THEORETICAL STUDIES ON THE BINDING ORIENTATIONS OF METHYL-β-D-GLUCOPYRANOSIDE AND METHYL-β-D-N-ACETYLGLUCOSAMINE TO CONCANAVALIN A.

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ABSTRACT

The possible modes of binding of methyl-β-D-glucopyranoside and methyl-β-D-N-acetyl-glucosamine to Concanavalin A have been determined using theoretical methods. While the former can align in three different modes in the carbohydrate binding site of Con A, the latter can align in only one mode. Hence, it is suggested that β-D-N-acetylglucosamine residue, either in simple glycosides or as a terminal residue in an oligosaccharide, may assume a similar orientation to interact with the combining site of Con A.

INTRODUCTION

CONCANAVALIN A (Con A), a plant protein, isolated from the jack bean belongs to the class of lectins. The unusual effects exhibited by this protein are related to its sugar binding properties. It binds to terminal glucose[1,2] and terminal[3] and internal mannose[4] residues of oligosaccharides and interacts preferentially with α-anomers[5]. Hence, it is widely used for the identification and isolation of cell surface carbohydrates[6]. The detailed knowledge of the binding mechanism of carbohydrates to Con A is important to understand its usefulness as a structural probe. Brewer and coworkers[7], using NMR technique, suggested the three-dimensional binding orientations of methyl-α-D-glucopyranoside (α-MeGlcP) and methyl-β-D-glucopyranoside (β-MeGlcP) with respect to the protein bound M_n^{++} only. Though NMR data, indicated two binding orientations for β-MeGlcP, these authors considered only one, while explaining the weak activity of β-MeGlcP over that of α-MeGlcP. Attempts have also been made using x-ray diffraction studies to determine the structure of Con A-sugar complex[8,9]. Owing to the experimental difficulties, these studies could not provide any information on the mode of binding of sugars to Con A. However, these studies helped in locating the approximate carbohydrate specific binding site of Con A. In this paper we report the theoretically determined possible binding orientations of β-MeGlcP and methyl-β-D-N-acetylglucosamine (β-MeGlcNAcP) to Con A. The latter compound is particularly interesting, since the glycopeptides with β-D-N-acetyl-glucosamine (β-D-GlcNAcP) as the non-reducing terminal residue exhibited very high binding affinity to Con A[10].

METHOD OF CALCULATION

The coordinates of various atoms of Con A were taken from the Protein Data Bank[8]. To model the active site of Con A, 70 amino acid residues of the protein which fall within 20 Å around the reference point (approximate centre of the pyranose ring in the active site of Con A) were considered. Sugars in $^4C_1$ (D) conformation[11,12] were placed in the carbohydrate specific binding site of Con A and were rotated through all the possible orientations using the rigid body rotation method[13]. Contact criteria[14] were used to identify the stereochemically allowed orientations. Then a search was made for possible non-covalent interactions such as hydrogen bonds and hydrophobic interactions between the sugar and the protein. Rotations through the exocyclic bonds of the sugar and translations from the initial reference point of the sugar were also considered in fitting sugars in the cabohydrate specific binding site of Con A. While fitting the sugar, the positions of the atoms of the side chains of protein (except those which are involved in the formation of coordination bonds or in dimerization) beyond C^β were also varied.

RESULTS AND DISCUSSION

The allowed regions for β-MeGlcP and β-MeGlcNAcP in the binding site of Con A are shown in figures 1 and 2. It can be seen that β-MeGlcP may be placed in three different regions of ϕ, θ and ψ space (figure 1), whereas β-MeGlcNAcP may be placed in only one region. Very few allowed points in figures 1

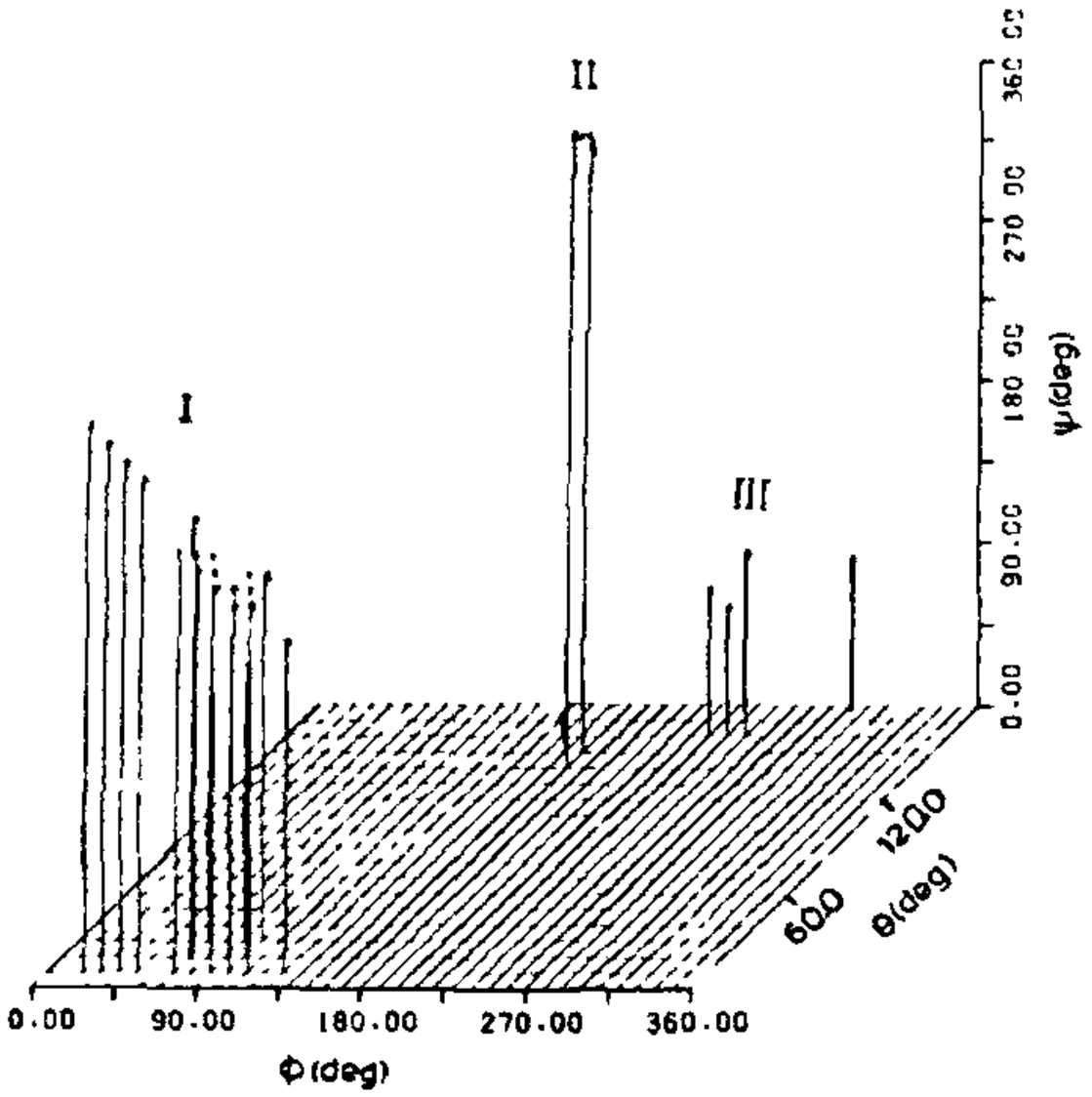

Figure 1. Steric map showing the allowed regions for methyl-β-D-glucopyranoside in the active site of Con A. Each of the allowed orientation is indicated by a dot '.'. The line joining the dot and a grid point gives the value of the angle ψ, whereas the point on the grid gives the values of the angles ϕ and θ.

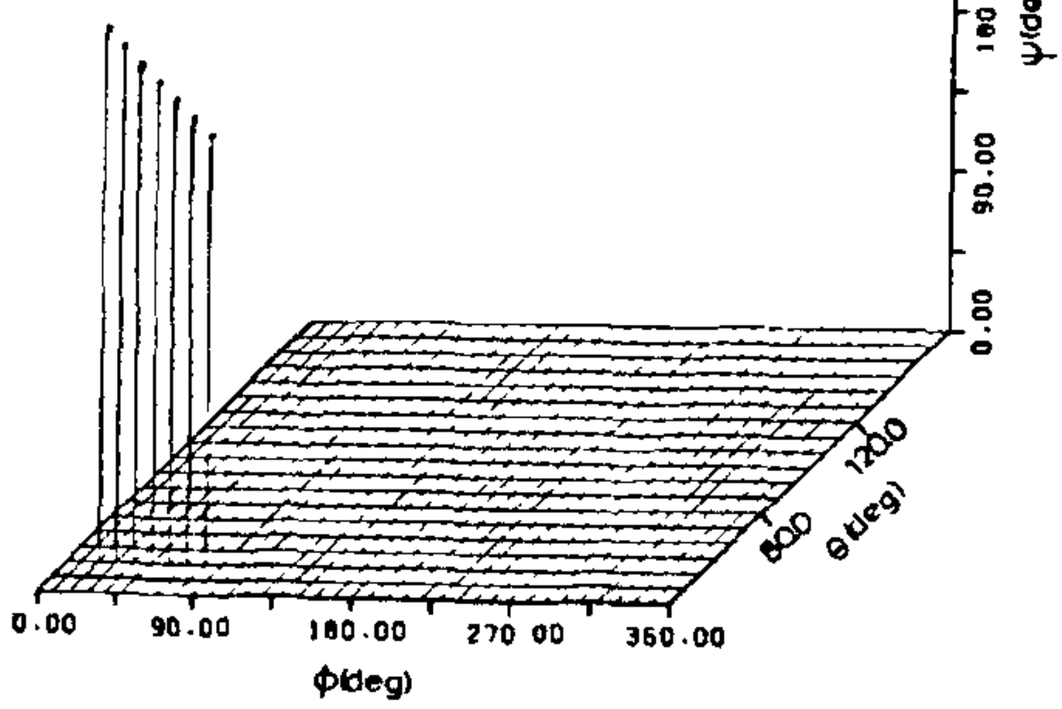

Figure 2. Steric map showing the allowed region for methyl-β-D-N-acetylglucosamine in the active site of Con A.

and 2 suggest that the allowed orientations of these sugars to reach the active site are very much limited. The region-I at the lower left hand bottom corner of the grid (figure 1) is relatively large and hence the most probable one for β-MeGlcP to reach the binding site. However, the existence of regions II and III indicates that β-MeGlcP can also bind to some extent in orientations corresponding to these regions also. In regions II and III the sugar reaches the binding site of Con A in inverted orientations (figures 3b, 3c) when compared to its orientations in region-I (figure 3a). In regions II and III the orientations of sugar are very similar. Thus, these results suggest more than one possible mode of binding for β-MeGlcP to bind to Con A which is consistent with NMR data but are in disagreement with the conclusions of Brewer *et al*[7]. Though NMR data gave an indication of two different binding orientations for β-MeGlcP, Brewer *et al* thought that it resulted from the inability to calculate unique distances for the C-3 and C-5 atoms of the pyranose ring from Mn^{++}, because of the overlap of their resonance signals.

In region I, the 0–2 hydroxyl of the sugar may, in some places, form a hydrogen bond with Leu(99). Two of the three amino acid residues, Leu(99), Tyr(100) and Asp(208) of the protein are always involved in the formation of good hydrogen bonds with the 0–3 hydroxyl of β-MeGlcP in region I. In fact, the 0–3 hydroxyl of β-MeGlcP may be a potential site of attachment for the protein. The 0–4 hydroxyl, in some orientations may form a weak hydrogen bond with Asp(208) and in other orientations with Tyr(12). The 0–1 and 0–6 hydroxyls may not be involved in the formation of good hydrogen bonds.

It is interesting to note that the substitution of the acetamido group at C-2 atom of β-MeGlcP further restricts the allowed region (figures 1 and 2). The presence of the finite allowed region in figure 2 suggests that β-GlcNAcP may also bind in the carbohydrate specific combining site of Con A (figure 4) and it can align in only one way in the binding site. This suggests that β-GlcNAcP residue, either in simple glycosides or as a terminal residue in an oligosaccharide, may assume a similar spatial orientation to interact with the combining site of Con A. The hydrogen bonding scheme for β-MeGlcNAcP is similar to that of β-MeGlcP in region I.

The 0–1 atom of both the sugars points outside onto the surface of the lectin and hence permits the extension of carbohydrate chain. There is enough evidence that the carbohydrate binding site of Con A may accommodate only one sugar residue[15]. When β-D-

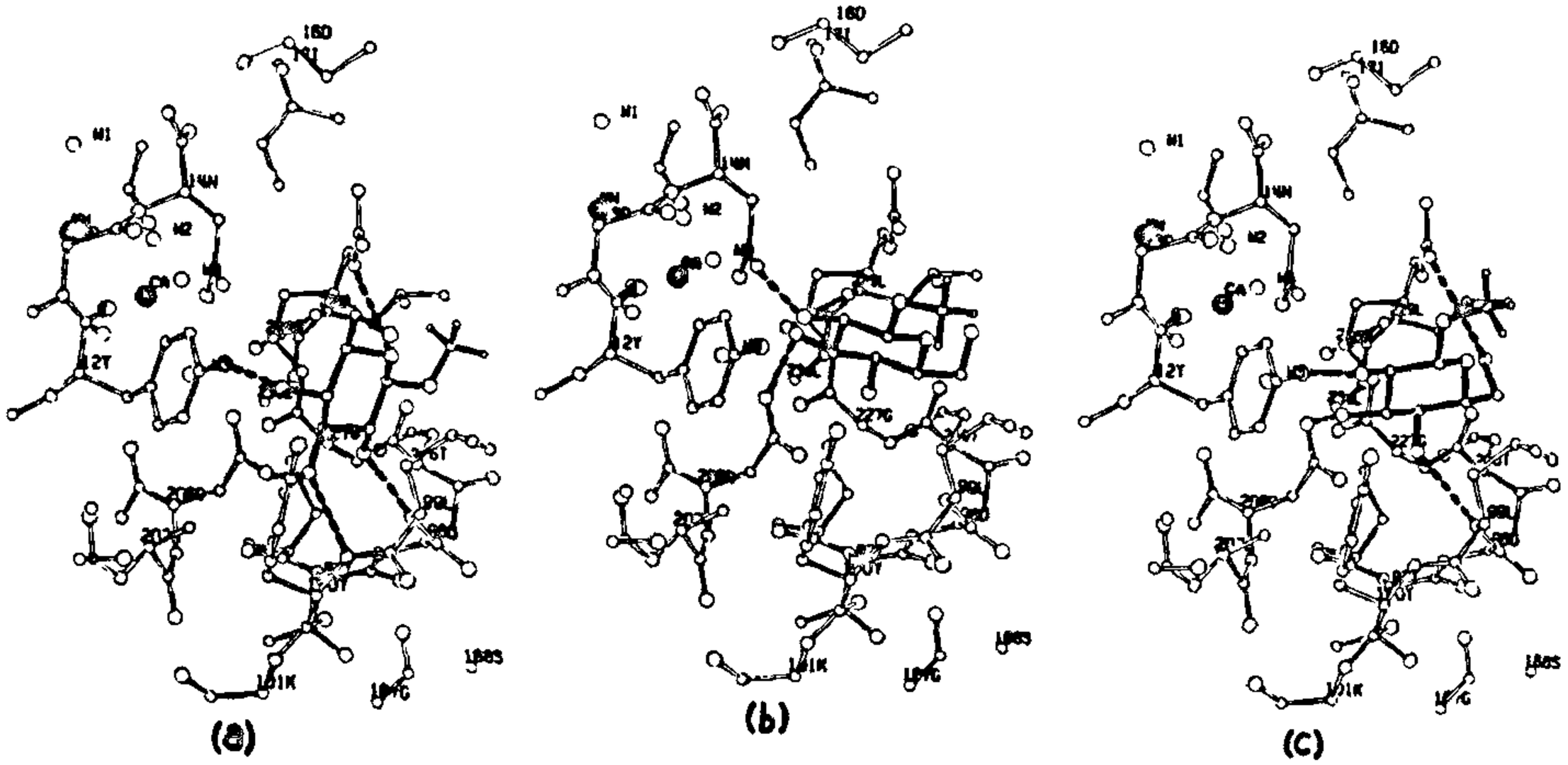

Figure 3. Projection of Con A-methyl-β-glucopyranoside complex in **a**. region-I ($\phi = 70$, $\theta = 20$, $\psi = 220$), **b**. region-II ($\phi = 160$, $\theta = 150$, $\psi = 20$), **c**. region-III ($\phi = 230$, $\theta = 160$, $\psi = 80$).

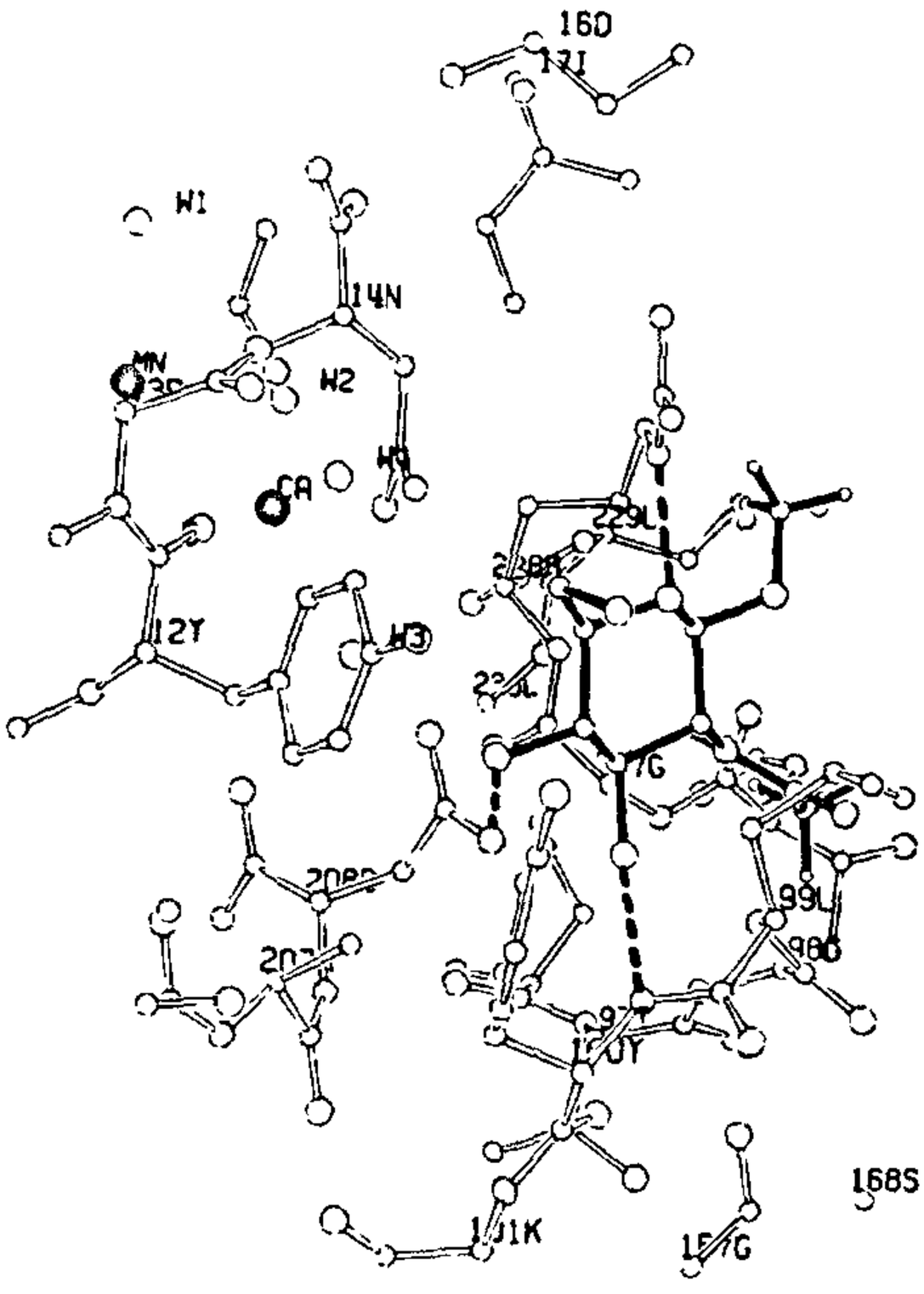

Figure 4. Projection of Con A-methyl-β-N-acetyl-glucosamine complex in one of the allowed orientations ($\phi = 70$, $\theta = 20$, $\psi = 250$).

GlcNAcP reaches the sugar binding site, the spatial orientation of the rest of the oligosaccharide (for which β-D-GlcNAcP is a non-reducing terminal residue) with respect to the protein depends on the shape of the molecule which in turn depends on the type of linkages in the oligosaccharide[16]. Hence, depending on the linkages the oligosaccharides with the same terminal sugar residue may align differently with respect to the protein. Such an alignment may lead to either favourable or unfavourable interactions and in the latter case the protein has to induce conformational changes in the oligosaccharide for proper alignment. Because of highly restricted number of allowed orientations, the high rigidity of the pyranose ring[17] and the limited freedom of rotation of the ring about the interunit glycosidic bonds[16], the protein has to spend large amounts of energy to induce any conformational change in the oligosaccharide. Hence, the binding potency of oligosaccharides to Con A depends not only on the nature of the terminal sugar residue but also on the overall shape of the oligosaccharide. This perhaps explains the fact that lectins with identical specificity with respect to the monosaccharides differ in their interactions with the oligosaccharide moieties of glycoconjugates[18] and thereby with cells.

This work was partially supported by the Department of Science and Technology, New Delhi.

13 January 1984

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NATURE OF CHLOROPHENOXY DERIVATIVES OF NIOBIUM (V)

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ABSTRACT

Compounds of composition $NbCl_n(OC_6H_4Cl)_{5-n}$, $n = 0 \rightarrow 4$, have been prepared by reacting niobium (V) chloride with *O*-chlorophenol in predetermined molar ratios in carbon tetrachloride. Based upon elemental analysis, conductance, infrared, magnetic and thermogravimetric analysis, their structures have been proposed.

INTRODUCTION

MALHOTRA and Martin[1] have recently reviewed physico-chemical properties and uses of metal phenoxides. A number of *bis* (substituted phenoxo) titanium (IV) complexes have been prepared and characterised by Harrord and Taylor[2]. Though compounds of composition $VCl_2(OC_6H_4Cl)_2$ and $VCl_2(OC_6H_4NO_2)$ with *O*-chloro and nitrophenol respectively have been isolated, the product obtained from VCl_4-phenol mixture has not been well characterised[3]. A series of *bis* (aryloxo) iron (II) complexes of the type $Fe(OC_6H_4X)_2.(bipy)_n$ have also been prepared and characterised[4]. In the present studies we report the synthesis of chlorophenoxy derivatives of niobium (V) and propose a possible structure on the basis of elemental analysis, molar conductance, TGA and magnetic and infrared spectral studies.

EXPERIMENTAL

O-Chlorophenol (AR) was purified by distillation under reduced pressure. Niobium pentachloride was of Fluka, analytical grade and was used as such without further purification. The organic solvents used were of AnalaR grade and were dried by appropriate drying agents, distilled and finally preserved over activated molecular sieves.

Compounds of composition $NbCl_4(OC_6H_4Cl)$ and $NbCl_3(OC_6H_4Cl)_2$ were obtained by stirring a suspension of niobium pentachloride with *O*-chlorophenol