732	$J_{R3-R4} = 8.80$
R4	4.120	$J_{R4-R5B} = 3.90$
R5A	3.864	$J_{R4-R5A} = 2.89$
R5B	3.740	$J_{R5A-R5B} = 12.89$

TABLE 4

The Chemical Shifts and Coupling Constants of the Protons Associated with the Propanolamine Moiety for 2′,5′–Dideoxyadenosylcobalamin

Atom	δ(ppm)	J(Hz)
Pr1A	3.534	$J_{Pr1A-Pr2} = 2.77$
Pr1B	3.124	$J_{Pr1A-pr1B} = 14.37$
Pr2	4.314	$J_{Pr1B-Pr2} = 7.35$
Pr3	1.198	$J_{Pr2-Pr3} = 6.46$

fashion as assigning the protons of upper axial deoxyadenosyl ribose ring, the protons, R2, R3, R4, R5A, R5B, of the ribose ring of the nucleotide can be conveniently assigned as shown on Fig.2. R4H is coupled by R3H, but also by two unequal protons of R5H2 in which the two proton resonances show strong cross peaks . The assignments of this part was given in Table 3.

The Propanolamine Moiety

The methyl group, Pr3, was readily indentified as a doublet at 1.198ppm ($J_{Pr2-Pr3}$ = 6.46Hz), and because it presented a strong cross peak caused by coupling with Pr2H (4.314ppm). The remaining two protons were assigned to Pr1HA, Pr1HB which also have very strong cross peaks, as shown on Fig.2. .

On J–resolved spectum, Pr2H shows octet, this reflected that it is coupled by three kinds of protons, Pr1HB , Pr1HA, and Pr3H3, but its weak coupling with Pr1HA($J_{Pr2-Pr1A}$ = 2.77Hz) couldn't be found in 2D COSY. The assignment of this part was summarized on Table 4.

The 5,6–Dimethylbenzimidazole Moiety

The two methyl groups , B10H3 and B11H3, overlap with each other at 2.189ppm. In Fig.1, the weak relayed cross peaks can be indentified clearly between B4H(6.215ppm) and B10H3 as well as B7H(7.149ppm) and B11H3, but B2H(6.922ppm) doesn't couple with other protons. The aromatic B4, B7 and B2 protons were located in low field.

The Corrin Ring and its Side Chains

On 2D COSY, the protons of the corrin ring and its side chains can be partially assigned as follow: C3H(4.040,$J_{C3-C30A}$ = 2.48,$J_{C3-C30B}$ = 9.50), C8H (3.282,$J_{C8-C41A}$ = 5.12,$J_{C8-C41B}$ = 11.69),C13H(2.934,$J_{C13-C48A}$ = 3.06,$J_{C13-C48B}$ = 10.64),C18H(2.612),C19H(4.204),C30H2(1.945,2.048),C41H2(0.825,1.767),C48H2(2.048,2.426), their coupling constants can't be determined entirely due to methine, methylene and methyl protons overlapping with each other. Based on the same reason, the remaining fourteen protons in its side chains can't be assigned only by 2D COSY. The remaining downfield singlet (5.993ppm) is assigned to C10H, since this is the only remaining proton at a conjugated site in the molecular and also it lacks J_{H-H} coupling to other protons.

The methyl groups on the corrin ring of the analogue can be easily recognized due to their similarities to that of other cobalamins[2,4], they were assigned to C35H3(2.411ppm), C53H3(2.402), C36H3(1.707), C54H3(1.317), C25H3(1.317), C47H3(1.342),C46H3(0.926) and C20H3(0.435).

It can be seen from the obtained data that the chemical shifits and coupling constants of the nucleotide, the corrin ring and its side chains of 2′,5′–dideoxyadenosylcobalamin are strikingly close to that of Coenzyme B_{12} [4,9]. Differences between them are found in those protons of the axial deoxyadenosyl ligands, the obvious changes are in the case of R′2 protons. Because the deoxyadenosyl ribose ring of the analogue is lack of 2′–hydroxyl group , the chemical shifts of R′2H2 are shifted to upfield (2.265, 2.731 ppm respectively) relative to that of R′2H(4.54 ppm) in Coenzyme B_{12} [9].

Besides, compared with the deoxyadenosyl group in uncoordinated 5′–*O*–*p*–tosyl–2′–deoxyadenosine, it was found that all the ^1H chemical

shifts of the upper axial deoxyadenosyl ligand in 2′,5′–dideoxyadenosyl cobalamin were unequally moved to upfield, the alteration decreases graduatedly in accordance with the distance from near to far from the coordination site Co atom, this is because Co–C bond formation makes π electrons of the conjugated corrin ring move onto the upper deoxyadenosyl group through Co atom, resulting in increasing electron density on the deoxyadenosyl ligand in a different degree . R′5H2(directly linked to Co) were influenced most markedly and shifted to high field by approximatedly 3.1–4.3 ppm . On the other hand, because of the large steric hindance between the deoxyadenosyl ligand and the corrin ring and its side chains, the restricted rotation about Co–C bond lead to the nonequivalence in ^{1}H resonances of R′5H2. Lastly, due to the action of the corrin and side chains, the two protons of R′2H2 in the compound have separate positions(2.265, 2.731 ppm) relative to the two equal protons (2.384ppm) of R′2H2 in 5′–*O*–*p*–tosyl–2′–deoxyadenosine.

CONCLUSION

Complete ^{1}H assignments and coupling constant measurements were carried out about the upper axial 2′,5′–dideoxyadenosyl ligand and 5,6–dimethylbenzimidazole nucleotide side chain of 2′,5′–dideoxyadenosyl cobalamin by 2D COSY and J–resolved experiment. This work obtained some structural information about the analogue in solution. As for the side chains of the corrin, ^{1}H assignments and coupling constants couldn't be entiredly resolved due to being overlapped by other methine, methelene and methyl groups at the same region. Gernerally, the chemical shifts and coupling constants of 2′,5′–dideoxyadenosylcobalamin and Coenzyme B_{12} are remarkedly similar, differences are only in R′2 protons of their different axial deoxyadenoxyl ligands.

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Date Received: 08/19/92
Date Accepted: 09/23/92