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PROTON AND CARBON-13 NMR STUDIES OF METHYL-(6-O-L-PHENYLALANYL)-α-D-GLUCOSIDE AND METHYL-(6-O-D-PHENYLALANYL)-α-D-GLUCOSIDE

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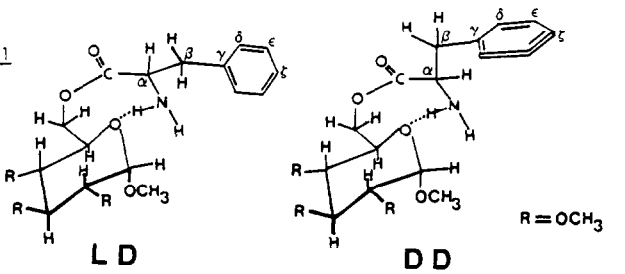
ABSTRACT: Study of methyl-(6-O-L-Phenylalanyl)-α-D-glucopyranoside and methyl-(6-O-D-phenylalanyl)-α-D-glucopyranoside by ^{1}H and ^{13}C nmr showed that the LD compound is more flexible than DD and that DD probably assumes a folded conformation.

Among the four major building blocks of life, viz. nucleic acid bases, fatty acids, amino acids and carbohydrates, only the latter two categories are chiral. The interesting fact that an overwhelming majority of metabolically active sugars are in the D-configuration and that all the protein amino acids are in the L form has stimulated a number of interesting theories.[1] Here we would like to look at the steric interactions of amino acids and carbohydrates as a possible reason for co-evolution of L-amino acids and D-sugars. Keeping in mind that the common natural hexoses are locked in the C1(D) conformation and that the protein amino acids are fixed in the L configuration at C_α, would there be a preferential binding (covalent or non-covalent) between amino acids and hexoses in L-D couples(or D-L , as the mirror image) rather than D-D (or L-L for that matter), leading to the prevalence of the existing forms in the biosystems? As an example, with the most abundant hexose,D-glucose and the amino acid,D or L phenylalanine, hydrogen bonds and/or ester bonds can be built around the stereodeterminant carbon atoms of the two species as depicted in Scheme 1:

SCHEME 1

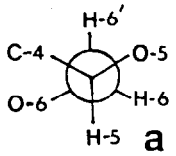


LD DD

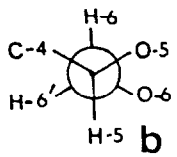
With such coupled species, the LD compound would have the bulky side chain of the amino acid freely rotating while rotation in the DD compound would be restricted by the steric interaction of the side chain and the pyranose ring.

Methyl-(6-O-L-phenylalanyl-2,3,4-tri-O-methyl)-α-D-glucopyranoside(1, LD) and its diastereomer, methyl-(6-O-D-phenylalanyl-2,3,4-tri-O-methyl)-α-D-glucopyranoside(2, DD) synthesized by identical procedures[2] were found to be similar in their mobilities on tlc, reactions towards ninhydrin and sulfuric acid sprays, and infrared spectra(cm^{-1},$CHCl_3$): 3380,2920, 2840, 1730-35. DD could be easily crystallized in CH_2Cl_2/petroleum ether, yielding fine needles, m.p. 178-180°, $[\alpha]_D$ = +58°(c 0.01, EtOH); while LD, $[\alpha]_D$ = +116°(c 0.033, EtOH), remained as a gum not crstallizable in all common solvents attempted, for reasons that may be evident later.

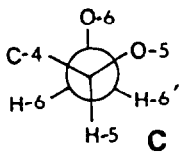
Proton nmr showed that both LD and DD retain the Cl(D) conformation of the glucose moiety[3] as observed from $J_{1,2}$, $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$. (Table 1) Calculation of the ratio of the rotamers a, b, and c:



a



b



c

by method of Streefkerk et al.[4] showed that a:b:c = 6:37:57.

TABLE 1 COUPLING CONSTANTS FOR THE SKELETAL PROTONS ON THE GLUCOSE MOIETY OF LD AND DD.≠

	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
1 (LD)	3.5	9.5	8.9	10.1	5.1	2.4	-11.8
					2.9*	2.9*	----*
2 (DD)	3.7	9.4	8.9	10.0	5.4	2.4	-11.7

≠ All coupling constants were measured at 45°($CHCl_3$) on a Varian XL-200 instrument, except the ones with * , which were measured at -30°. Under the latter conditions, $J_{6,6'}$ was not observable.

Temperature dependence studies of the chemical shifts for the skeletal protons on the glucose moiety of LD and DD showed that 1-OCH_3, H-3 and H-5 have identical chemical shifts in both compounds while a small but consistent upfield shift of H-1 and H-2(0.03 and 0.05 ppm respectively) were observed for DD, presumably resulted from shielding effect of the phenyl ring. For LD, the chemical shifts for H-6 and H-6' separated by 0.13 ppm at 45° merge at -30° when $J_{5,6}$ and $J_{5,6'}$ become 2.9 Hz, indicating some kind of conformational changes. This was absent in DD, which showed a uniform temperature dependence for all protons throughout the temperature range.

A comparison of the ^{13}C chemical shifts for LD and DD revealed significant upfield shifts of the carbonyl carbon(5.67 ppm), C_α(1.02 ppm), C_β(4.15 ppm) and C_γ(2.69 ppm) in DD, as a result of possible shielding through "steric crowding" of these atoms in the compact and folded structure of DD.[5] (Table 2)

The motional freedom of the phenylalanine side chain in LD was most obvious in the ^{13}C spin-relaxation time studies. (Table 3) The T_1 values for all the carbon nuclei of DD ranged from 0.11(for the carbonyl carbon) to 0.52(for one of the O-CH_3's) of those on LD, the most prominent differences being those of the entire amino acid side chain. This provides strong support for the possible free rotation of the phenyl ring of LD along the C_γ----C_ξ axis, as well as rotation of the side chain around C_α---C_β. The overall data are consistent with a rather rigid and folded conformation for DD which tumbles as an entirety with little relative motion of the amino acids and the carbohydrate moieties while LD has segmental movements of the two limbs independent of each other. The significance of the NH---$O_{pyranose}$ hydrogen bond in restricting conformational changes of the molecules is further demonstrated by comparing the T_1's of LD with those of methyl-(O-6-phenylpropanoyl-2,3,4-tri-O-methyl)-α-D-glucopyranoside (5) which does not have the hydrogen bond. A further increase of T_1 of 10% to 162% for the phenylpropanoyl side chain was observed.

TABLE 2 ^{13}C CHEMICAL SHIFTS[a] OF LD(1) AND DD(2) COMPARED TO PHENYLALANINE METHYL ESTER(3) AND METHYL-(2,3,4-TRI-O-METHYL)-α-D-GLUCOPYRANOSIDE(4)

	LD	DD	LD - DD	3	4
C=O	174.19	168.52	+5.67	174.87	
C_α	55.14	54.12	+1.02	52.38	
C_β	40.30	36.15	+4.15	37.80	
C_γ	136.51	133.82	+2.69	135.61	
C_δ	128.88	129.53	-0.65	129.30	
C_ε	128.14	128.80	-0.66	128.78	
C_ζ	126.41	127.53	-1.12	127.26	
C-1	96.91	97.12	-0.21		97.45(98.16)[b]
C-2	81.25	81.27	-0.02		81.77(82.58)
C-3	82.99	83.26	-0.27		83.33(84.28)
C-4	79.27	79.09	+0.18		79.53(80.61)
C-5	63.28	64.78	-1.50		61.79(70.98)
C-6	68.03	67.99	+0.04		70.58(72.41)
1-OCH_3	54.73	55.21	-0.48		55.10(55.24)
2-OCH_3	58.51	58.77	-0.26		58.97(58.38)
3-OCH_3	60.38	60.68	-0.30		60.80(60.68)
4-OCH_3	60.04	60.51	-0.47		60.49(60.49)

[a] Determined at 50.309 MHz in $CDCl_3$, values in ppm relative to $(CH_3)_4Si$.

[b] Values in parentheses were reported by Haverkamp et al.[3] at 25.2 MHz for solutions in acetonitrile-d_3.

In conclusion, the nmr studies of the aminoacyl esters LD and DD have demonstrated significant conformational differences as governed by the intramolecular hydrogen bonding and the ester bond. Both LD and DD were quite easily hydrolysed in aqueous solutions, which may be why such natural 6-O-aminoacyl hexoses have not been isolated from nature. Nevertheless, formation of such esters of primary alcohols, albeit short-lived, should be reasonably feasible in nature. Common hexoses such as D-galactose, D-mannose can also participate in similar interactions as long as a free 6-hydroxy group is available. If one pictures the occurrence of the interaction on or within the primordial membrane with surfactant-like lipids, the methylated glucoside model would be even closer to reality than the unprotected one. Such differences in steric interactions of amino acids and carbohydrates may have some bearing on the co-evolution of L amino acids and D sugars in biological systems. Although large number of glycoproteins have been isolated, the function of carbohydrates on glycoproteins remains obscure. It is also known that L and D amino acids have different tastes.[8] The model described might also give some insight as to the function of the carbohydrate moiety of glycoproteins on cell membranes in terms of steric recognition in metabolism and chemoreception.

TABLE 3 ^{13}C SPIN-LATTICE RELAXATION TIME (T_1)[a] FOR LD,[b] DD[c] AND METHYL-(6-O-PHENYLPROPANOYL-2,3,4-TRI-O-METHYL)-α-D-METHYLGLUCOPYRANOSIDE(5)

	LD	DD	5	DD/LD	5/LD
C=O	9.09(0.75)[d]	1.21(0.777)	12.6	0.13	1.39
C_α	1.19(1.744)	0.19(1.572)	1.3	0.16	1.09
C_β	0.61(1.84)	0.19(1.931)	1.16	0.31	1.90
C_γ	12.94(0.619)	1.49(0.69)	19.7	0.11	1.52
C_δ	1.50(1.538)	0.35(1.595)	3.56	0.23	2.37
C_ε	1.49(1.478)	0.37(1.664)	3.90	0.25	2.62
C_ζ	0.96(1.633)	0.24(1.306)	1.38	0.25	1.44
C-1	0.89(1.80)	0.23(1.54)	0.97	0.26	1.09
C-2	0.79(1.862)	0.18(2.369)	0.88	0.23	1.11
C-3	0.81(1.914)	0.16(1.704)	0.86	0.19	1.06
C-4	0.77(1.892)	0.23(1.542)	0.81	0.30	1.05
C-5	0.43(2.028)	0.13(1.339)	0.49	0.31	1.13
C-6	0.74(1.932)	0.25(1.775)	0.82	0.34	1.11
1-OCH_3	2.19(1.396)	1.03(1.803)	2.33	0.47	1.06
2-OCH_3	3.20(1.018)	1.69(2.064)	3.36	0.52	1.05
3-OCH_3	2.81(1.173)	1.09(1.692)	3.05	0.40	1.08
4-OCH_3	2.97(1.118)	1.01(1.647)	2.97	0.33	1.00

[a] Determined at 50.309 MHz in $CDCl_3$ using saturation-recovery method,[6] recorded as seconds. A second determination using inversion-recovery method[7] gave essentially the same values.

[b] LD = Methyl-(6-O-L-phenylalanyl-2,3,4-tri-O-methyl)-α-D-glucopyranoside.

[c] DD = Methyl-(6-O-D-phenylalanyl-2,3,4-tri-O-methyl)-α-D-glucopyranoside.

[d] Values in parentheses are NOE factors.

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