CONFORMATION OF PYRANOSE RING IN MONO, DI- AND POLYSACCHARIDES BY NUCLEAR MAGNETIC RESONANCE*

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ABSTRACT

Nuclear magnetic resonance studies on the determination of pyranose ring conformation in mono, di- and polysaccharides have been briefly reviewed. These data have been compared with the recent theoretical studies and the limitations of Karplus equation for the precise determination of dihedral angles in carbohydrates are mentioned.

Key Words : Conformation, NMR, Mono-saccharides, Di-saccharides, Polysaccharides.

INTRODUCTION

Conformational studies of six-membered rings dates back to the work of Sachse [1] in 1890, who pointed out that six-membered rings (cyclohexane) can exist in two puckered forms, the flexible or unsymmetrical form (skew or boat) and the rigid or symmetric form (chair) (Fig. 1). About forty years later Haworth (1929) [2] extended these ideas to the field of carbohydrates. Twenty years later, proper experiments [3] were designed to study the conformation of simple sugars and their derivatives in solution. Haworth pointed out that each pyranose sugar is capable of existing in several strainless conformations and noted the possibility of two chair and six boat conformation (Fig. 2) for each sugar, *i.e.*, *D* or *L*. It can be seen with the help of models that all these conformations can be realized by making rotations about the bonds in the ring, without being broken. The word conformation was first coined by Haworth to describe the various shapes of molecules. This word is now commonly used to describe the different spatial arrangements of atoms or atomic groups produced by rotations about the bonds, without

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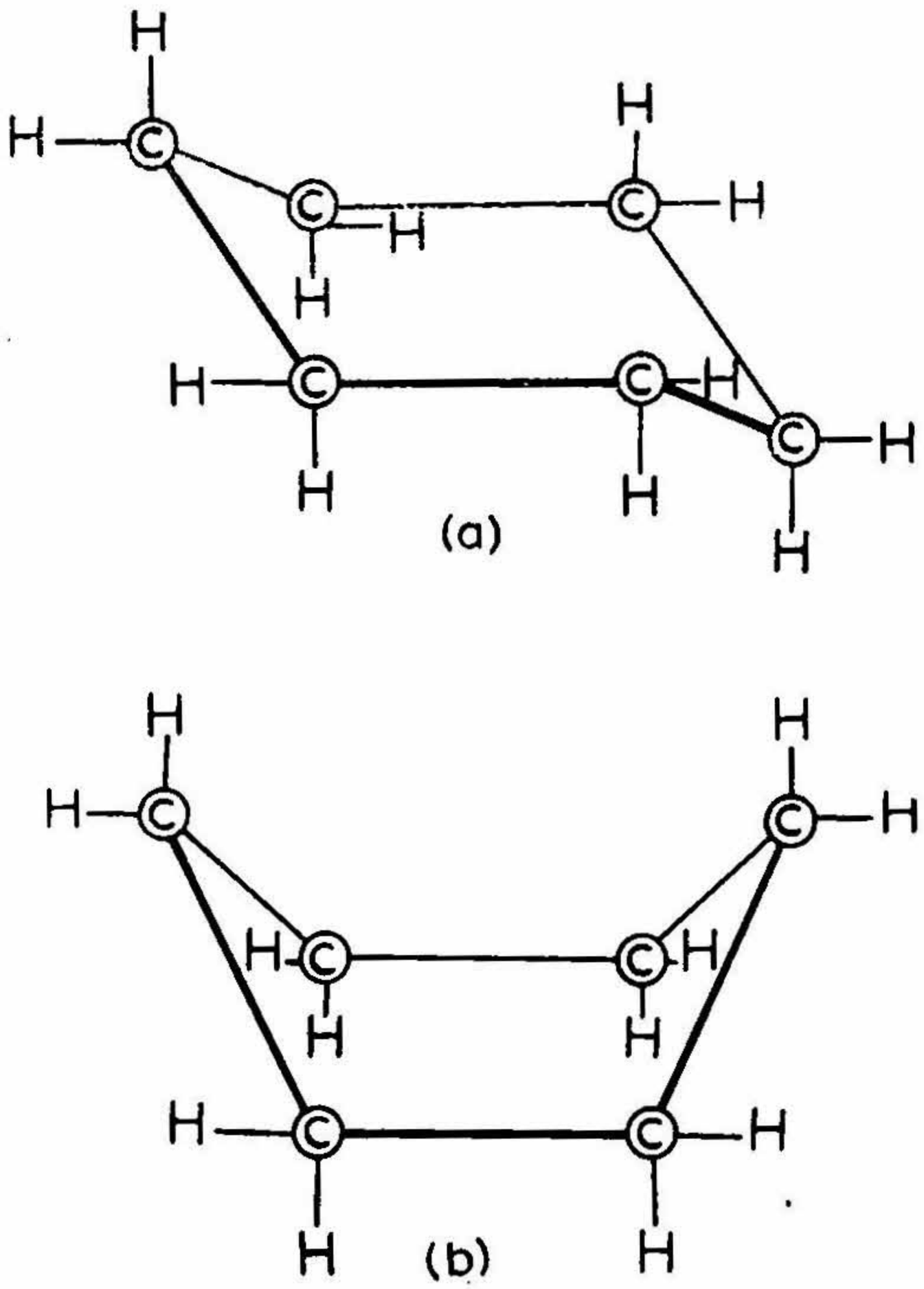


FIG. 1. (*a*) Chair and (*b*) boat conformations of cyclohexane.

the bonds being broken. The two chair conformations were designated by Reeves [4] as C1 and 1C and boat conformations as B1, B2, B3, 1B, 2B and 3B (Fig. 2). In the chair conformation (Fig. 3) alternate ring atoms C (1), C (3), C (5) and C (2), C (4) and O lie in two parallel planes. When the plane containing the oxygen is above the other, the numbering of atoms is in clockwise direction for C 1 (*D*) conformation and *vice versa* for 1C (*D*). The bonds extending from these carbon atoms are termed equatorial, when they are approximately inplane and when nearly perpendicular they are termed axial. The various arrangements of the side groups in C1 and 1C conformations for various aldopyranoses are shown in Tables I and II. The two chair forms differ not only in the spatial arrangements of the ring atoms, but also in the orientations of the side groups. The interconversion from one chair form to another changes the equatorial substituents to axial position or *vice versa*. Similarly the orientations of C—H

bonds and their relative orientations with respect to hydrogens on the adjacent carbons also change during this interconversion. For example, in the case of β-*D*-glucose, the hydrogens at C (1), C (2), C (3), C (4) and C (5) atoms are in axial orientations (Fig. 3). In 1C conformation they are in equatorial orientation. The relation between adjacent C—H bonds are in axial-axial orientation in C1 (*D*) whereas in 1C (*D*), they are in equatorial-equatorial orientation. These changes may affect the chemical shifts and also the vicinal coupling constants of the protons attached to the ring carbon atoms. Hence Nuclear Magnetic Resonance spectroscopy is an ideal method for the determination of ring conformation in mono-, oligo- and polysaccharides in solution. In the *D* series, C1 is the most prevalent form.

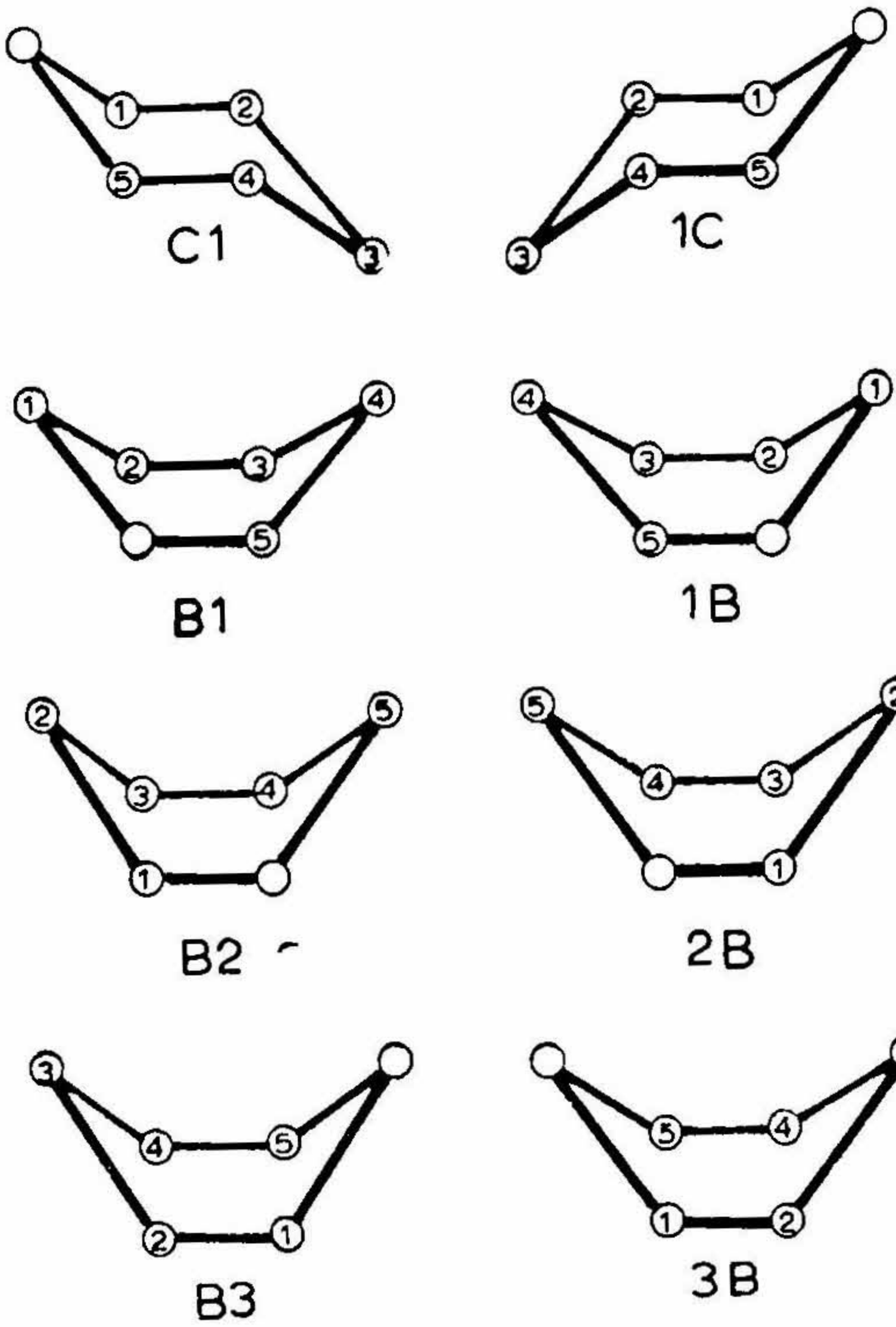
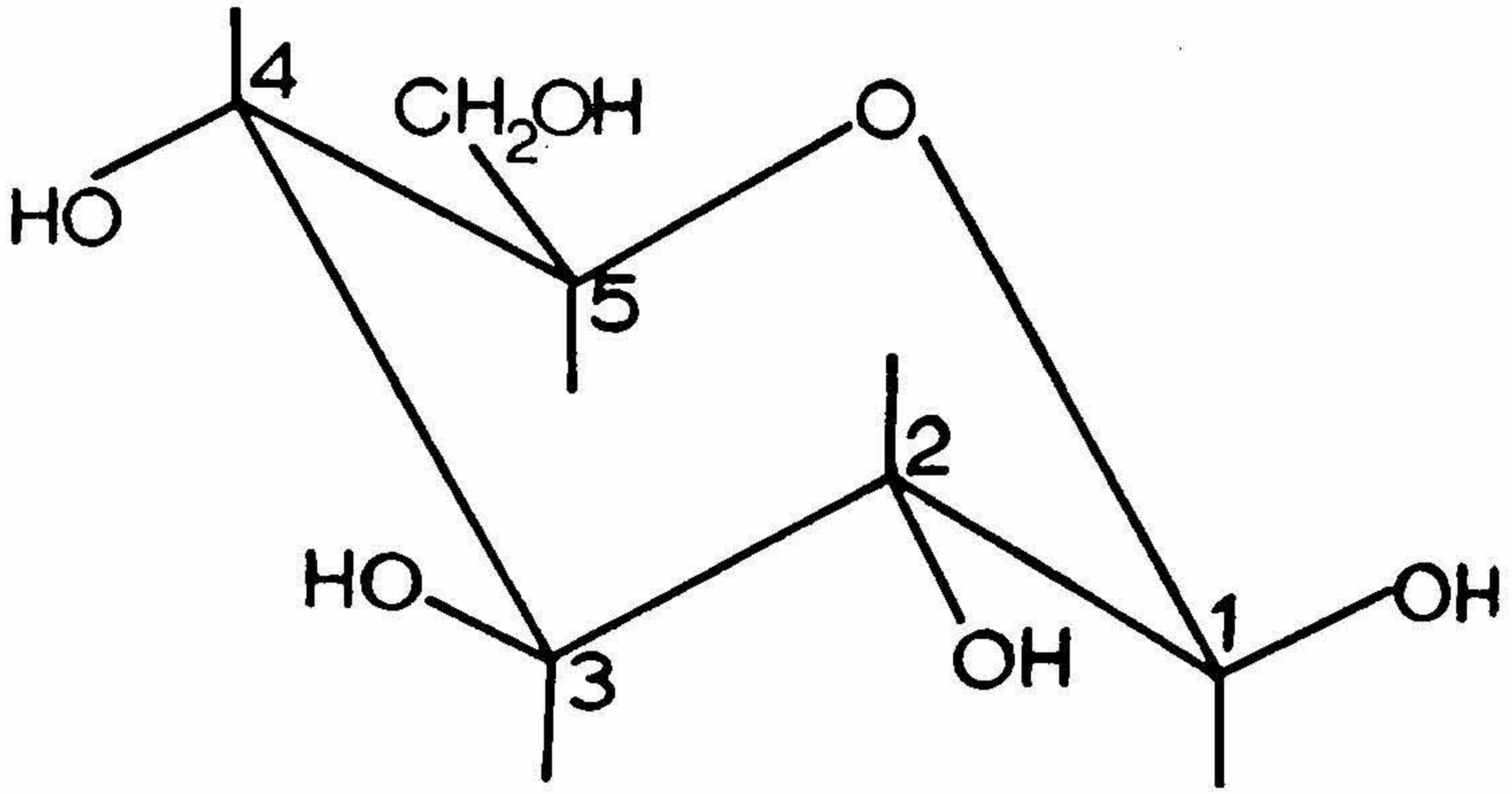


FIG. 2. The eight pyranose ring conformations.



C 1

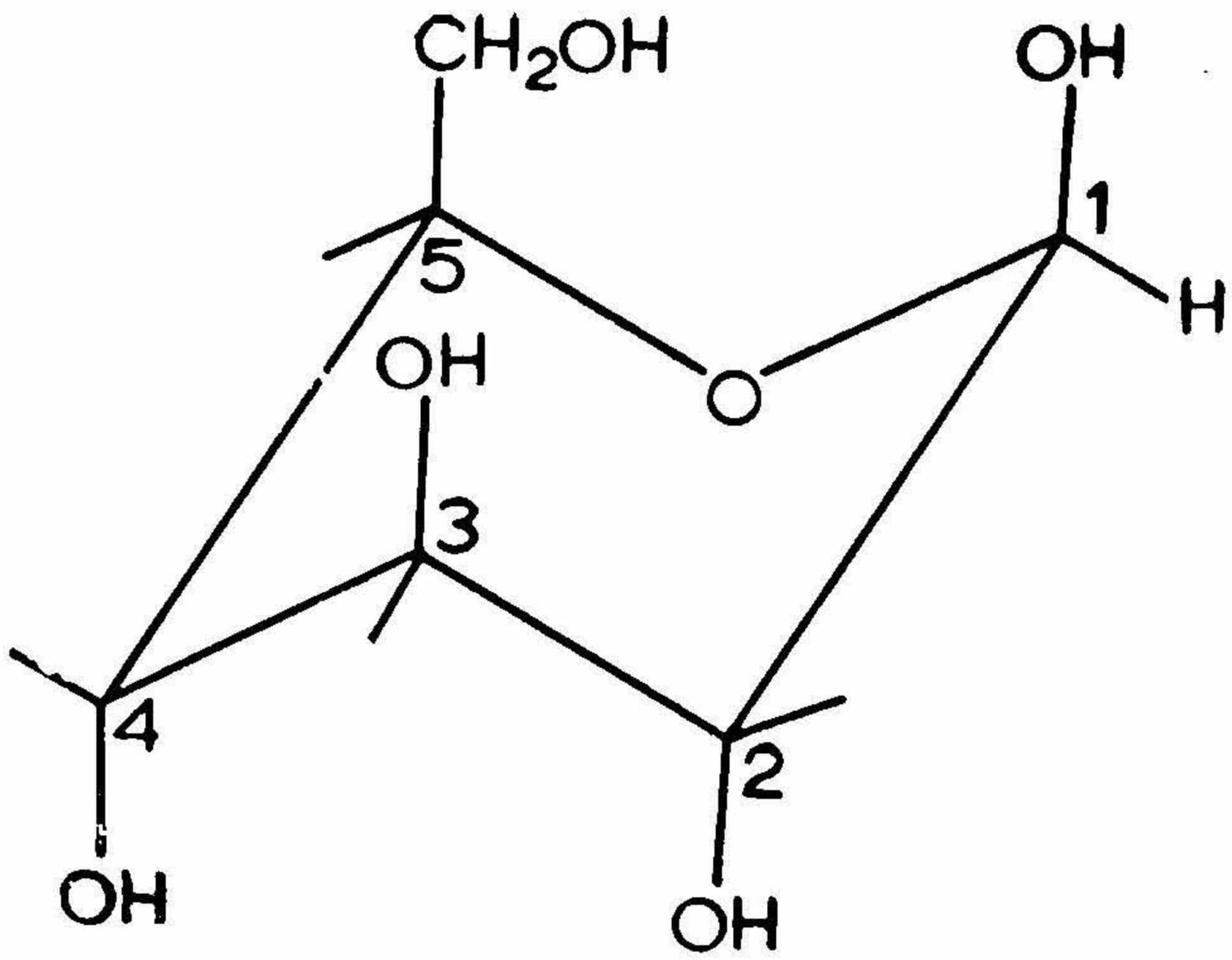


FIG. 3. C1 and 1C conformations of *β-D*-glucose.

TABLE I

Orientations of —OH and —CH_2OH groups in C1 and 1C conformations for Hexoses

Name of Compound	C1					1C				
	Hydroxyl group at				CH_2OH	Hydroxyl group at				CH_2OH
	C_1	C_2	C_3	C_4		C_1	C_2	C_3	C_4	
α-D-Allose	A	E	A	E	E	E	A	E	A	A
β-D-Allose	E	E	A	E	E	A	A	E	A	A
α-D-Altrose	A	A	A	E	E	E	E	E	A	A
β-D-Altrose	E	A	A	E	E	A	E	E	A	A
α-D-Galactose	A	E	E	A	E	E	A	A	E	A
β-D-Galactose	E	E	E	A	E	A	A	A	E	A
α-D-Glucose	A	E	E	E	E	E	A	A	A	A
β-D-Glucose	E	E	E	E	E	A	A	A	A	A
α-D-Gulose	A	E	A	A	E	E	A	E	E	A
β-D-Gulose	E	E	A	A	E	A	A	E	E	A
α-D-Idose	A	A	A	A	E	E	E	E	E	A
β-D-Idose	E	A	A	A	E	A	E	E	E	A
α-D-Mannose	A	A	E	E	E	E	E	A	A	A
β-D-Mannose	E	A	E	E	E	A	E	A	A	A
α-D-Talose	A	A	E	A	E	E	E	A	E	A
β-D-Talose	E	A	E	A	E	A	E	A	E	A

A denotes axial orientation.

E denotes equatorial orientation.

Since the *L*-series are mirror images of the former, 1C conformation is favoured in most cases. Since boat and skew conformations are very rare, a detailed discussion of these is omitted here.

Empirical rules to assign the favoured conformations

High resolution NMR spectroscopy has been widely used to study the structural problems in the field of carbohydrates, since the pioneering studies of Lemieux [5] on sugar acetates.

TABLE II

Orientations of —OH groups in C1 and 1C conformations of Pentoses

Name of Compound	C1				1C			
	Hydroxyl group at				Hydroxyl group at			
	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4
α-*D*-Arabinose	A	A	A	E	E	E	E	A
β-*D*-Arabinose	E	A	A	E	A	E	E	A
α-*D*-Lyxose	A	A	E	E	E	E	A	A
β-*D*-Lyxose	E	A	E	E	A	E	A	A
α-*D*-Ribose	A	E	A	E	E	A	E	A
β-*D*-Ribose	E	E	A	E	A	A	E	A
α-*D*-Xylose	A	E	E	E	E	A	A	A
β-*D*-Xylose	E	E	E	E	A	A	A	A

A denotes axial orientation.
E denotes equatorial orientation.

The first observations or empirical rules which have continued to be used in assigning favoured conformations are: (*i*) Equatorially oriented protons (usually) appear at lower fields compared to the chemically similar but axially oriented protons. (*ii*) The coupling constant between protons in axial-axial orientation is about two to three times greater than either axial-equatorial or equatorial-equatorial protons.

The difference in chemical shifts between axial and equatorial protons has its origin in a long range shielding effect (σ) associated with the diamagnetic anisotropy of carbon-carbon single bonds. The long range shielding due to carbon-carbon or carbon-oxygen bonds can be expressed as [6],

$$\sigma_{ave} = \frac{(3\cos^2\theta - 1)}{3r^3}(X_L - X_T) \qquad (1)$$

where r is the distance from the centre of the bond to the proton, X_L and X_T longitudinal and transverse susceptibilities. For example, in the case of cyclohexane the C (1)—C (2) and C (1)—C (6) bonds (Fig. 4) are symmetrically oriented with respect to protons at C (1) and will contribute equally

to the shielding of both. On the other hand, the C (2)—C (3) bond has different spatial relations with respect to the axial and equatorial protons attached to C (1) atom. Since $X_T > X_L$ for C—C bonds, using the above relation, it can be shown that C (2)—C (3) or C (5)—C (6) bond deshield the equatorial proton and shield the axial proton. Similarly C (3)—C (4) and C (4)—C (5) bonds also may shield these protons to different extent, but their contribution is quite small. In this treatment the carbon-hydrogen bonds were neglected because their diamagnetic susceptibility was considered to be too small to have a significant differential effect on the shielding of the equatorial and axial protons.

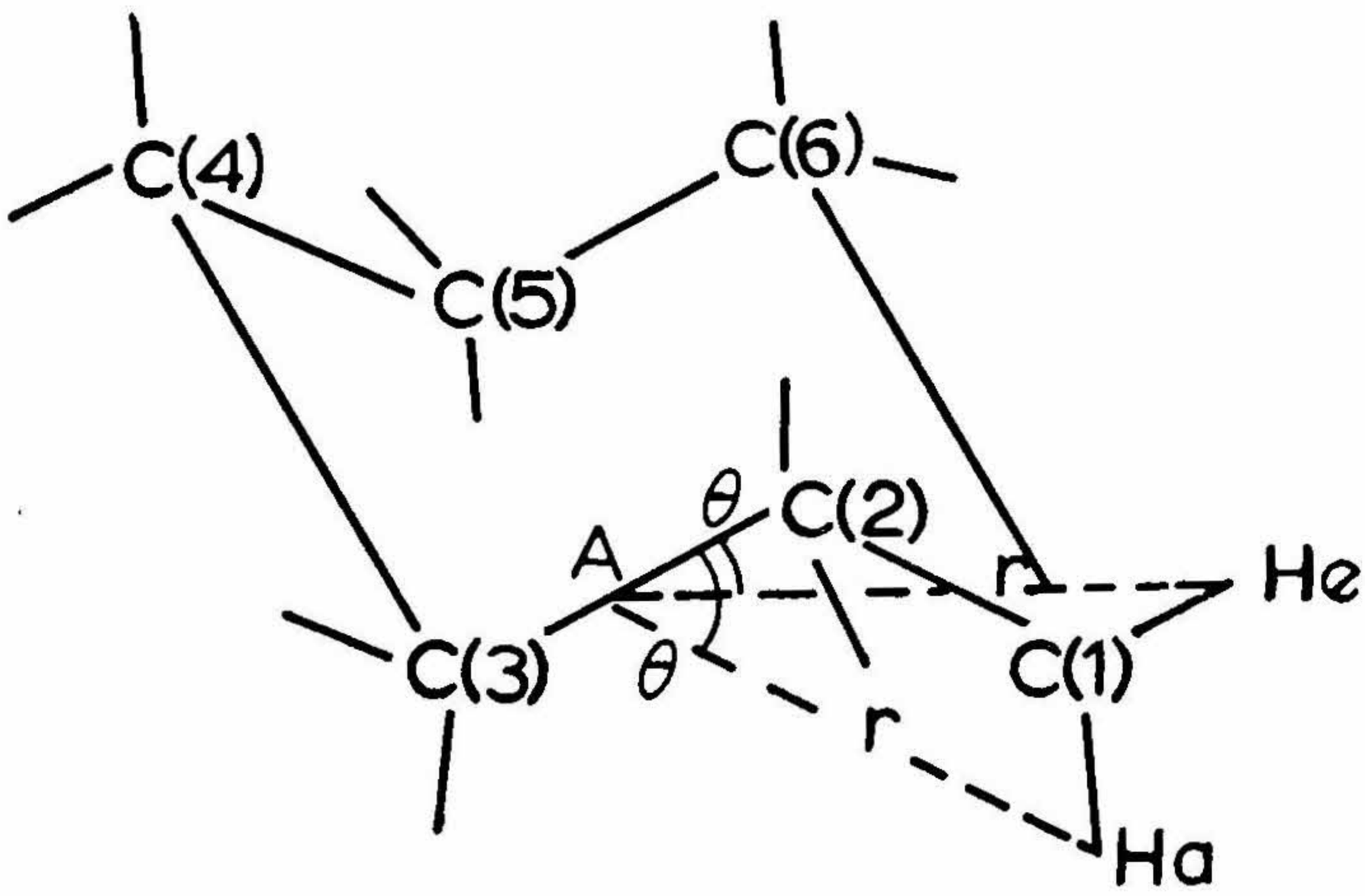


FIG. 4. Cyclohexane chair conformation. r is the distance between the centre (A) of the bond to the proton. θ is the angle which the line joining A and Ha or A and He makes with the C–C bond.

Lemieux and Stevens [7, 8] also formulated empirical rules for estimating long-range shielding that occur with changes in conformation and configuration of aldopyranoses and their derivatives. In the case of free sugars these correlations were made by using chemical shifts of *β-D*-xylose and *β-D*-glucose as reference points respectively for aldopentopyranoses and aldohexopyranoses; the chemical shift of any proton relative to the respective reference compound can be estimated using the following empirical rules;

1. For obtaining the chemical shift of an equatorially oriented proton, subtract 0·6 ppm (τ values).

2. For obtining the chemical shift of an axially oriented proton (*a*) subtract 0·3 ppm for each neighbouring axial —OH group and (*b*) subtract 0·35 ppm for each axial —OH group, which is in opposition to the axial proton.

Karplus [9, 10] provided the theoretical basis for the variation of the vicinal coupling constant ($J_{H,H}$) with dihedral angle (ϕ) between adjacent C—H bonds in saturated systems and showed that

$$J_{H,H} = A + B\cos\phi + C\cos 2\phi \tag{2}$$

where A, B and C are constants for a given system, and their values are 7·8, — 1·0 and 5·6 [11] respectively. Equation 2 has been modified as

$$J_{H,H} = (A + B\cos\phi + C\cos 2\phi)(1 - M\triangle X) \tag{3}$$

in order to take into account the electronegativity of the substituent; M is a constant and $\triangle X$ is the difference in the electronegativity of the substituent and hydrogen. A value of 0·1 has been assigned for M for studies in carbohydrates. The values for the coupling constants calculated by Durette and Horton [11] of various sugar derivatives are shown in Table III. It can be seen from the table that according to the theory the greater the number of electronegative substituents on the H—C—C—H unit, the smaller the vicinal spin coupling which is in agreement with experiments.

$$J_{4,5}(60°) > J_{3,4} \quad \text{or} \quad J_{2,3}(60°) > J_{1,2}$$
$$J_{4,5}(180°) > J_{3,4} \qquad J_{2,3}(180°) > J_{1,2}$$

The vicinal coupling constant tends to increase as the electronegativity of the R group decreases. It can also be seen from Table III, that the agreement between the calculated and observed values are very poor for $J_{1,2}(60°)$ and $J_{4,5}(60°)$ coupling constants. This is due to the fact that the vicinal coupling constants are found to be sensitive not only to the magnitude of electronegativity of the substituent but also to its configuration [12, 13] besides other factors. The vicinal coupling constant will be affected maximum when the electronegative group is trans-coplanar to one of the coupled protons. On the other hand, when the electronegative substituents are not in such a trans-coplanar relationship, with respect to the coupled protons, the magnitude of a vicinal, gauche coupling increases instead of decreasing. These points are illustrated in Fig. 5. It will be seen from the figure that

TABLE III

Calculated and observed coupling constants in 2, 3, 4-*tri*-o-*acetyl*-D-*aldopentopyranose derivatives of* α-D-*xylose**

Substituent at C (1)	Electronegativity of the substituent at C (1)	$J_{1,2}$		$J_{2,3}$ or $J_{3,4}$		$J_{4,5}$	
		60°	180°	60°	180°	60°	180°
OAc	3·72	2·32 (3·5)	7·42	2·68	8·58 (9·8, 9·6)	3·04 (5·5)	9·75 (11·6)
OMe	3·31	2·50 (3·5)	8·01	2·68	8·58 (9·3, 9·2)	3·04 (5·7)	9·75 (11·1)
Cl	3·25	2·53 (3·9)	8·09	2·68	8·58 (9·6, 9·5)	3·04 (5·7)	9·75 (12·1)
Br	2·96	2·66 (4·0)	8·51	2·68	8·58 (9·6, 9·5)	3·04 (5·8)	9·75 (11·5)

The values within brackets are observed J values.

• Data taken from ref. [11].

J^{ε} varies from 1 to 6 Hz depending on the configuration of the electronegative substituents. Until the effect of the configuration of the electronegative substituent on J is taken into account, the generalised Karplus equation 2 cannot be used for the precise determination of dihedral angles. In spite of these difficulties in estimating the dihedral angles precisely, the magnitudes of observed coupling constants, can often be employed to assign the favoured chair conformations since J (180°) is still much higher than J (60°).

Estimation of the components present in the equilibrium mixture

It is well known that when the energy difference between two forms of a particular molecule is small it may exist in conformational equilibrium. If the interconversion between the two forms is slow, on the NMR time scale, superimposed spectra of the two chair conformations would be obtained and from the area of the peaks the percentage of proportion of both conformations present may be estimated. Alternatively, if the interconversion is rapid, a time average spectrum would be observed. The observed (J_{obs}) coupling constant or the chemical shift (ν_{obs}) in the averaged

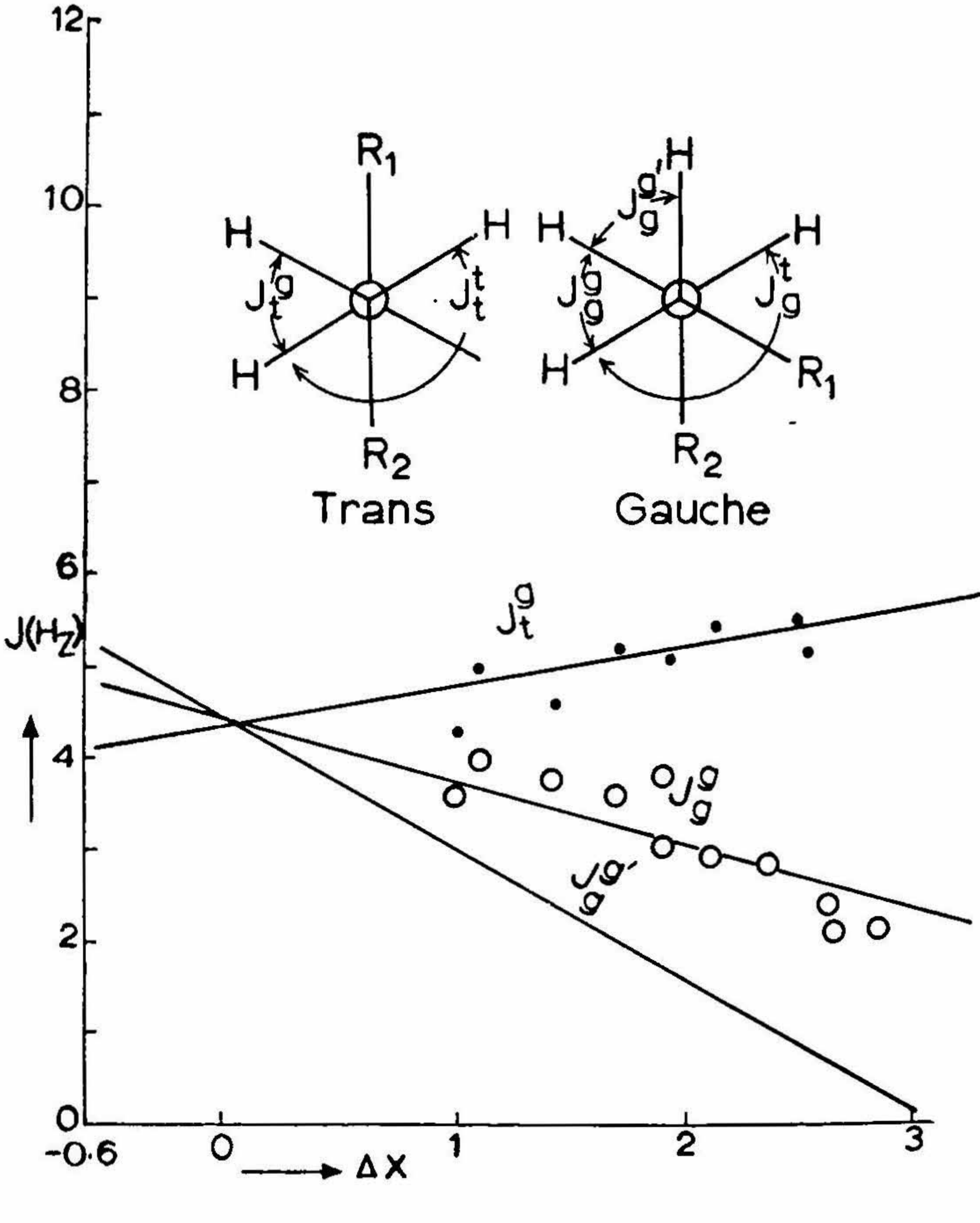


FIG. 5. Variation of the vicinal coupling constant (J) with ΔX the difference in the electronegativity of the substituents and the hydrogens for different configurations. The configuration of the protons is represented by the superscript and the substituents by the subscript. For example J_t^g denotes that the coupling protons are in ganche position and the substituents are in transposition. J_g^g and $J_g^{g'}$ denote that the coupling protons and substituents are in gauche position, but in the former only one of the coupling protons is transcoplanar with one of the substituents whereas in the latter both the coupling protons are transcoplanar with the substituents. (Data taken from Ref. [13]).

spectrum can be related to the mole fractions (N_1) of the C1 (*D*) form and the mole fraction N_2 of 1C (*D*) form by the equations

$$J_{obs} = N_1 J_1 + N_2 J_2 \tag{4}$$

$$\nu_{obs} = N_1 \nu_1 + N_2 \nu_2. \tag{5}$$

From the knowledge of J_1 and J_2 or ν_1 and ν_2 it is possible to estimate the mole fractions N_1 and N_2 from the observed J_{obs} or ν_{obs} in the equilibrium mixture. These values of N_1 and N_2 may be used to estimate the standard free energy difference ($\triangle G$) and the equilibrium constant (K).

$$\triangle G = - RT \ln K = - RT \ln \frac{N_2}{N_1} \tag{6}$$

It has been shown, both theoretically [14, 15] and experimentally [11, 16–18] that the proton-proton geminal coupling coupling constant $J_{5e,5a}$ in pentopyranose derviatives is also conformational dependent. $J_{5e,5a}$ in C1 (*D*) conformation is found to be numerically less than in 1C (*D*) conformation. The geminal coupling constant increases algebraically with an increase in the equilibrium amount of conformation in which O (4)—C (4)—C (5) plane bisects the H—C (5)—H angle.

Conformational energy calculations

Before going to discuss the results of NMR, a brief mention of conformational energy calculations will be made since we are going to compare the NMR data with theoretical studies to have a better idea about the stereochemistry of these molecules. As pointed out earlier, the various shapes of pyranose ring can be derived by making rotations about the bonds within the ring. Because of these rotations, the various side groups will be placed in different orientations in different conformations of the sugar. Hence the problem of ring conformations requires a knowledge of the forces that exist between atoms and atomic groups in the molecule. Hence a quantitative study can be made by calculating the potential energy of various possible conformations by using the appropriate expressions for different types of interactions. At present semi-empirical approaches [19–24] are most widely used for computing the energies of simple sugars and their derivatives. In these procedures the potential energy of a system is divided into several individual contributions, such as Van der Waal's energies (V_{nb}), electrostatic interactions (V_{es}), torsional strain (V_τ), hydrogen bond formation (V_{hb}), bond angle (V_θ) and bond length distorsion (V_l). Since the energy difference

between conformations is the determining factor for predicting relative stability, the study of absolute energy is not necessary. It is sufficient if the energy calculation takes into account the parameters which vary when going from one conformation to another. In the classical treatment [25] the conformational potential energy is expressed as the sum of the energies in the form

$$V = V_{nb} + V_{es} + V_r + V_{hb} + V_l + V_\theta$$

The details of the expressions used to calculate the above energies have been discussed in the literature [19, 25].

Because of low barrier to rotation about the C—O bond, the hydroxyl group is less rigid and the O—H bonds in sugar molecule can assume various orientations. If one wants to explain the experimental results on simple sugars the entropy that arises due to possible rotations of the side groups has to be computed in order to obtain free energy. If S is the conformational entropy which arises due to the possible orientations of the side groups, the free energy (G) of a conformation can be expressed as,

$$G = \Sigma\ N_i V_i - TS \tag{7}$$

where N_i is the mole fraction and V_i its potential energy.

Proton magnetic resonance studies on simple sugars

PMR spectra of α and β-*D*-glucose were obtained in D_2O at 220 MHz by Koch and Perlin [26] and these data are given in Tables IV and V. As we have noticed earlier, all the ring protons (Fig. 3) are axially placed for β-*D*-glucose in C1 conformation. Hence large values protens are expected for $J_{1,2}$, $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$. The observed values of 8·1–9·0 Hz for the above coupling constants are consistent with the C1 conformation for β-*D*-glucose. Similarly the values of the coupling constants observed for α-*D*-glucose are also consistent with C1 conformation. However, the observed values of $J_{2,3}$ and $J_{3,4}$ for α-*D*-glucose are higher than those of β-*D*-glucose. These differences in J values need not signify substantial differences in the ring conformations of α and β-*D*-glucopyranoses, since a small change in geometry (*i.e.*, of H—C—C bond angle) may have a large effect on the J value [9, 10]. Both X-ray [27] and theoretical studies [21] also do not predict any significant deviations from C1 (*D*) conformation, in both the cases. Similarly, as expected from the empirical rules [7] the protons at C (1), C (2), C (3) and C (5) atoms for α-*D*-glucose appear at

TABLE IV

*Chemical shifts (τ) for protons in glucose anomers dissolved in D_2O**

Compound	C–1	C–2	C–3	C–4	C–5	C–6
α-*D*-glucose	4·73	6·44	6·25	6·56	6·19	5·95; 5·88
β-*D*-glucose	5·37	6·71	6·41	6·55	6·48	6·09; 5·78

* Data taken from ref. [26].

TABLE V

*Proton coupling constants (Hz) for α and β, D-glucose in D_2O**

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
α, *D*-glucose	3·8	10·0	9·5	9·0	2·5	4·0	−12·0
β, *D*-glucose	8·1	8·5	8·8	9·0	2·0	5·0	−12·2

*Data taken from ref. [26].

low fields, than the corresponding protons in β-*D*-glucose. The experimentally determined chemical shifts of some aldopyranoses in D_2O together with the calculated values using the empirical rules are shown in Table VI. Unless otherwise stated, the calculated chemical shifts are for the sugar in C1 (*D*) conformation. The close agreement of the chemical shifts of protons at C (1) and C (2) atoms of β-*D*- lucose and β-*D*-xylose indicate that the presence of the hydroxymethyl group has little effect on these chemical shifts. There is also a good agreement between the observed and calculated chemical shifts for H (1) and H (2) based on a C1 (*D*) conformation for hexopyranoses, whereas the results for pentopyranoses are reasonably good except for α-*D*-lyxose, α-*D*-arabinose and α-*D*-ribose. The reason might be that these latter compounds exist in considerable amounts, both in C1 (*D*) and 1C (*D*) conformations. Hence the observed value is intermediate between those obtained for C1 (*D*) and 1C (*D*) conformations. Table VII gives the theoretically calculated free energy values for various aldopyranoses. It can be seen that for the molecules known to be conformationally homogeneous from NMR data, the calculated energy differences of 1·0—6 kcal/

TABLE VI

Observed and calculated chemical shifts (τ values) of Aldopyranoses in Deuterium Oxide [7]

Compound	H–1		H–2	
	observed	calculated	observed	calculated
α, *D*-glucose	4·68 (3·5)	4·66	6·40	6·38
β, *D*-glucose	5·26 (7·5)	..	6·68	..
α, *D*-mannose	4·75 (1·7)	4·66	5·99	6·08
β, *D*-mannose	5·03 (1·0)	4·96	5·97	6·08
β, *D*-allose	5·00 (8·2)	4·91	..	..
α, *D*-galactose	4·66 (2·8)	4·66	6·13	6·03
β, *D*-galactose	5·32 (7·1)	5·26	6·45	6·33
α, *D*-talose	4·63 (<1)	4·66	..	..
β, *D*-talose	5·12 (<1)	4·96	..	..
α, *D*-xylose	4·74 (3·1)	4·75	6·37	6·44
β-*D*-xylose	5·35 (7·4)	..	6·74	..
α-*D*-lyxose	4·92 (4·2)	(5·00+; 4·75)	6·1	(6·09+; 6·14)
β-*D*-lyxose	5·06 (1·5)	5·05	5·96	6·14
α, *D*-arabinose	5·4 (7·2)	5·35+	6·42	6·39+
β, *D*-arabinose	4·66 (2·7)	(4·75+; 4·70)	6·07	(6·09+; 6·14)
α, *D*-ribose	5·09 (2·1)	(5·05+; 4·75)	6·15	(6·14+; 6·14)
β-*D*-ribose	5·01 (6·4)	5·00	6·4	6·44

The values within brackets are approximate. $J_{1,2}$ coupling constants; + 1C conformation.

mole between the C1 (*D*) and 1C (*D*) conformations also support the former assignments. On the other hand, the calculated free energies on aldopentapyranoses reveal that except for α and β-*D*-xylopyranose that energy differences between the two chair forms are less than a kcal/mole. Hence these molecules exist in both C1 (*D*), 1C (*D*) conformations in considerable amounts in solution. It thus explains the intermediate chemical shifts observed for H (1) and H (2) from the expected values computed assuming C1 and 1C conformations for most of the aldopentopyranoses.

TABLE VII

Favoured conformations of D-*aldopyranoses in aqueous solution*

Aldopyranose	Conformation favoured by NMR [28]	Calculated energy differences between C1–1C (in k. cal. mole^{-1}) [22]
α-*D*-allose	C1	−2·74
β-*D*-allose	C1	−3·80
α-*D*-altrose	1C⇌C1	−1·06
β-*D*-altrose	C1	−2·12
α-*D*-galactose	C1	−2·62
β-*D*-galactose	C1	−4·11
α-*D*-glucose	C1	−4·45
β-*D*-glucose	C1	−5·97
α-*D*-gulose	C1	−1·42
β-*D*-gulose	C1	−2·86
α-*D*-idose	1C⇌C1	0·02
β-*D*-idose	C1	−0·9
α-*D*-mannose	C1	−3·87
β-*D*-mannose	C1	−2·5
α-*D*-talose	C1	−1·94
β-*D*-talose	C1	−3·26
α-*D*-arabinose	1C	0·96
β-*D*-arabinose	1C⇌C1	0·26
α-*D*-lyxose	1C⇌C1	0·11
β-*D*-lyxose	C1	−0·76
α-*D*-ribose	1C⇌C1	−0·79
β-*D*-ribose	1C⇌C1	−1·36
α-*D*-xylose	C1	−1·79
μ-*D*-xylose	C1	−2·43

The preference for C1 (*D*) conformation in aldohexopyranoses appears to be controlled by the tendency of the hydroxymethyl group at the C (5) atom to be equatorial. In 1C (*D*) conformation, the —CH_2OH group is axially

oriented and hence is placed in close proximity with the axial hydrogens or —OH groups on the same side of the ring, which makes this particular steric arrangement unstable and favours the C1 (*D*) conformation for most of the aldohexopyranoses. Since the bulky —CH_2OH is absent in the aldopentopyranoses the preferred conformation is determined mainly by the relative orientations of the hydroxyl groups. Thus α and β-*D*-xylose exist predominantly in C1 conformation in solution, whereas α-*D*-arabinose exists in 1C conformation. The other pentoses contain substantial amounts of both conformations.

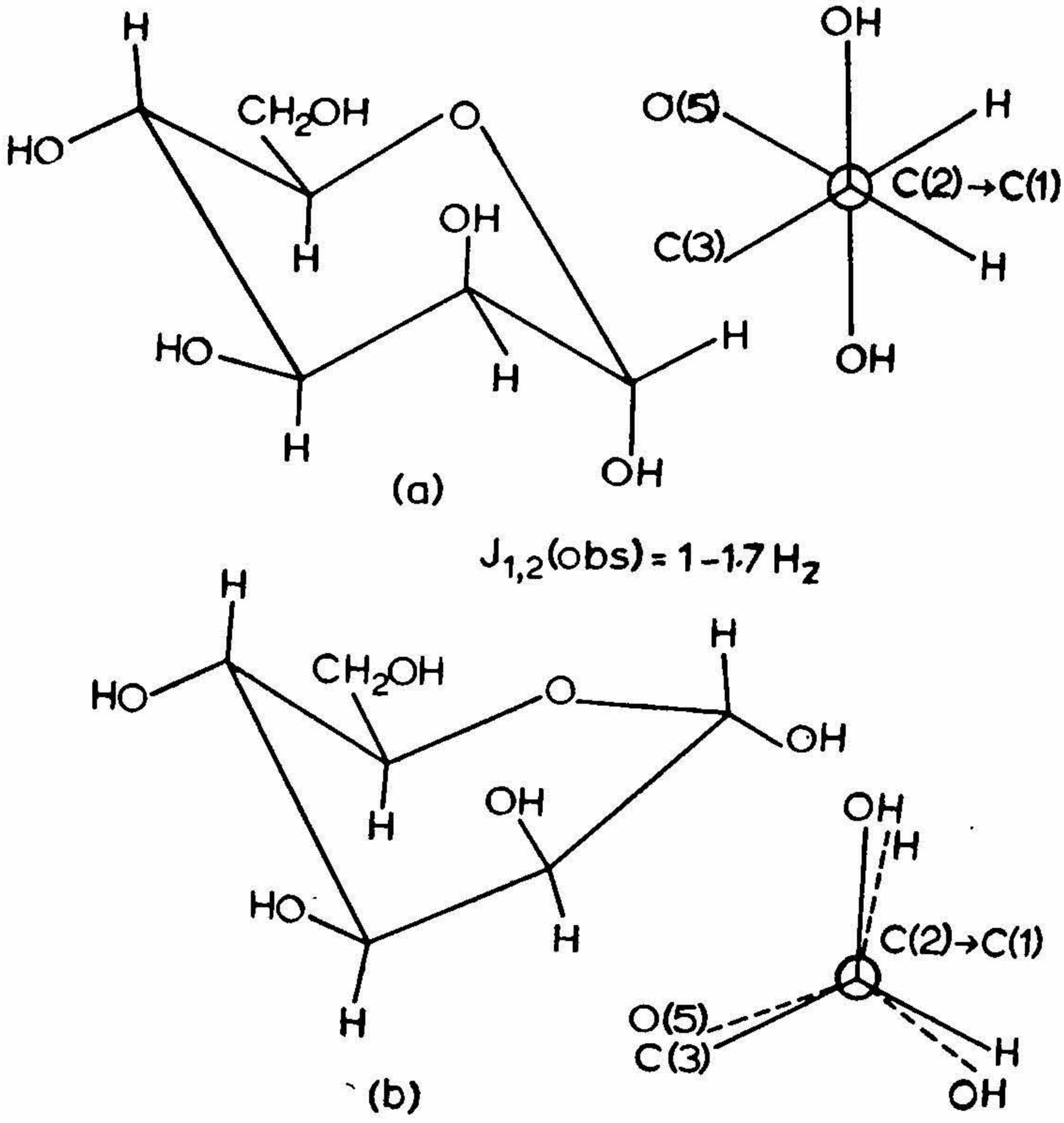


FIG. 6. (*a*) Perfect chair (C1) and (*b*) Couch conformations of β-*D*-mannose. Newman projections along C(2)—C(1) bond are also shown.

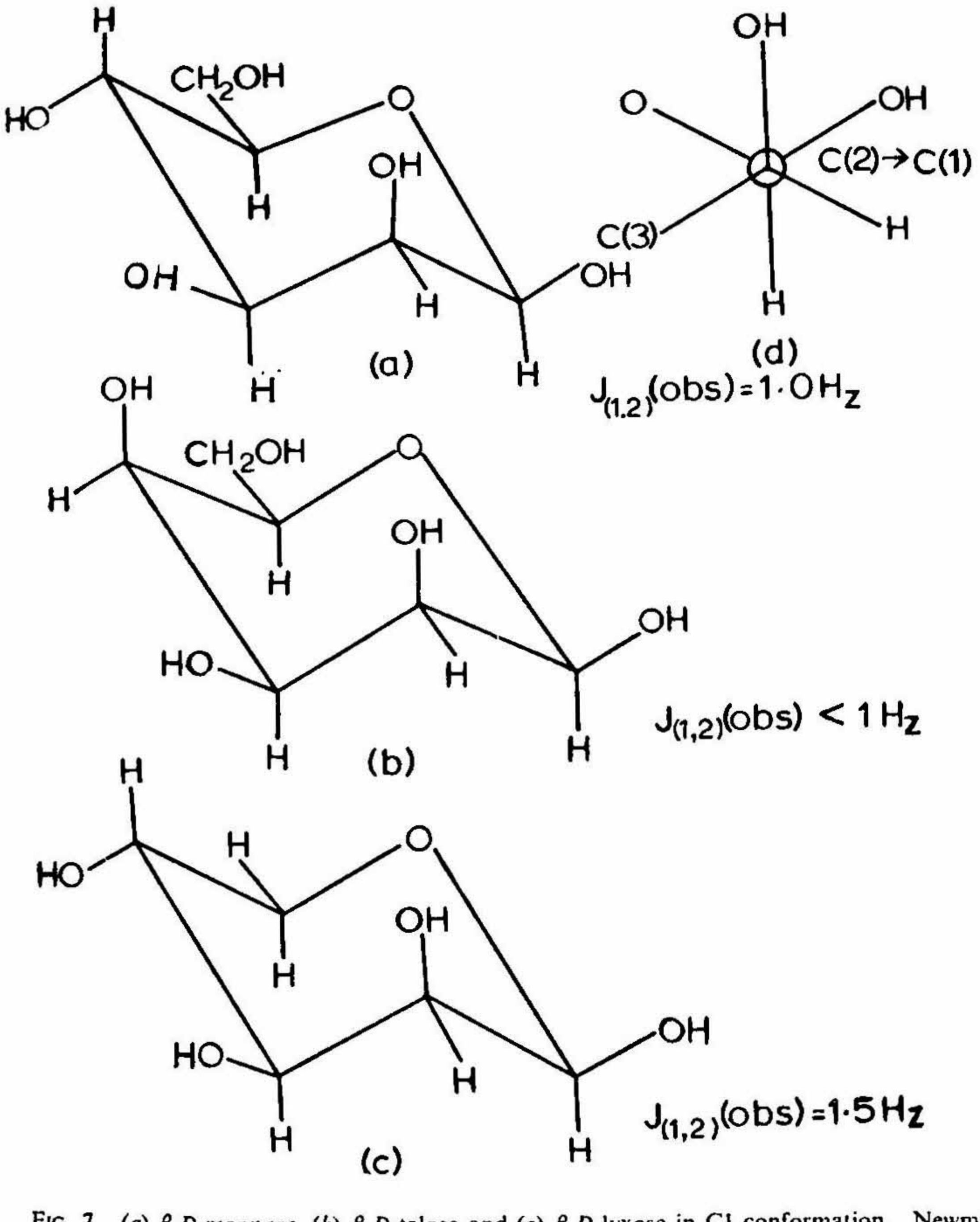


FIG. 7. (*a*) *β-D*-mannose, (*b*) *β-D*-talose and (*c*) *β-D*-lyxose in C1 conformation. Newman projection along C(2)—C(1) bond is also shown.

Table VI shows the $J_{1,2}$ coupling constants obtained for *α* and *β-D*-mannose, *α* and *β-D*-talose are much smaller than 3·5 Hz observed for *α-D*-glucose, though one expects from Karplus equation about the same value for all the cases. These variations between the observed $J_{1,2}$ values may lead as much as 30% variation in ϕ, when equation 3 is used. Such

differences have been interpreted in terms of major ring distortions by the earlier investigators [29], on the assumption that exact dihedral angles could be obtained from Karplus relation. In fact for α-*D*-mannose, the small value observed for $J_{1,2}$ has been rationalized by suggesting a couch form (Fig. 6). However, the recent theoretical studies [21, 22] have indicated only small deviations of the order of 1–2° in bond angles of C1 (*D*) conformations of all hexopyranoses. Such small deviations in bond angles may not distort the ring significantly. In other words, for all the hexopyranoses, the favoured C1 (*D*) conformation is very close to the ideal model except for the distortion introduced due to shortness of C—O compared to C—C bonds. The low $J_{1,2}$ coupling constants obtained for β-*D*-mannose, β-*D*-talose and β-*D*-lyxose may be rationalised from the earlier discussion in the sense that for these cases the coupled protons in C1 (*D*) conformations have ring oxygen atom and the hydroxyl group at C (2) in trans-coplanar arrangement (Fig. 7). On the other hand, the low values observed for α-*D*

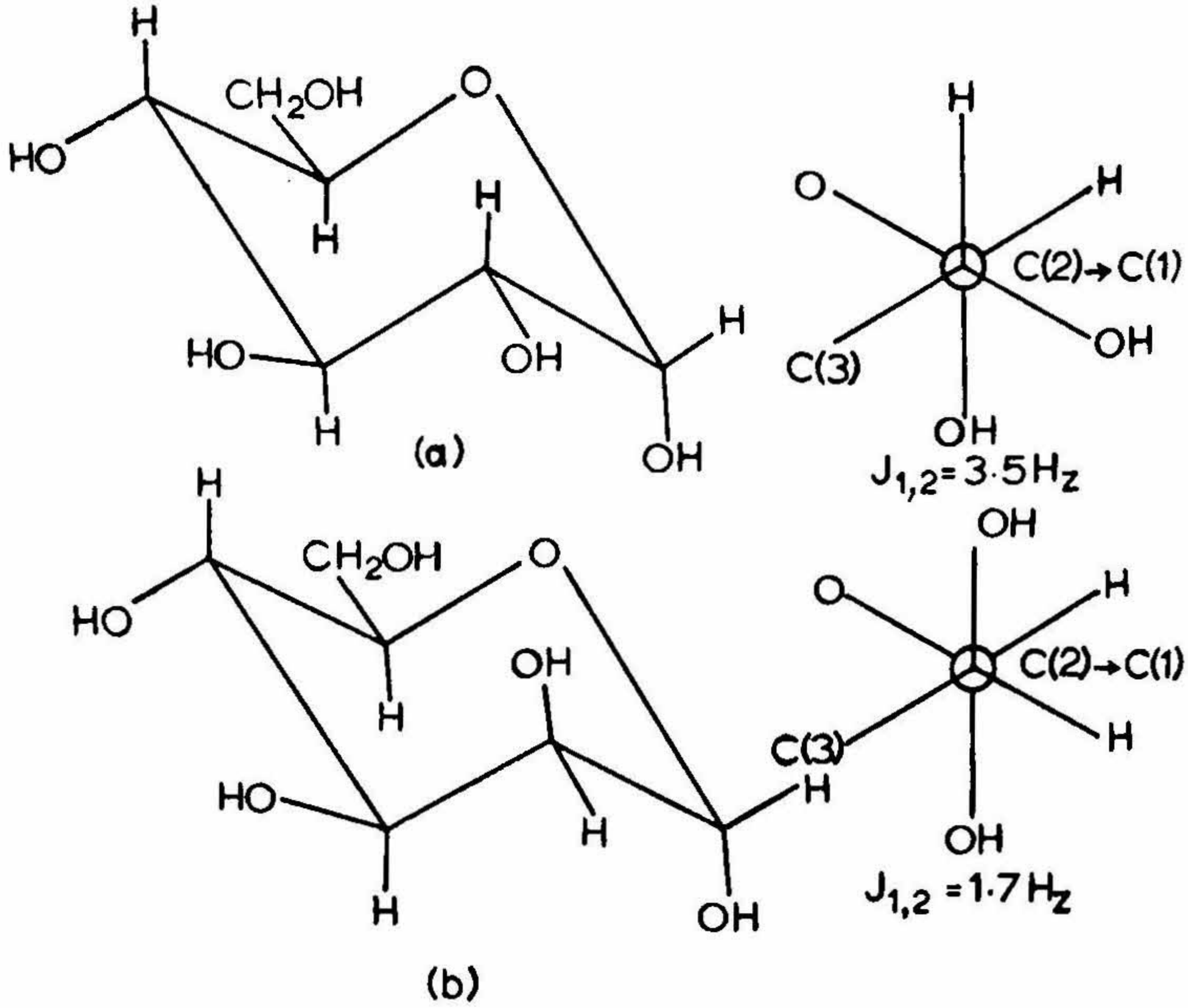


FIG. 8. α-*D*-glucose and (*b*) α-*D*-mannose in C1 conformation. Newmann projections along C (2)—C (1) bond are also shown.

mannose and α-*D*-talose compared to α-*D*-glucose cannot be explained from the consideration of Karplus equation and the trans-coplanar electronegativity effect, since in all the three cases only one of the coupled protons is in trans-coplanar arrangement to the ring oxygen or hydroxyl oxygen atom (Fig. 8) and the other to ring carbon atom. Since theoretical studies eliminated the possibility of large distortions in the ring, probably some other factors such as ring strain which is expected to be higher in the former two molecules because of diaxial substituents on the adjacent carbon atoms, might be affecting the coupling constants besides the factors discussed earlier. Similar discrepancies have also been noted in the conformational studies of certain sugar derivatives [11, 16, 30].

Orientation of hydroxyl groups

In recent years, the use of dimethyl sulfoxide as solvent has been increasing; the advantage being that the hydroxyl protons signals are generally displayed, as well resolved peaks at fields below $\tau = 6$ which provides information on the stereochemistry of —OH protons. The chemical shifts and the coupling constants obtained for some of the sugars

TABLE VIII

Chemical shifts and coupling constants**, $J_{H-1,\ H-2}$ of the C-1—H Resonance of some Deuterated Monosaccharides in DMSO and D_2O* [29, 31 *b*, 32]

Monosaccharide	Equatorial		Axial	
	DMSO	D_2O	DMSO	D_2O
α-*D*-glucose	5·06 (3·0)	4·68–4·84 (2·4–3·0)		
β-*D*-glucose			5·68 (6·5)	5·36–5·42 (6·8–7·5)
α-*D*-galactose	5·05 (3·0)	4·66–4·77 (1·8–2·7)		
α-*D*-xylose	5·14 (3·5)	4·83 (2·2–2·6)		

* Chemical shifts are given in [τ] ppm relative to internal TMS.

** Coupling constants in Hz are given in parentheses.

TABLE IX

Chemical shifts and coupling constants of some Monosaccharides in DMSO* [31]

Monosaccharide	O–1—H doublets		O–2—H O–3—H O–4—H doublets	O–6—H triplet
	Eq.	Ax.		
α, *D*-glucose		3·85 (4·5)	5·28 (5·0) 5·40 (4·5) 5·60 (6·5)	5·70 (5·0)
β, *D*-glucose	3·50 (6·5)		5·25 (3·5)	5·58 (5·5)
α, *D*-galactose		3·95 (4·5)	5·57 (5·0) 5·74 (7·0) 5·78 (4·0)	5·54 (5·0)
α, *D*-xylose		3·90 (4·5)	5·24 (4·0) 5·36 (3·5) 5·60 (6·5)	
Methyl α, *D*-glucopyranoside	..	..	5·22 (5·0) 5·32 (3·5) 5·40 (6·5)	5·62 (5·5)

* Chemical shifts (τ) are given relative to internal TMS.

** Coupling constants (Hz) are given in parentheses.

[31] are shown in Table VIII. The data suggest that in DMSO also, α and β-*D*-glucose, α-*D*-galactose and α-*D*-xylose exist in C1 (*D*) conformation as in D_2O. It has also been shown by PMR that *D*-glucose retains the same conformation, *i.e.*, C1 in alkaline solution also [32]. It is interesting (Table VIII) to note that the values of the O (1)—H/C (1)—H coupling constant obtained for α-anomer (4·0–4·5 Hz) are much smaller than those of β-anomer (6–7 Hz). It has been shown recently that Karplus type of relation for H—C—C—H fragment (Fig. 9) is also applicable for H—O—C—H fragment [33, 34], *i.e.*, the coupling constants are higher for anti-conformer than gauche. In a simplified model the O—H bonds in a simple sugar molecule may be arranged in any one of the three staggered positions. If the rate of rotation of the O—H, about C—O is greater than the chemical shifts

between the hydroxyl protons of the three isomers, the coupling constant varies in proportion to the mole fraction according to the equation,

$$J = N_1J_1 + N_2J_2 + N_3J_3$$

Using methane diol as a model compound, Jeffrey *et al.* [35] suggested that the O—H bond at anomeric carbon favour an anti and gauche conformation for β-anomer, and only a gauche form for α-anomer in C1 ring form. Since $J_{anti} > J_{gauche}$ a larger coupling constant for a β-anomer than α-anomer [in C1 (*D*)] is expected because of the contribution of anticonformer in the former. The observed values are consistent with this model.

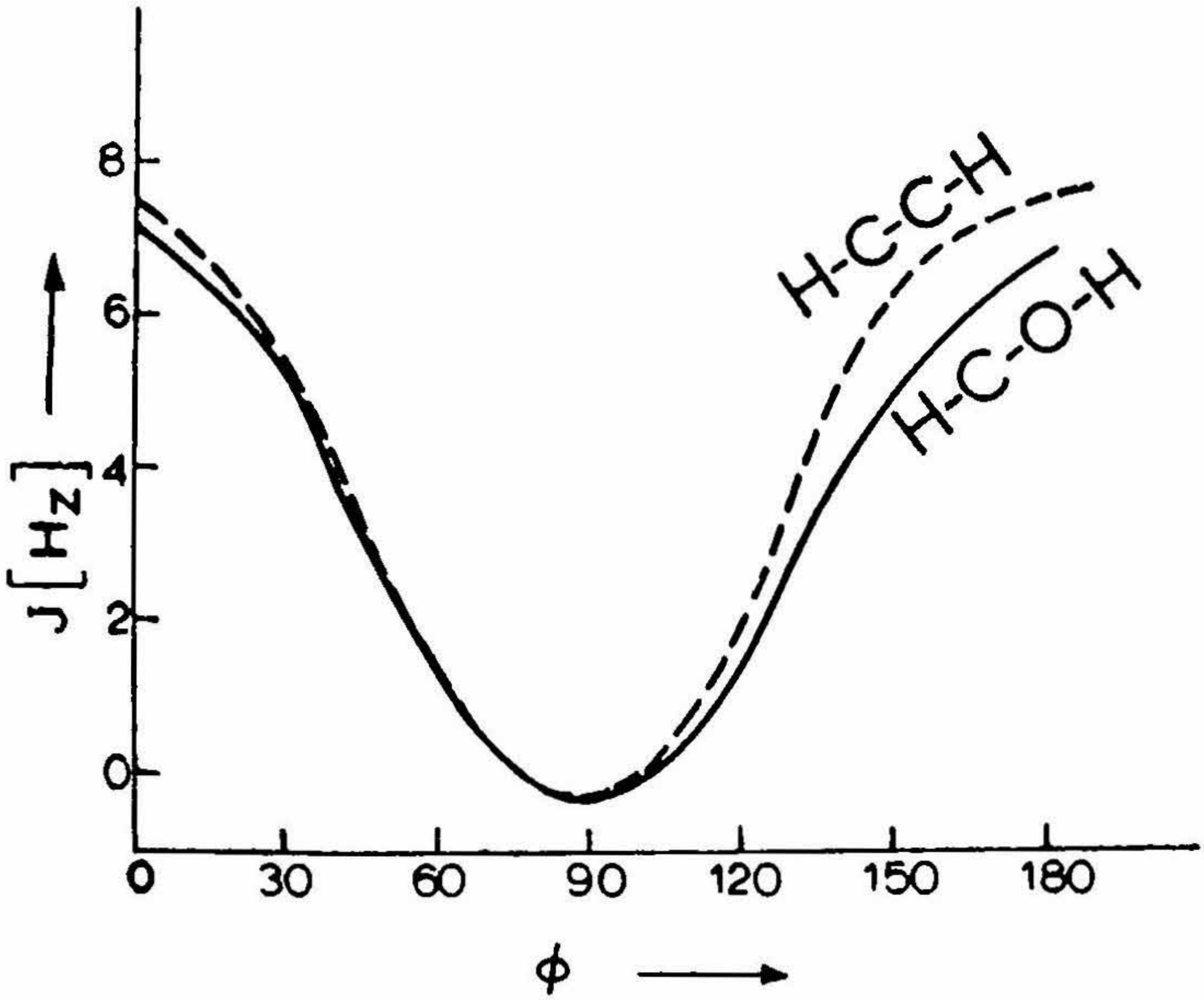


FIG. 9. Variation of the coupling constant (J) with dihedral angle (ϕ) for H—C—C—H and H—O—C—H fragments [34].

Conformation of pyranose ring in the derivatives of simple sugars

Unlike free sugars, the PMR spectra of fully acetylated sugars are generally well resolved and provide information about the ring conformations. The theoretically computed energy values obtained for aldohexo-

pyranose penta-acetates are displayed in Table X. Except for β-*D*-idose penta-acetate, in all other cases C1 (*D*) has at least 1·5 kcal/mole lower energy than the alternate form suggesting C1 (*D*) is the favoured conformation in agreement with NMR experiments [5, 7, 36–39]. Hence the calculated free energy difference of the order of 0·5 kcal/mole between the two chair forms of β-*D*-idose penta-acetate suggest that this molecule exists in conformational equilibrium in agreement with NMR studies [39].

For tetra-acetates the calculated free energy differences $\triangle G$ between the C1 (*D*) and 1C (*D*) conformations [24] and the values derived by NMR [40] are shown in Table XI. The calculated $\triangle G$ values agree fairly well

TABLE X

Conformational-energy values calculated for aldohexopyranose pentaacetates (in kcal . mole^{-1})* [23]

Aldohexopyranose pentaacetate	Calculated free energies	
	C1	1C
α-*D*-allo	−0·31	2·47
β-*D*-allo	0·57	1·93
α-*D*-altro	−0·34	2·45
β-*D*-altro	0·79	2·35
α-*D*-galacto	−0·12	2·70
β-*D*-galacto	0·41	2·98
α-*D*-gluco	−0·74	2·93
β-*D*-gluco	0·00	3·49
α-*D*-gulo	−0·13	2·73
β-*D*-gulo	0·80	1·82
α-*D*-ido	−0·46	1·80
β-*D*-ido	0·83	1·30
α-*D*-manno	−0·44	2·40
β-*D*-manno	0·65	2·46
α-*D*-talo	0·50	2·19
β-*D*-talo	1·15	2·08

* The excess energy of a particular conformation in each set, over that of β-*D*-glucopyranose pentaacetate in the C1 (*D*) conformation in that set, is given.

with the experimental values for all the molecules except for α-*D*-arabinose tetra-acetate and β-*D*-xylose tetra-acetate. Even though the theoretical studies are slightly at variance in these two cases, they correctly predict that these (α, *D*-arabinose tetra-acetate and β-*D*-xylose tetra-acetate) exist as Cl ⇌ 1C conformational mixture in solution in accordance with NMR data. It is also interesting to note that in all these cases theory predicts that the favoured conformation is very close to the ideal form, *i.e.*, no significant deviations in the pyranose ring conformations. But the observed $J_{4,5}$ (60) coupling (0·8 Hz) for the conformationally homogeneous β-*D*-arabinopyranose tetra-acetate is much smaller than the value (5·5 Hz)

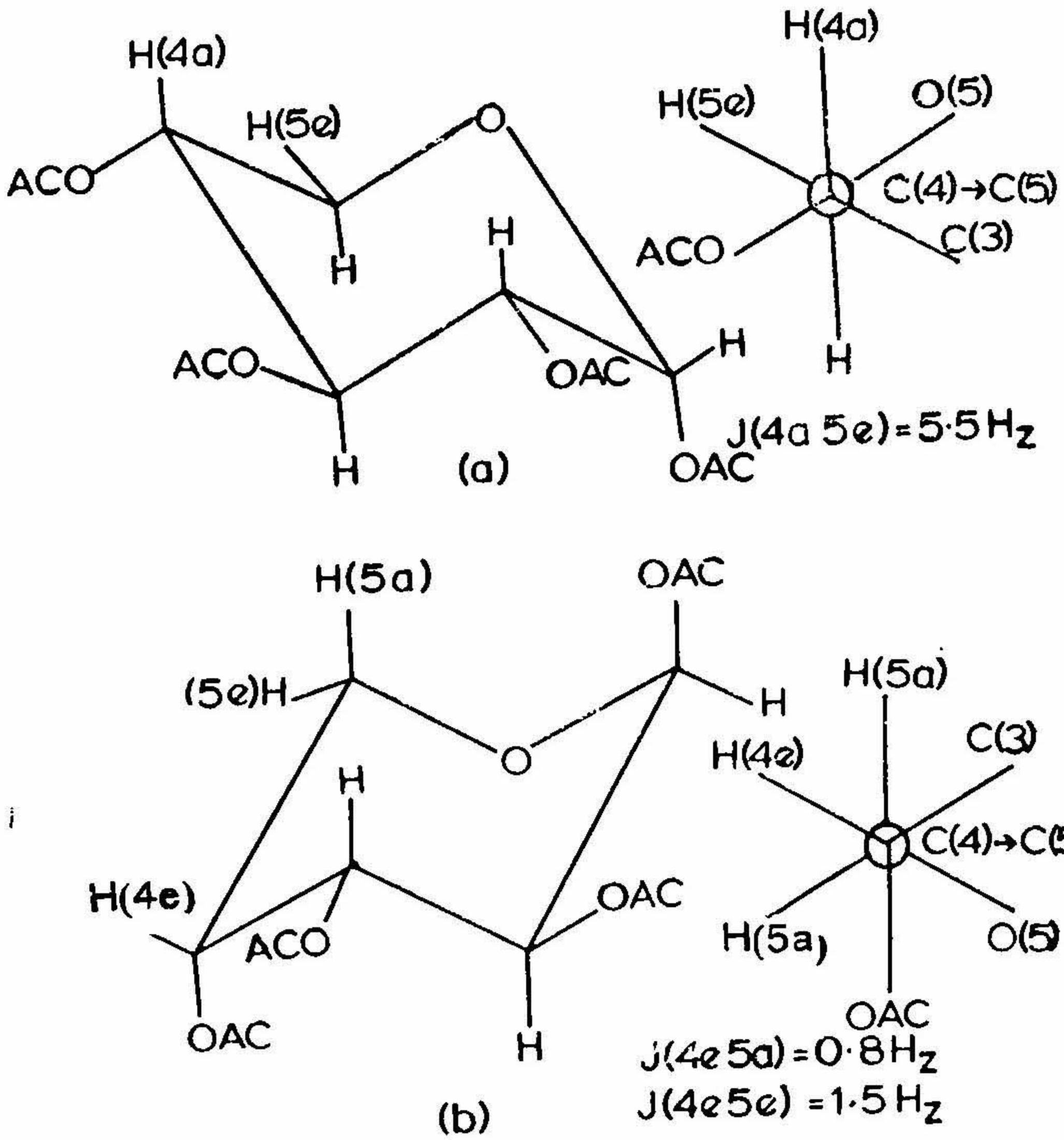


FIG. 10. (*a*) α-*D*-xylopyranose tetra-acetate in C1 conformation and (*b*) β-*D*-arabinopyranose tetra-acetate in 1C conformation. Newman projections along C (4)– C (5) bonds are also shown.

observed for α-*D*-xylopyranose tetra-acetate (Fig. 10). These differences have also been mainly attributed to the configuration of electronegative substituents on carbon atoms (4) and (5). The recent theoretical studies also suggested that these molecules exist in chair conformations close to

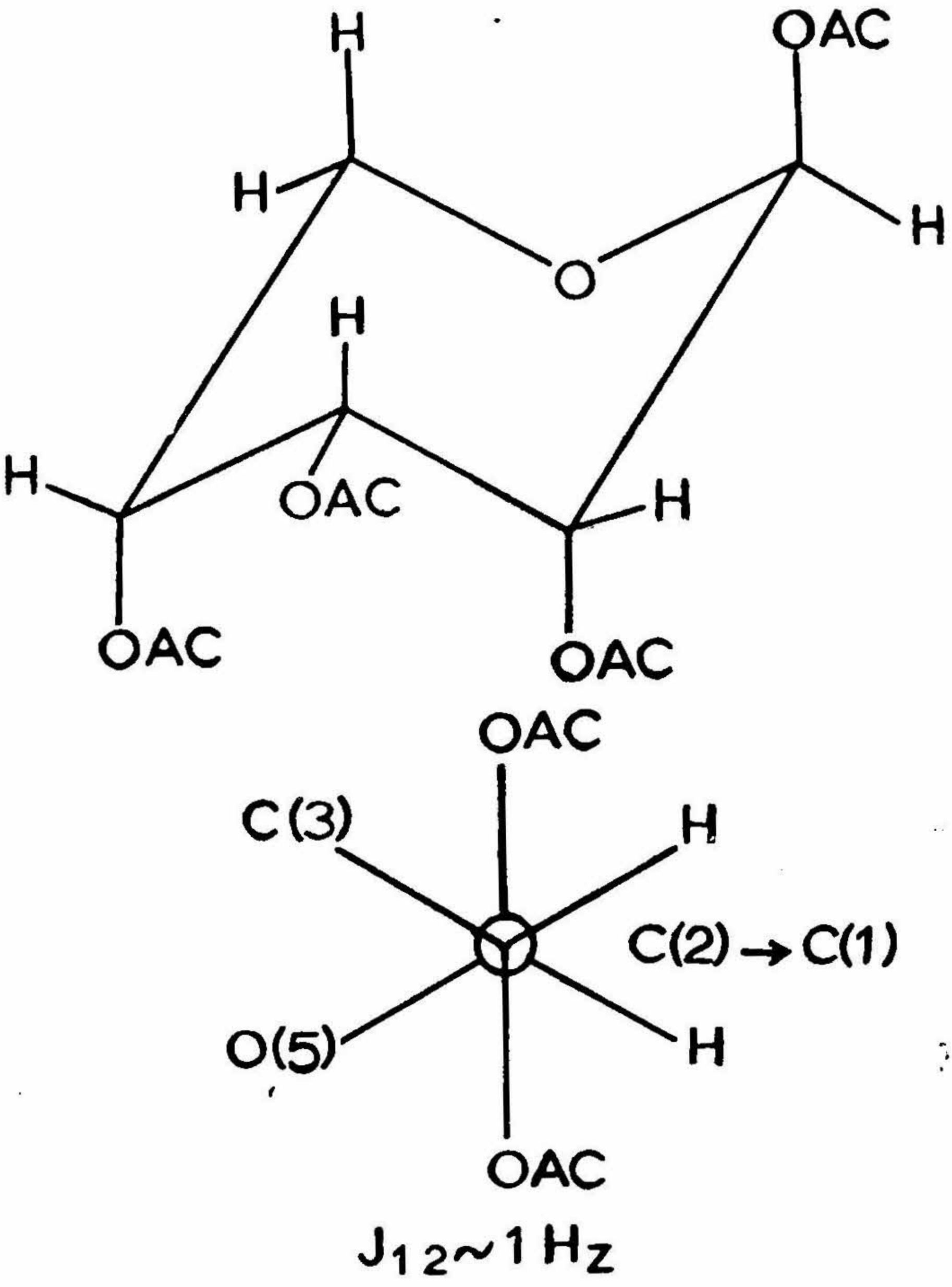


FIG. 11. β-*D*-ribopyranose tetra-acetate in 1C conformation. Newman projection along C(2)—C(1) bond is also shown.

TABLE XI

Conformational energies of aldopentopyranose tetraacetates (in kcal . mole^{-1})

Aldopentopyranose tetraacetate	Calculated free energy difference/ ΔG C1–1C [24]	ΔG from NMR for C1–1C [40]
α-*D*-arabino	−0·51	+0·81±0·34
β-*D*-arabino	+0·86	+1·9±1·0
α-*D*-lyxo	−0·60	−0·55±0·30
β-*D*-lyxo	+0·80	+0·28±0·27
α-*D*-ribo	−0·19	−0·74±0·33
β-*D*-ribo	+0·93	+0·18±0·26
α-*D*-xylo	−1·03	<−2·4
β-*D*-xylo	+0·38	−0·58±0·30

ideal ones. In β-*D*-arabinose tetra-acetate (1C) (Fig. 11) configuration the atoms or groups at C (4) and C (5) atoms are in $J_g{}^{g'}$ relation, whereas in β-*D*-xylose tetra-acetate (C1) they are in $J_t{}^{g}$ relation. Hence a high value for $J_{4,5}$ (60) is expected for the latter compared to the former. On the other hand, the observed differences between $J_{1,2}$ (60) coupling (3·5 Hz) for α-*D*-xylopyranose-tetra-acetate in the C1 conformation and (~ 1 Hz) β-*D*-ribopyranose tetra-acetates in 1C conformation cannot be accounted merely from a consideration of the Karplus equation and the trans-coplanar, electronegativity effect. Similar anomalies have also been noted in other ribose derivatives. These large changes in $J_{1,2}$ coupling in β-*D*-ribopyranose tetra-acetate [11] have been explained by assuming substantial flattening of the tetrahydropyran ring as a result of axial orientation of the acetoxy groups at C (1) and C (2) and the syn-diaxial disposition of O (2) and O (4). However, conformational energy calculations do not support this view. In fact, these studies indicate that the ring conformation of β-*D*-ribopyranotetra-acetate is close to the ideal form.

Conformation of pyranose ring in oligo and polysaccharides and the nature of intramolecular hydrogen bonds

Although the PMR spectra of oligo and polysaccharides in D_2O, KOD and DMSO were not well resolved, the trends in chemical shifts and coupling

constants of anomeric protons have some similarity to that observed in monosaccharides. Rao and Foster [32, 41] by means of PMR suggested that the pyranose ring exists in C1 (*D*) conformation in methyl *D*-glucopyranosides, cellobiose, maltose and γ-cyclodextrin. Based on these studies, it was suggested that the pyranose units exist in C1 (*D*) conformation for the high polymers of *D*-glucose including amylose. These studies were also extended to alkaline solutions and showed that the D-glucopyranose ring in methyl, α- and methyl, β-*D*-glucopyranosides, *D*-glucose, sucrose, *D*-maltose and amylose exist in the C1 (*D*) conformation in alkaline as well as in neutral aqueous solution, thus ruling out the boat conformations [32]. Glass [42] not only supported these conclusions but also extended the PMR studies in D_2O to nine other carbohydrates, including hydrolysed starch' commercial dextrans, laminaran and crowngall polysaccharides and assigned the C1 (*D*) conformation for pyranose unit in all these compounds. The PMR data obtained by various investigators [32, 41–44] on oligo- and polysaccharides in D_2O are shown in Table XII. It is interesting to note that glycosidic anomeric proton signal appears at about $4{\cdot}8 \pm 0{\cdot}3$ ($J_{H-1,\,H-2} = 3{\cdot}0 \pm 0{\cdot}3$ Hz), in all the α-linked compounds ($1 \rightarrow 1$; $1 \rightarrow 2$; $1 \rightarrow 3$;

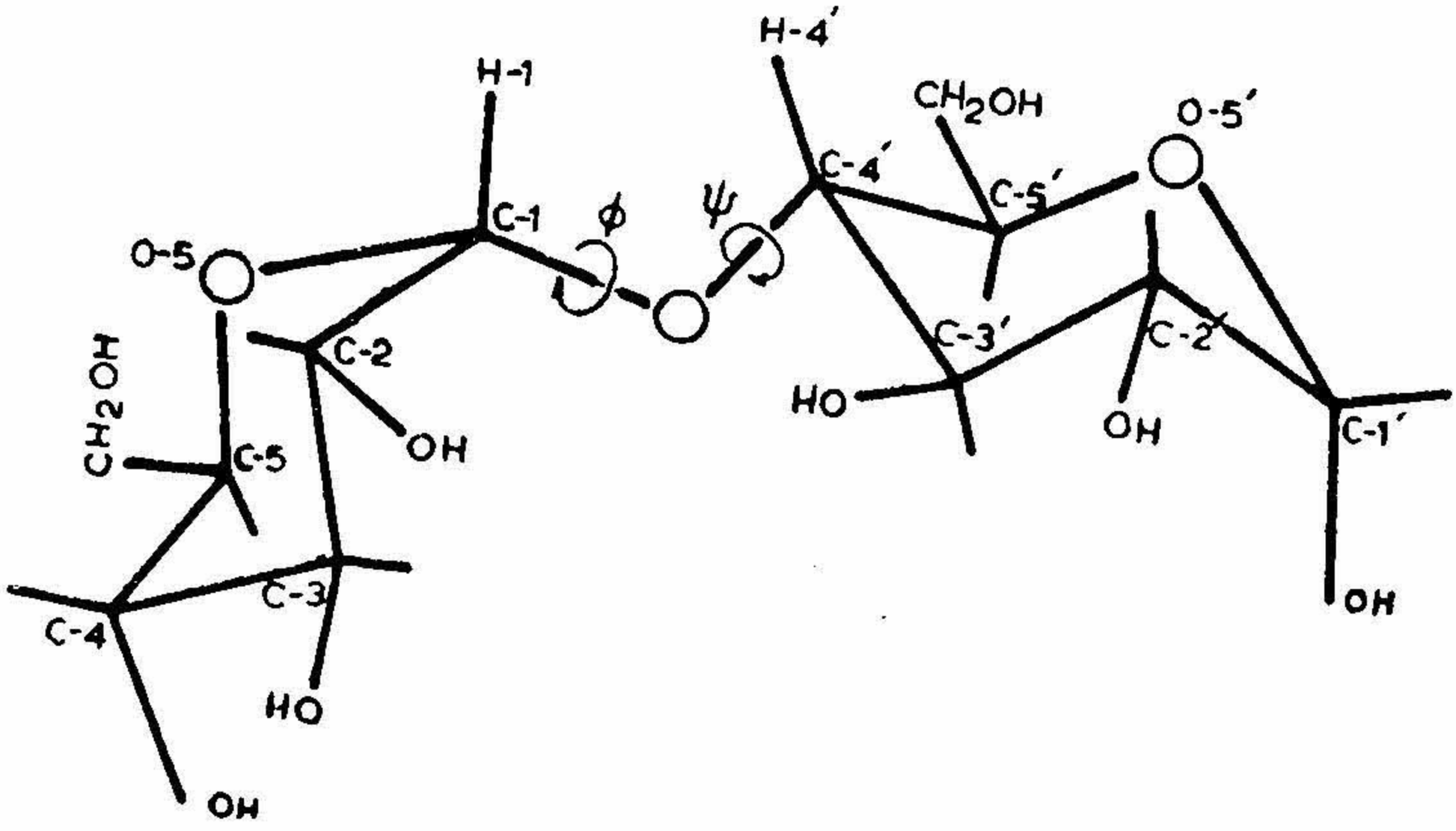


FIG. 12. Pair of *D*-glucose residues joined through α-(1—4) linkage in their initial conformation. Rotations ϕ and ψ about the single bonds C (1)—O and O—C (4) are marked. When viewed from C (1) towards O, rotation of the reducing residue as a whole in a clockwise direction about the C (1)—O bond, with the non-reducing residue being held stationary, gives an increase in the value of ϕ. Similarly, when viewed from O towards C (4), rotation of the reducing residue in the clockwise direction about O—C (4) bond, with the non-reducing unit being held stationary, gives an increase of the value ψ.

TABLE XII

Chemical shifts, τ (ppm), and coupling constants (Hz, in parenthesis) of oligo and polysaccharides in D_2O [32, 39, 41–44]

Name of the compound	Anomeric protons at the glycosidic link		Anomeric protons at reducing end	
	H eq.	H axial	H eq.	H axial
α, α-trehalose	4·81 (3·2)			
Turanose	4·70 (3·2)			
Methyl, α, *D*-glucose	5·2–5·25 (3·0–3·3)			
D-maltose	4·6 (2·8–3·4)		4·75–4·8 (3·0–3·4)	5·3–5·7 (7·3–8)
Methyl, β-maltoside	4·62 (3·2)			5·62 (7·6)
Sucrose	4·52–4·59 (3 to 3·2)			
Maltrotriose	4·63 (3·5)		4·8 (3·7)	5·37 (7·3)
Raffinose	5·02 (2·7)			
	4·59 (2·9)			
Melibiose	5·04 (2·9)		4·79 (2·9)	5·04 (2·9)
Cyclohexa amylose	4·95 (2·7)			
Cycloocta amylose	4·8–4·9 (2·7–3·5)			

TABLE XII—*Contd.*

Name of the compound	Anomeric protons at the glycosidie link		Anomeric protons at reducing end	
	N eq.	H axial	H eq.	H axial
Amylose	4·7+			
Hydrolysed Starch	4·62 (3·0)			
Dextran B 512E	5·09 (2·3)			
Dextran B 1355	5·07 (4·73)			
Methyl β-*D* glucopyranoside		5·54–5·65 (7·4–7·7)		
Cellobiose		5·38–5·5 (7·0–8·8)	4·7–4·78 (2·9–3·3)	5·35–5·38 (7·4–8·0)
Lactose		5·58 (7·1)	4·78 (3·7)	5·36 (7·3)
Gentibiose		5·50 (7·7)	4·78 (3·5)	5·34 (7·6)
Laminaran		5·25 (6·7)		
Crowngall polysaccharide		5·12 (6·2)		
Digalacturonic acid	4·93			
Trigalacturonic acid	4·94			
Tetragalacturonic acid	4·94			

1 → 4 and 1 → 6) which is close to the values observed for α *D*-glucose in C1 (*D*) conformation. Similarly, the glycosidic anomeric proton signals in all the β-linked compounds appear at about $5{\cdot}3 \pm 0{\cdot}1$ ($J_{H\text{-}1,\,H\text{-}2} = 7{\cdot}0 \pm 0{\cdot}7$) which are close to the values observed for β, *D*-glucose in C1 conformation. From these similarities it has been concluded that glucose

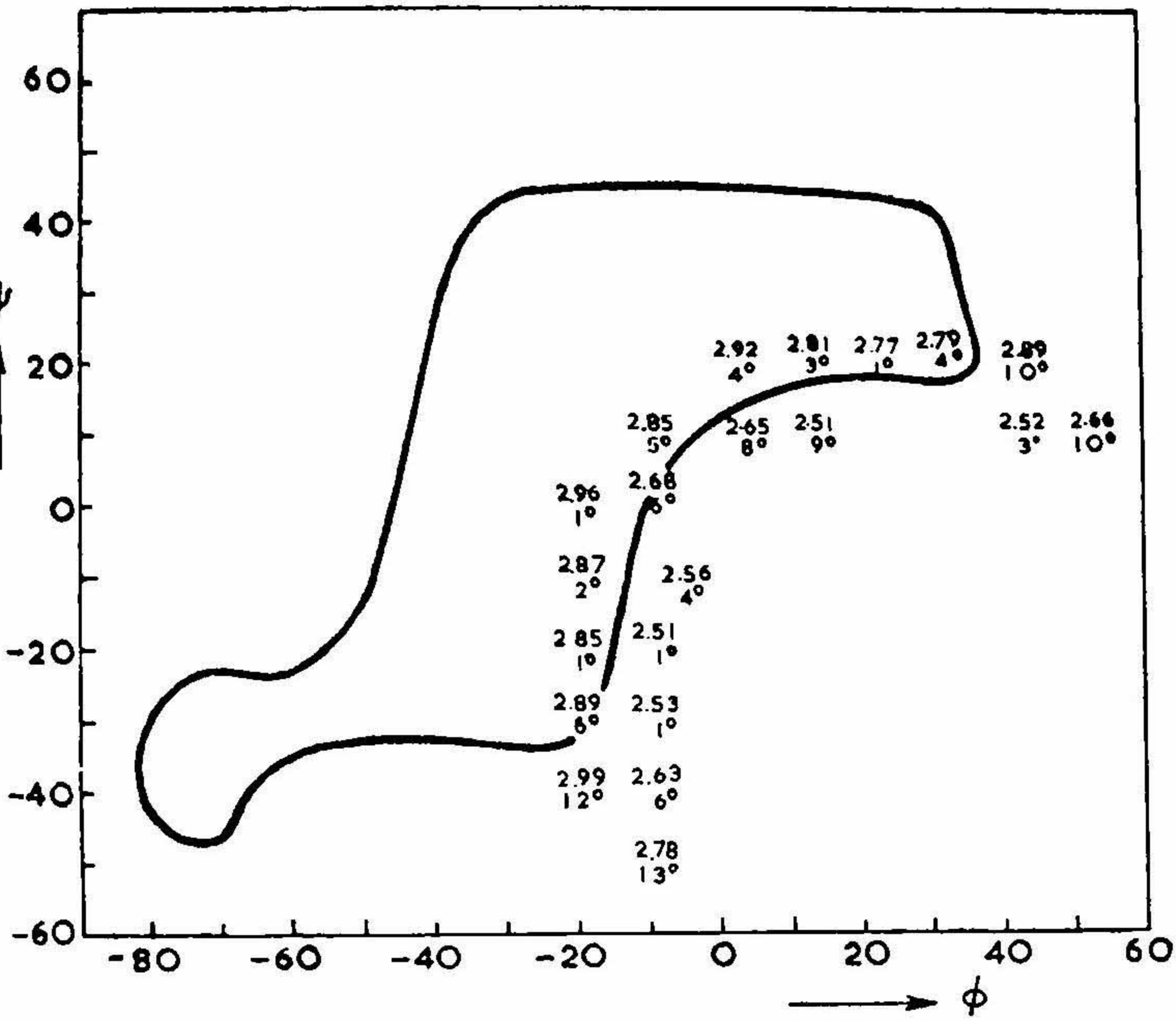


FIG. 13. Steric map for a pair of *D*-glucose units joined through α-(1–4) linkage. Conformations, which fall with the solid contour are permitted from stereochemical criteria. The O (2) - - - O (3′) type hydrogen bond distances and angles are marked.

residues in all the compounds exist in C1 (*D*) conformation; and the ring conformation is not significantly effected by the type of linkage. In methylated derivatives also, Casu *et al.* [45] observed the equatorial proton signal at lower field than the axial one. The coupling constant of the anomeric proton is of the order of 3 Hz for α (1 → 4); 7 Hz for β (1 → 4) compounds both in $CDCl_3$ and DMSO suggesting that conformation of glucose units of O-methylated amylose, cyclodextrin and O-methylated glucose and diglucoses are in C1 (*D*) conformation. Even in acetylated derivatives of cyclodextrins and amylose, the spin-spin coupling constants of the ring protons indicated the C1 conformations for the *D*-glucopyranose units [46]. From the comparison of PMR spectra of oligosaccharides with that of monomer, Rees and Wright [44] have concluded that *D*-galacturonic residues in (1 → 4)

chains also exist in C1 conformation. It thus seems that C1 conformation is more prevalent also in di, oligo and polysaccharides of *D*-sugars.

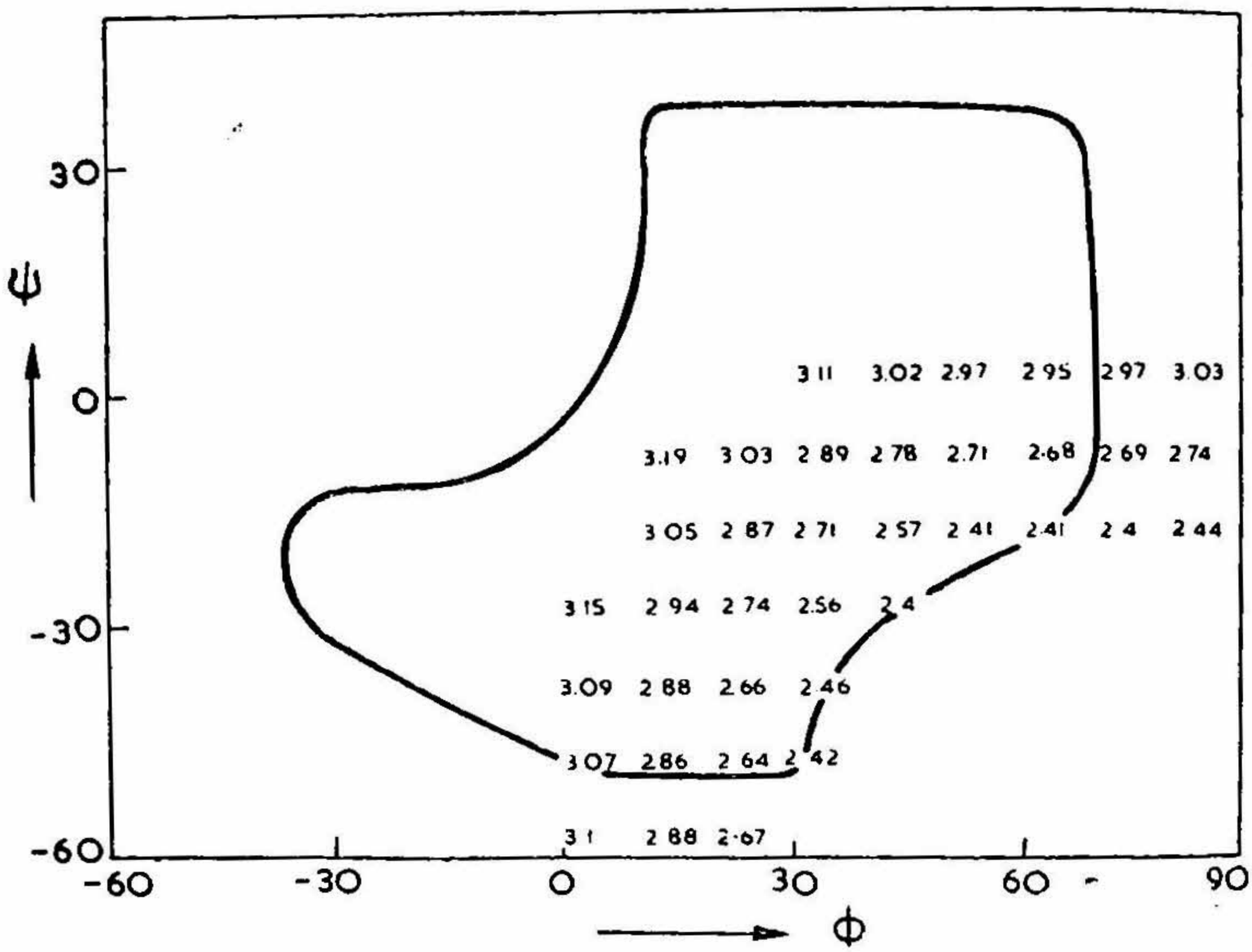


FIG. 14. Steric map for a pair of *D*-glucose units joined through β-(1–4) linkage. Conformations which fall within the solid line are permitted from stereochemical criteria. The O (3)- - - O (5) type of hydrogen bond distances are marked.

PMR spectral data of oligo and polysaccharides obtained in DMSO [31] (Table XIII) also showed trends in chemical shifts and coupling constants of anomeric protons similar to that observed for monosaccharides, indicating that the pyranose units in these compounds exist essentially in C1 (*D*) conformation as in D_2O. The signals of the anomeric protons in cyclohexa- and cyclooctaamylose appear at higher fields than those in maltose and amylose in both D_2O and DMSO. These shifts of the anomeric proton signals to higher fields in cycloamyloses have been attributed to the magnetic anisotropy effect of the O (1)—C (4′) bond, which shields the anomeric protons in cyclic than in linear structure.

In DMSO, some of the non-anomeric hydroxyl protons in both α, D (1 → 4) and β, D (1 → 4) linked di- and polysaccharides appear on the low field side (τ 4·3—4·8) compared to their counterparts in simple sugars.

TABLE XIII

Chemical shifts, τ (ppm) and coupling constants (c/s, given in parentheses) of some di-, oligo- and polysaccharides in DMSO [31]

Di-, oligo- and polysaccharides	O_1H doublet		C_1H doublet		OH's under 5τ doublets	
	equat.	axial	equat.	axial		
α, α' Trehalose 1–O (α-*D*-glucopyranosyl) α-*D*-glucose			5·15 (3·0)			
α-Maltose 4–O (α-gluco-pyranosyl) α-*D*-glucose		3·70 (4·5)			4·75	
β-Maltose 4-O (α-*D*-gluco-pyranosyl) β-*D*-glucose	3·40 (6·5)		5·01 (3·0)		5·70 (6·5; 6·5) pair of doublets	4·63 (<2); 4·66 (6·5)
α-Cellobiose 4-O (β-*D*-glucopyranosyl) α-*D*-glucose		3·72 (4·5)				
β-Cellobiose 4-O (β-*D*-glucopyranosyl) β-*D*-glucose	3·42 (7·0)				5·60 (7·0; 7·0) pair of doublets 5·71 (6·5)	4·82 (3·5)
α-Gentibiose 6-O (β-*D*-glucopyranosyl) α-*D*-glucose		3·84 (4·0)				
β-Gentibiose 6-O (β-*D*-glucopyranosyl) β-*D*-glucose	3·50 (7·0)					
α-Melibiose 6-O (α-*D*-galactopyranosyl) α-*D*-glucose		3·88 (4·0)			5·00–5·22	
β-Melibiose 6-O (α-*D*-galactopyranosyl) β-*D*-glucose	3·54 (6·5)					

TABLE XIII—*Contd.*

Di–, oligo- and polysaccharides	O_1H doublet		C_1H doublet		OH is under 5*r* doublets
	equat.	axial	equat.	axial	
Maltotriose			(5·00)		~4·60; ~4·68
Isomaltotriose			5·10		
Maltotetraose			5·00		4·60
Maltopentaose			5·00		4·60
Maltohexaose			5·00		4·60
Amylose			4·90 (3·0)		4·55; 4·61
α-Cyclodextrin			5·19 (3·0)		4·55 (6·0); 4·59 (<2·0)
β-Cyclodextrin			5·17 (3·0)		4·33 (6·0); 4·38 (<2·0)
Laminaran				5·56 (7·0)	4·75
Linear dextran			5·40 (2·5)		
Branched dextran			~5·10		
			~5·35		

The low field signals in α, (1 → 4) linked glucose products have been assigned to the internal hydrogen bonds between O (2)—H and O (3′) —H groups of adjacent residues. The strength of such an intra-hydrogen bond depends on the proximity of the groups involved and their relative orientations. It is seen from Figs. 12 and 13 that a slight rotation of the glucose residues about the glycosidic bonds effect the distance between O (2)—H and O (3)—H groups significantly and make them accessible to the solvent. Such rotational freedom about the glycosidic linkage is possible in maltose and amylose but not in cycloamyloses, hence closer proximity of these hydroxyl groups is expected in the latter which results in stronger intramolecular hydrogen bonds compared to maltose and amylose. These studies also indicated that the intramolecular hydrogen bonds are stronger in cycloamyloses compared to amylose and maltose. Among cycloamyloses, the intrahydrogen bond is stronger in cyclohepta-amylose compared to cyclohexa-amylose.

The absence of any signal (Table XIII) due to hydroxyl proton in the low field side in the spectra of linear dextran, has been suggested as an

evidence for the absence of intramolecular hydrogen bonds. On the other hand, the presence of the signal at around τ 4·8 for cellobiose and laminaran has been indicated as an evidence for the presence of intramolecular hydrogen bonds. From the peak area it has been concluded that the hydrogen bonds in these compounds involve one — OH group and an acceptor non-hydroxyl group. Casu *et al.* further suggested that the cellobiose conformation involves O (3′)—H...O (5) and laminaran O (2′)—H...O (5) or O (4′)—*H*...O (5) type hydrogen bonds between contiguous residues in DMSO. Such hydrogen bonds have also been suggested from theoretical studies [47]. It is seen from Figs. 14 and 15 that intramolecular hydrogen bonds of the type O (3′)...O (5) and O (4′)...O (5) are possible in the allowed conformations of cellobiose and laminaran.

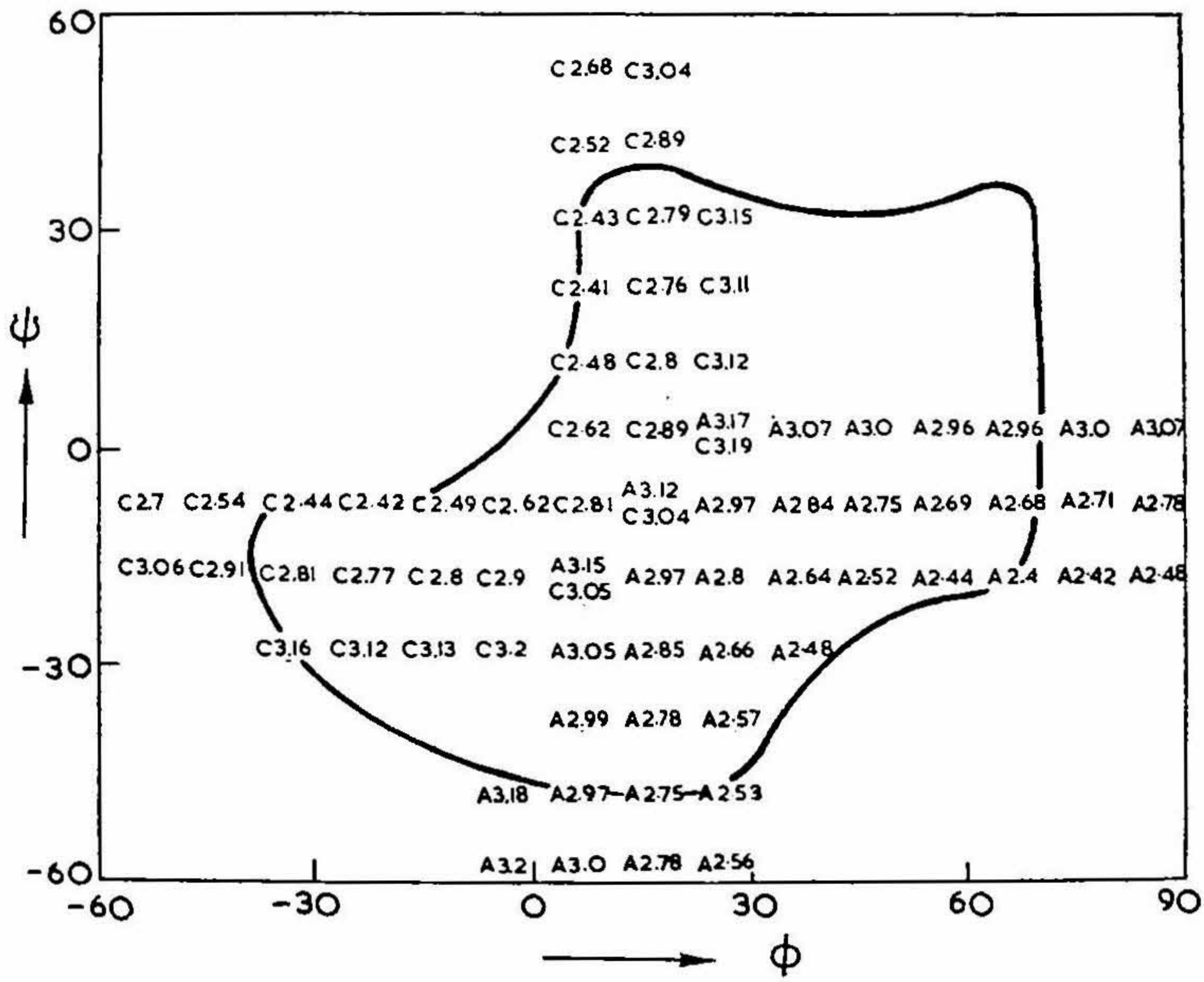


FIG. 15. Steric map for a pair of *D*-glucose units joined through β-(1–3) linkage. The types of hydrogen bonds are indicated together with the O—O bond lengths. A denotes an O (4′)- - - O (5) type of hydrogen bond. C denotes an O (2)—O (2) type of hydrogen bond.

CONCLUSIONS

NMR has been developed into a powerful and direct technique for the solution of structural problems in the field of carbohydrates. Valuable

information has already been obtained in many cases about the configuration of simple sugars, conformation of pyranose ring in mono, oligo- and polysaccharides; the inter and intramolecular hydrogen bonds; and also about the various conformers present in the equilibrium mixture. However in view of the limitations of Karplus type of equation, (particularly the trans-coplanar electronegativity effect on the coupling constant), in predicting the dihedral angles precisely, this method at present is not recommended to detect small conformational changes. However, this technique can be used more profitably in conformational studies, by comparing these data with conformational energy calculations.

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