Carbon-13 Nuclear Magnetic Resonance Spectra of Acyclic Carbohydrate Derivatives: Alditols, 1,2-Bis(phenylhydrazones), and Dithioacetals

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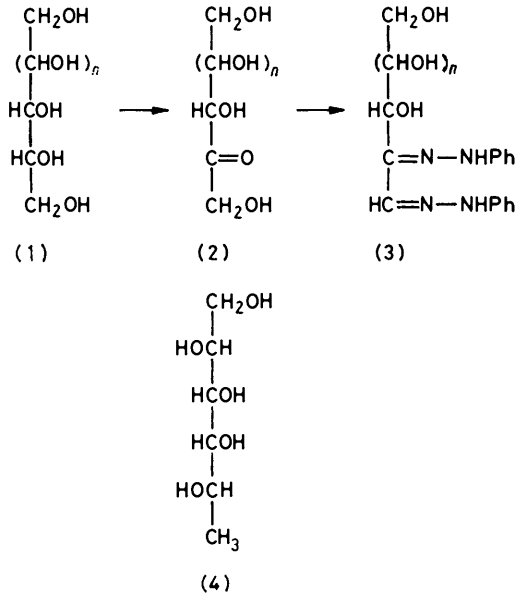
The carbon-13 n.m.r. spectra of acyclic carbohydrate derivatives, namely alditols, 1,2-bis(phenylhydrazones), dithioacetals, and related acyclic sugar derivatives, are reported. The study has corroborated earlier results, based on ^{1}H n.m.r. investigations, on the conformational behaviour of such compounds in solution. .Application of this technique in elucidation of regiospecificity of oxidation of alditols by *Acetobacter suboxydans* is discussed.

In the field of carbohydrate chemistry carbon-13 n.m.r. spectroscopy is now recognised [1] as one of the most versatile and sensitive spectroscopic techniques available for stereochemical assignment and structural elucidation. In studies where ^{1}H n.m.r. spectroscopy has been uninformative and of little utility, ^{13}C n.m.r. spectroscopy, besides corroborating and complementing ^{1}H n.m.r. results, has made its own unique contributions; the potential and capabilities of the technique have been elegantly exemplified in structural and conformational studies on a variety of compounds such as polysaccharides,[2] oligosaccharides,[3] antigenic determinants,[4] glycoside–protein complexes,[5] ketose sugars,[6] branched-chain sugars,[7] and chlorodeoxy sugars.[8] Prior to the present investigation there has been a dearth [8a,9] of accurate ^{13}C n.m.r. spectral data on acyclic carbohydrate derivatives, especially alditols and dithioacetals; this is in contrast to the wealth [10] of ^{1}H n.m.r. spectral data available on these compounds. Horton *et al.* have discussed the progress and research directions in ^{1}H n.m.r. spectroscopy and its implications in conformational analysis of these derivatives in various review articles.[11]

A major programme in this laboratory is concerned with the study of various aspects [12] of microbiological oxidations of carbohydrates by the acetic acid bacteria[13] *Acetobacter suboxydans*, one important aspect being concerned with the utility of the oxidative specificity of *Acetobacter suboxydans* towards alditols and other carbohydrate derivatives in general carbohydrate syntheses. This has been demonstrated in the stereospecific synthesis of L-dendroketose reported [14] earlier from this laboratory. The regiospecificity and stereospecificity of this microbial oxidation in the alditol series [15] has been formulated as the Bertrand–Hudson rule;[15a] polyols containing the D-*erythro* structural unit (1) are oxidised to the ketose product (2). In most of the earlier studies [14] the ketose products were characterised by making their crystalline 1,2-bis(phenylhydrazone) derivatives (3). However, in the case of L-fucitol (4) where an anomalous oxidation pattern was observed,[15a] lengthy chemical procedures were required [16] to unequivocally establish the identity of the two oxidation products.

The purpose of the present investigation is two-fold; firstly, to report ^{13}C n.m.r. spectroscopic data on alditols, dithioacetals, and 1,2-bis(phenylhydrazones), and secondly, to demonstrate the utility of ^{13}C n.m.r. spectroscopy in determining the oxidative site in the microbial oxidation of alditols by *Acetobacter suboxydans*, by correlation of the ^{13}C n.m.r. chemical-shift data of the 1,2-bis(phenylhydrazones) of the ketose products with those of the alditols. Where possible, an attempt has been made at interpreting the ^{13}C n.m.r. data of these acyclic derivatives, in terms of their preferred conformation in solution. Hitherto, ^{1}H n.m.r. spectroscopy has been of little utility in unravelling the conformations of alditols in solution; only occasionally has the use of lanthanide-shift reagents permitted a conformational



study.[17] Numerous *X*-ray crystallographic [18] and ^{1}H n.m.r.[10] studies on various acyclic sugar derivatives have indicated that these compounds exist predominantly in an extended planar zig-zag conformation, except where this would result in two oxygen atoms having a parallel 1,3-interaction. Thus, acyclic derivatives having *arabino*- and *lyxo*-configurations exist as planar zig-zag conformers in solution,[10a,c,d] as well as in the crystalline state.[18g] Similar conformational preference is also exhibited by derivatives with *galacto*- and *manno*-configurations in both solution [10c] and in the crystalline state.[18b-g] In contrast, D-glucitol, D-iditol, and allitol exist [18f-g] as the ' bent ' or ' sickle ' [10c] conformation in the crystalline state.

In the present investigation, to aid rational interpretation of the ^{13}C n.m.r. spectral data of 1,2-bis-(phenylhydrazones), their ^{1}H n.m.r. spectra were

recorded, and the preferred rotamer state in solution was deduced from the coupling-constant data, in a manner analogous to the previous related studies on phenylosotriazoles [10a] and quinoxaline [10b] derivatives.

RESULTS AND DISCUSSION

Alditols, Dithioacetals and Related Derivatives.—The ^{13}C chemical-shift data for alditols, dithioacetals, and glucitol-related acyclic derivatives, studied in this research are documented in Tables 1—3, respectively.

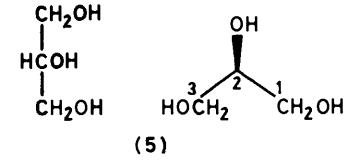
TABLE 1

^{13}C Chemical-shift data [a] of the alditols

Compound	C-1	C-2	C-3	C-4	C-5	C-6
(5)	63.0	72.4	63.0			
(6)	63.2	72.4	72.4	63.2		
(7)	63.0	71.8	71.8	63.0		
(8)	62.9	72.6	72.6	72.6	62.9	
(9)	62.6	72.4	70.1	72.4	62.6	
(10)	62.8	70.1 [b]	70.4 [b]	71.3	63.6	
(11)	63.7	71.3	69.6	69.6	71.3	63.7
(12)	62.4	73.5	68.8	72.1	71.3	63.3
(13)	63.1	70.1	69.2	69.2	70.1	63.1
(14)	62.8	70.5	70.9	71.8	73.0	62.8
(15)	62.8	73.1	72.5	72.5	73.1	62.8
(16)	62.8	72.0	71.0	71.0	72.0	62.8

[a] In p.p.m. downfield from internal $SiMe_4$ in $(CD_3)_2SO$.
[b] Assignments for these peak positions may be reversed.

Assignments for individual resonances are based on a number of considerations, which are discussed below. In the light of all the previous ^1H n.m.r. studies [10a,10b,11] on derivatives containing a polyhydroxyalkyl chain, it is assumed in the present investigation, that the predominant rotamer state of each polyol in $(CD_3)_2SO$ is the one illustrated. During the course of the discussion justification for these conformers will be provided where possible, based on their ^{13}C and ^1H n.m.r. spectral data.



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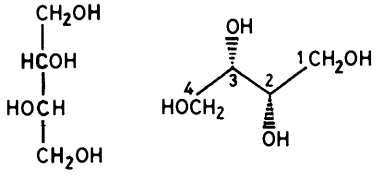

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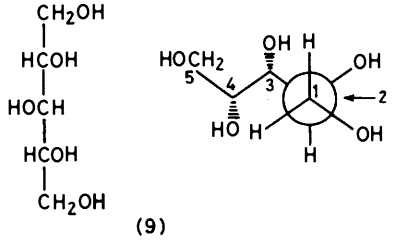

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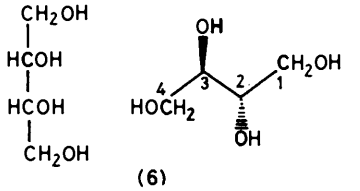

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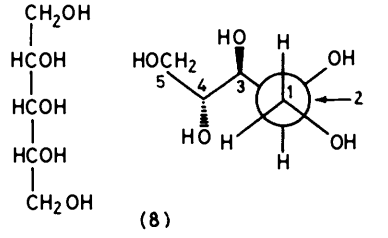

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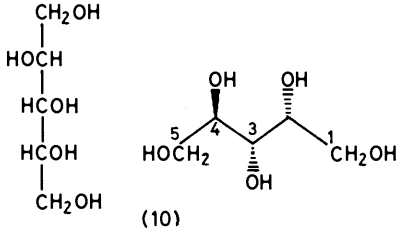

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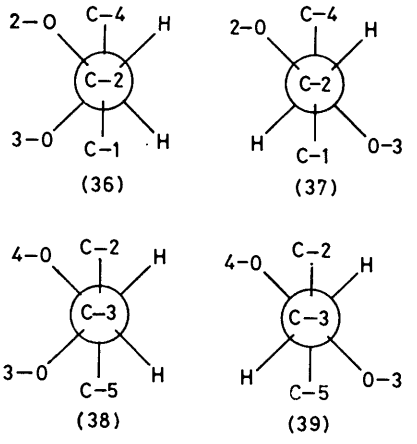
In the ^{13}C n.m.r. spectra of glycerol (5), erythritol (6), L-threitol (7), ribitol (8), and xylitol (9) the signal assignments to primary and secondary carbons were straightforward due to symmetry in these molecules, the signal intensities and their characteristic chemical shifts. Moreover, the present data are also consistent with those reported by Voelter *et al.*[9a] Comparison of the chemical-shift data of D-glyceraldehyde diethyl dithioacetal (17) and D-xylose diethyl dithioacetal (18) with those of glycerol (5) and xylitol (9), respectively, reveals that substitution effects are reflected in a significant change in shieldings at the substituted α-carbon and the adjacent β-carbon; remote carbons (γ, δ, or ε) experience negligible shielding changes (see Table 2). Accordingly, in the ^{13}C n.m.r. spectrum of D-arabinitol (10) a complete, unequivocal signal assignment was facilitated by a comparison with the chemical shift parameters for D-arabinose diethyl dithioacetal (19).

An examination of the ^{13}C n.m.r. chemical-shift data of the tetritols (6) and (7) and pentitols (8)—(10) revealed the following interesting features, which may partly be regarded as the manifestation of their conformational behaviour in $(CD_3)_2SO$. (*a*) In the spectrum of L-threitol (7), the C-3 signal was shifted upfield (0.6 p.p.m.), relative to the corresponding signal in the spectrum of erythritol (6). Considering projections (36) and (37) for (7) and (6), respectively, in their most preferred planar zig-zag conformations, the observed upfield shift of 0.6 p.p.m. could be attributed to the *gauche* and *anti*, respectively, orientations of O-2 and O-3. (*b*) The C-3 signal of xylitol (9) is shifted upfield (2.5 p.p.m.) with respect to the corresponding signal in the spectrum of ribitol (8). The magnitude of this shielding clearly suggests non-planar or ' sickle ' con-

formations for both xylitol and ribitol. If, however, both adopted the planar zig-zag conformations then the upfield shift should be *ca.* 1.2 p.p.m. This is evident by



consideration of projections (**38**) and (**39**) for xylitol and ribitol, respectively, in their planar zig-zag conformations; the difference in nuclear interactions in (**38**) and (**39**) is identical to that between (**36**) and (**37**). (*c*) C-3 in D-arabinitol (**10**) is shielded (2.2 p.p.m.) with respect to C-3 in ribitol (**8**) and shows nearly identical shielding with respect to C-3 in xylitol (**9**). If, however, xylitol and ribitol were to adopt the planar zig-zag conformation adopted by D-arabinitol (**10**) then the shielding order, based on consideration of the O-2–O-3 and O-3–O-4 interactions, would be C-3(xylitol) > C-3(D-arabinitol) >C-3(ribitol).

Carbon-13 signal assignments in the spectra of D-mannitol (**11**), D-glucitol (**12**), and D-galactitol (**13**) were readily made by comparing their spectral data with those of the diethyl dithioacetals of D-mannose (**20**), D-glucose (**21**), and D-galactose (**22**), respectively. The chemical-shift correlations (see Table 2) are in agreement with those observed earlier for compounds (**17**)—(**19**). In the case of D-glucitol (**12**), the signal assignments were further corroborated by the availability of ^{13}C n.m.r. spectral data on 6-deoxy-D-glucitol (**23**), 2-deoxy-D-*arabino*-hexitol (**24**), D-glucose dimethyl acetal (**25**), and 2-acetamido-2-deoxy-D-glucitol (**26**). Examination of the chemical shift correlations (Table 3) reveals that substitution effects are manifested at the substituted α-carbon, and the adjacent β- and γ-carbons; remote carbons (δ and ε) experience negligible shielding changes.

D-Arabinitol (**10**), D-mannitol (**11**), and galactitol (**13**) all adopt the planar zig-zag conformation, whereas D-glucitol (**12**) adopts the ' sickle ' conformation with C-1 *exo*-planar. In these compounds, conformational and configurational identity of a certain fragment of the molecule may be reflected in identical chemical shifts for carbons experiencing identical nuclear interactions. Thus, considering the C-2—C-5 fragment of D-arabinitol (**10**) and the C-3—C-6 fragment of D-mannitol (**11**) and D-glucitol (**12**), the environment for C-4 in (**10**) is identical to that of C-5 in both (**11**) and (**12**); this is manifested in their ^{13}C shieldings being identical (see Table 1). The nearly identical chemical shifts for C-3 in the spectra of D-mannitol (**11**) and galactitol (**13**), and of C-2 in galactitol (**13**) and D-arabinitol (**10**) are further examples emphasizing that in the present series of acyclic poly-

TABLE 2

^{13}C Chemical-shift data [a] for the aldose dithioacetals

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-other
(17)	54.2	74.9	63.2				24.8 (CH_2); 14.6 (CH_3)
	(+8.8) [b]	(−2.5)	(−0.2)				
(18)	54.4	74.2	70.8	72.6	62.8		24.4 (CH_2); 14.4 (CH_3)
	(+8.2)	(−1.8)	(−0.7)	(−0.2)	(−0.2)		
(19)	54.5	71.6 [c]	70.5	71.4 [c]	63.6		23.8, 24.2 (CH_2); 14.4 (CH_3)
	(+8.3)	(−1.5)	(−0.1)	(0.0)	(0.0)		
(20)	55.0	73.8	69.8 [c]	69.4 [c]	71.4	63.8	24.9 (CH_2); 14.6 (CH_3)
	(+8.7)	(−2.5)	(−0.2)	(+0.2)	(−0.1)	(−0.1)	
(21)	54.1	75.3	69.8	72.1	71.4	63.4	24.4 (CH_2); 14.5 (CH_3)
	(+8.3)	(−1.8)	(−1.0)	(0.0)	(−0.1)	(−0.1)	
(22)	54.7	71.6	69.7 [c]	69.4 [c]	70.0	63.2	23.8, 24.2 (CH_2); 14.4 (CH_3)
	(+8.4)	(−1.5)	(−0.5)	(−0.2)	(+0.1)	(−0.1)	

[a] In p.p.m. downfield from internal $SiMe_4$ in $(CD_3)_2SO$. [b] Values in parentheses denote shielding, with respect to the corresponding carbon of the parent alditol; (+) denotes shielding and (−) denotes deshielding. [c] Assignments for these peak positions may be reversed.

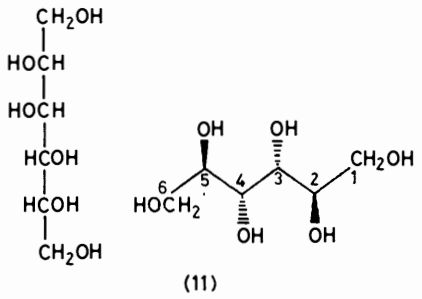
TABLE 3

^{13}C Chemical-shift data [a] of acyclic sugar derivatives (related to D-glucitol)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-other
(23)	62.5	73.5	68.9	75.9	66.4	20.1	
	(−0.1) [b]	(0.0)	(−0.1)	(−3.8)	(+4.9)	(40.2)	
(24)	58.1	36.6	66.7	73.3	71.5	63.6	
	(+4.3)	(+36.9)	(+1.1)	(−1.2)	(−0.2)	(−0.3)	
(25)	104.1	73.6	67.6	72.5	71.3	63.2	53.1, 54.5 (OCH_3)
	(−41.7)	(−0.1)	(+1.2)	(−0.4)	(0.0)	(+0.1)	
(26)	60.7	53.9	68.1	71.7	71.7	63.4	22.8 (CH_3); 169.6 (CO)
	(+1.7)	(+19.6)	(+0.7)	(+0.4)	(−0.4)	(−0.1)	

[a] In p.p.m. downfield from internal $SiMe_4$ in $(CD_3)_2SO$. [b] Values in parentheses denote shielding with respect to the corresponding carbon of D-glucitol.

hydroxy-compounds, carbon nuclei with nearly identical magnetic environments exhibit nearly identical shielding properties. In the light of the present investigation,



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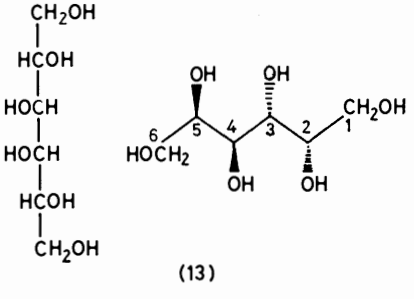

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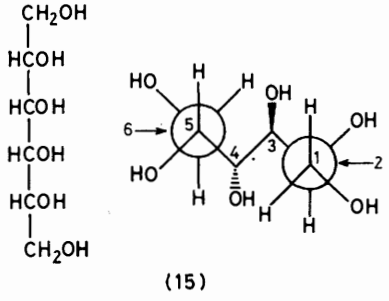

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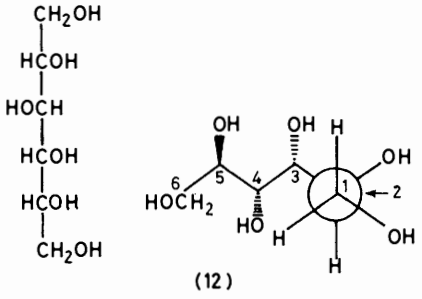
previous chemical-shift assignments to resonances in the ^{13}C n.m.r. spectrum of galactitol (13) made by Voelter *et al.*,[9d] on the basis of methylation studies, seem erroneous.

In D-altritol (14) and allitol (15) the configurational and conformational identity of the C-3—C-6 fragments facilitated signal assignments to C-5 and C-6 in their ^{13}C n.m.r. spectra. Thus, resonances at *ca.* 73.0 and 62.8 p.p.m. were assigned to C-5 and C-6, respectively. Consequently, a complete spectral analysis of allitol (15) was accomplished, because of symmetry in the molecule. Assignments to C-2, C-3, and C-4 in the spectrum of (14) were possible on the basis of the following reasoning. The resonance at 70.5 p.p.m. was assigned to C-2 since it has the same magnetic environment as carbon-2 in D-arabinitol (10) and galactitol (13). On the basis of similar reasoning the C-4 shielding in D-glucitol (12) should be similar to the C-3 shielding in D-altritol (14); thus resonances at 70.9 and 71.8 p.p.m. were assigned to the C-3 and C-4 signals, respectively, in the ^{13}C n.m.r. spectrum of D-altritol (14).

Conformationally L-iditol (16) provides an interesting case, since the more highly populated rotamer state could either be one where C-4, C-5, and C-6 are *exo*-planar (as shown) or one where C-1 and C-6 are both *exo*-planar



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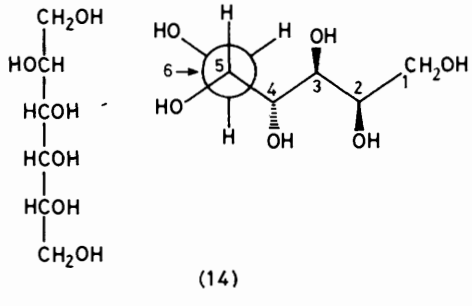

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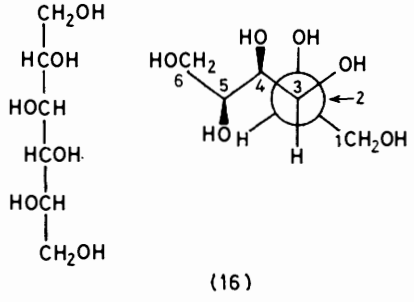
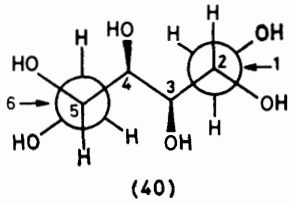


(16)

as shown in (40). An *X*-ray crystallographic study [18f] has clearly shown the preference for the former. Carbon-13 chemical-shift data (Table 1) also point towards this



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conformational behaviour in $(CD_3)_2SO$ as discussed below. Examination of the ^{13}C n.m.r. spectral data of xylitol (9), D-glucitol (12), and L-iditol (16) reveals that the C-1 and C-2 resonances in the spectrum of xylitol (9) closely match the resonances at 62.8 and 72.0 p.p.m., respectively, in the spectrum of L-iditol (16); this reflects configurational and conformational identity of the C-1—C-4 fragment of xylitol with the corresponding fragment of L-iditol. The complete signal assignment for (16) is documented in Table 1.

The conformational flexibility of the acyclic poly-

hydric alcohols in $(CD_3)_2SO$ is clearly manifested in the present investigation, in the observation of some atypical shielding trends in contrast to the trends observed in pyranose sugars [19] and anhydroalditols [6d,20] which possess fixed conformer geometry. Thus, for example, a chemical-shift difference of only 0.6 p.p.m. between the C-2 and C-3 signals in the spectra of L-threitol (7) and erythritol (6) is not comparable to the 3—4 p.p.m. chemical-shift difference observed with similar configurational inversions in the cyclohexane series [21] and pyranose sugars.[19] This discrepancy could partly be attributed to the freely rotating terminal hydroxymethyl groups. Furthermore, observations of a near identity of the chemical shifts for C-2 (erythritol and ribitol), C-2 (L-threitol and xylitol), and C-3 and C-4 (ribitol and allitol) are incompatible with the shielding associated with the *γ-gauche* interaction between 1,3-diaxially disposed H and Me, and H and OH groups in both pyranoid sugars [7a,19] and cyclohexane derivatives.[21,22] A more remarkable violation of this is reflected in the observations that C-3 in D-altritol (14) and C-4 in D-glucitol (12) are both *deshielded* with respect to the C-3 resonance in the spectrum of D-arabinitol (10), in spite of the fact that in both D-altritol and D-glucitol there exists in the predominant rotamer state a *γ-gauche* interaction between H-4 and CH_2OH groups. To account for these observed atypical trends a number of factors may be operative, for example, antiperiplanar nuclear interactions,[23] δ-effects,[24] and rotational freedom of terminal groups.[10e]

1,2-*Bis*(*phenylhydrazones*) *or Phenylosazones*.—The ^{13}C-chemical shifts of the sugar 1,2-bis(phenylhydrazones) studied in this research are documented in Table 4. The

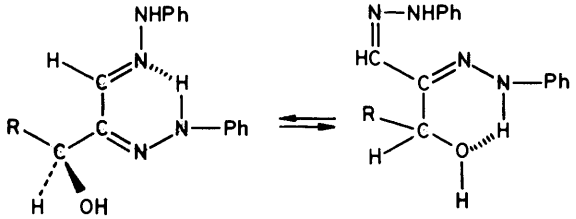
TABLE 4

^{13}C Chemical-shift data [a,b] of sugar 1,2-bis(phenlhydrazones)

Compound	C-1	C-2	C-3	C-4	C-5	C-6
(27)	133.6	136.2	64.1			
(28)	133.3	136.3	74.3	65.0		
(29)	134.1	136.8	74.3 [c]	74.1	63.5	
(30)	134.1	137.1	74.6	73.3	62.7	
(31)	134.5	136.7	74.7	74.2	73.0	63.0
(32)	134.2	136.7	74.2	72.8	71.8	62.7
(33)	133.8	137.5	73.4	71.8	70.2	62.8
(34)	134.5	137.7	74.5	72.2	71.4	63.4
(35)	133.7	136.8	77.4	70.8	36.4	58.1

[a] In p.p.m. downfield from internal $SiMe_4$ in $(CD_3)_2SO$. [b] The chemical shift (p.p.m.) ranges for phenyl carbons are: C-1′, C-1″ (143.9—144.4); C-2′, C-2″ (118.8—112.7); C-3′, C-3″ (129.2—129.4); C-4′, C-4″ (119.6—120.2). See ref. 28. [c] Assignments for these peak positions may be reversed.

signal assignments were made by comparison of their spectra with each other and by considering general shielding properties associated with the various nuclear interactions as discussed above in the alditol series. In the present investigation, spectra of all the 1,2-bis(phenylhydrazones) were recorded prior to the occurrence of any significant amount of mutarotation [25] to the *O*-chelated



structure (42); this phenomenon has been extensively studied by ^1H n.m.r. spectroscopy.[26] Thus, it was safely assumed on the basis of a preliminary investigation * that all the 1,2-bis(phenylhydrazones) in $(CD_3)_2SO$ exist predominantly as the *N*-chelated ring structure (41).

In the ^{13}C n.m.r. spectra of the 1,2-bis(phenylhydrazones) of glyceraldehyde (27), *glycero*-tetrose (28), *erythro*-pentose (29), and *threo*-pentose (30), the most readily assigned signals were those of the terminal primary carbon of the hydroxymethyl group, C-1 and C-2 (easily identified in their coupled spectra) and the phenyl-ring carbons. Signal assignments to C-1 and C-2 are consistent with a ^{13}C n.m.r. study of compounds containing C=N bonds [27] and, moreover, phenyl-carbon resonances were carefully assigned using aniline as a model.[28] Assignment of a resonance at 73.3 p.p.m. in the spectrum of (30) to C-4 was based on the assumption that the *gauche* O-3–O-4 interaction in (30) would shield C-4 with respect to the corresponding carbon of (29); this is in analogy with C-2 and C-3 shieldings in erythritol (6) and L-threitol (7).

In the case of 1,2-bis(phenylhydrazones) (31)—(34), in order to assign ^{13}C chemical shifts in their n.m.r. spectra on the basis of nuclear interactions, it was essential to determine, or infer, the conformation the polyhydroxyalkyl chain would adopt in $(CD_3)_2SO$. Such conformational information was extracted from their ^1H n.m.r. spectra, by measuring the coupling constant ($J_{3.4}$) from the signal of the most deshielded methine proton at C-3,

TABLE 5

Correlation of ^1H coupling constant $J_{3.4}$ (Hz) in 1,2-bis(phenylhydrazones) [a] to conformation of polyhydroxyalkyl chain

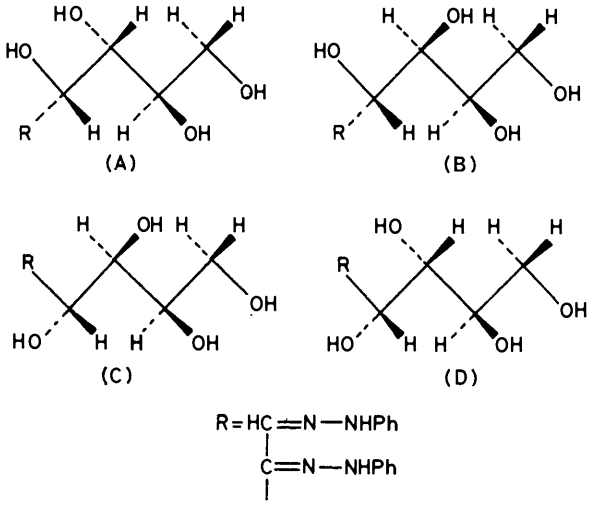
Compound	Phenylosotriazoles [b] $J_{1.2}$	Quinoxaline derivatives [c] $J_{1.2}$	1,2-Bis(phenylhydrazones) [d] $J_{3.4}$
L-*ribo*-Hexose (31)			*ca.* 4.0 (A)
L-*xylo*-Hexose (32)	5.6	3.5	*ca.* 6.0 (B)
D-*lyxo*-Hexose (33)	8.4	8.0	*ca.* 9.0 (C)
D-*arabino*-Hexose (34)	<2.0	*ca.* 1.0	<2.0 (D)

[a] Spectra recorded in $(CD_3)_2SO$–D_2O (this work). [b] Spectra recorded in $(CD_3)_2SO$–HCl (ref. 10*a*). [c] Spectra recorded in $(CD_3)_2SO$ (ref. 10*h*). [d] Letters correspond to the following preponderant rotamer states.

in a manner analogous to that employed in a parallel study on acyclic phenylosotriazoles [10g] and quinoxaline derivatives.[10h] Coupling constants ($J_{3.4}$) were readily

* In every case mutarotation was detectable by recording their ^1H [26] and ^{13}C n.m.r. spectra after allowing the $(CD_3)_2SO$ solution to stand at room temperature over a period of 2—4 weeks. From the complexity of the ^{13}C n.m.r. spectrum it could be inferred that in some cases more than two equilibrating species were present, which made unambiguous signal assignments difficult.

measured from their ^1H n.m.r. spectra, which were recorded after an overnight exchange with D_2O in



$(CD_3)_2SO$. A correlation between coupling constant ($J_{3,4}$) and the most highly favoured rotamer state is tabulated in Table 5. The results are in good agreement with related studies on phenylosotriazoles and quinoxaline derivatives. In the case of (33) and (34) coupling constants ($J_{3,4}$) of *ca.* 9.0 and <2.0 Hz, respectively, clearly reflect the planar zig-zag conformations in solution, whereas in (31) and (32) the magnitudes of *ca.* 4.0 and *ca.* 6.0 Hz, respectively, for the coupling constant ($J_{3,4}$) imply minor contributions from the other rotamer state where C-6 is *exo*-planar and the C-1–C-2 fragment is in the plane of C-3, C-4, C-5.[10e] The above results suggest that the polyhydroxyalkyl chain in compounds (31)—(34) are conformationally similar to the C-3–C-6 fragments of allitol (15), L-iditol (16), galactitol (13), and D-mannitol (11), respectively.

In the ^{13}C n.m.r. spectra of 1,2-bis(phenylhydrazones) (31)—(34) the most readily assigned signals were those of C-1 and C-2 (easily distinguished in their ^1H-coupled spectra), C-6, and the phenyl-ring carbons, since they have characteristic chemical shifts. Assignment of C-3, C-4, and C-5 in the ^{13}C n.m.r. spectra of both phenylosazones (31) and (32) were consistent with the nuclear interactions as discussed previously for xylitol and ribitol. It is interesting to note that C-3 in (31) and (32) is *deshielded* with respect to other carbons irrespective of configurational dissimilarity of the C-3–C-5 fragment. Since both *lyxo*- (33) and *arabino*-phenylosazones (34) adopt planar zig-zag conformations, the C-4 and C-5 signals in the ^{13}C n.m.r. spectrum of the former would appear at highest field with respect to the corresponding signals in (34) because of the more shielding *gauche* O-4–O-5 interaction present in (33). Assignments based on these considerations (see Table 4) are further corroborated by the observation of identical C-5 chemical shifts in diethyl dithioacetals (22) and (20) compared with those of (33) and (34), respectively. Furthermore, the availability of a ^{13}C n.m.r. spectrum of the 1,2-bis(phenylhydrazone) (35) of 5-deoxy-D-*threo*-hexose further substantiates the chemical shift assignments in (32) and (34), on the basis of the observed α-, β-, and γ-shifts of approximately +35.0, −2.0, and +3.0 p.p.m., respectively, experienced on replacement of a hydroxy group by a hydrogen.

TABLE 6

Correlation of ^{13}C-chemical shifts of signals due to C_3—C_6 of the 1,2-bis(phenylhydrazones) with corresponding carbons of the hexitols

1,2-Bis(phenylhydrazone) derivative	Carbon	Δδ (p.p.m.) * D-Mannitol (11)	D-Glucitol (12)	Galactitol (13)	D-Altritol (14)	Allitol (15)	L-Iditol (16)
L-*ribo*-Hexose	C-3	−5.4	−5.9	−5.5	−2.9	−2.2	−3.7
	C-4	−4.6	−2.1	−5.0	−3.3	−1.7	−3.2
	C-5	−1.7	−1.7	−2.9	−0.0	+0.1	−1.0
	C-6	+0.7	+0.3	+0.1	−0.2	+0.2	+0.2
L-*xylo*-Hexose (32)	C-3	−4.6	−5.4	−5.0	−2.4	−1.7	−3.2
	C-4	−3.2	−0.7	−3.6	−2.9	−0.3	−2.8
	C-5	−0.5	−0.5	+1.7	+1.2	+1.3	−0.2
	C-6	+1.0	+0.6	+0.6	+1.1	+0.1	+0.1
D-*lyxo*-Hexose (33)	C-3	−3.8	−4.6	−4.2	−1.6	−0.9	−2.4
	C-4	−2.2	+0.3	−2.6	−1.9	+0.7	−0.8
	C-5	+1.1	+1.1	−0.1	+2.8	+2.9	+1.8
	C-6	+0.9	+0.5	+0.3	0.0	0.0	0.0
D-*arabino*-Hexose (34)	C-3	−4.9	−5.7	−5.3	−2.7	−2.0	−3.5
	C-4	−2.6	−0.1	−3.0	−1.3	+0.3	−1.2
	C-5	−0.1	−0.1	−1.0	+1.6	+1.7	+0.6
	C-6	+0.3	−0.1	−0.3	−0.6	−0.6	−0.6

* (+) Denotes shielding and (−) denotes deshielding.

The possible application of ^{13}C n.m.r. spectroscopy in determining the regiochemistry of microbial oxidation of alditols by *Acetobacter suboxydans* was evident from the chemical-shift correlations (Table 6) between shieldings of the C-3—C-6 carbons in the 1,2-bis(phenylhydrazones) and the corresponding carbons of alditols (11)—(16). This chemical-shift correlation (Table 6) is based on the assumption that in a hypothetical transformation of a hexitol to a hexose 1,2-bis(phenylhydrazone), in the ^{13}C n.m.r. spectrum of the latter only C-3 (α) and C-4 (β) would experience significant deshielding, whereas the more remote C-5 (γ) and C-6 (δ) would remain almost unaffected, provided the C-3—C-6 polyhydroxy-alkyl

chain in both the compounds is conformationally identical in solution. The validity of this assumption is further provided in the previously discussed correlations regarding diethyl dithioacetals (see Table 2). Accordingly, examination of Table 6 reveals that in the ^{13}C n.m.r. spectra of 1,2-bis(phenylhydrazones) (31)—(34) the C-5 and C-6 chemical shifts are almost identical to those of the corresponding carbons in allitol (15) [or D-altritol (14)], L-iditol (16), galactitol (13), and D-mannitol (11), respectively. These results are in agreement with the preceding ^1H n.m.r. observations (see Table 5) which also indicated that the C-3—C-6 polyhydroxyalkyl chain in 1,2-bis(phenylhydrazones) (31)—(34) is conformationally identical to the corresponding fragments in alditols (15) [or (14)], (16), (13), and (11), respectively, in solution. In conclusion, the above correlation facilitates the determination of the regiospecificity of *Acetobacter suboxydans* oxidation in unsymmetrical alditols such as D-glucitol (12), D-mannitol (11), and D-altritol (14). However, ^{13}C n.m.r. spectroscopy fails to unequivocally establish the site of oxidation in symmetrical alditols such as allitol (14).

EXPERIMENTAL

Carbon-13 n.m.r. spectra were recorded in $(CD_3)_2SO$, unless otherwise stated, on a Bruker HX-60 spectrometer equipped with an FT60M Fourier-transform accessory at 15.09 MHz, with tetramethylsilane as an internal standard; chemical shifts are given in p.p.m. downfield from $SiMe_4$. ^{13}C-^1H-coupled spectra were recorded using the gated decoupling technique. The ^1H n.m.r. spectra were recorded on a Varian EM-360 spectrometer; all samples were run as 10—20% solutions in $(CD_3)_2SO$ containing $SiMe_4$ as internal standard.

Glycerol (5), erythritol (6), ribitol (8), D-mannitol (11), D-glucitol (12), and galactitol (13) were obtained commercially. Xylitol (9), D-arabinitol (10), allitol (15), 2-acetamido-2-deoxy-D-glucitol (26), 2-deoxy-D-*arabino*-hexitol (24), and 6-deoxy-D-glucitol (23) were prepared from the corresponding aldoses according to the procedure of Wolfrom and Thompson.[29] Published procedures were employed in the preparation of D-altritol (14),[30] L-iditol (16),[31] the 1,2-bis(phenylhydrazones),[32] and the aldose diethyl dithioacetals.[33] L-Threitol (7) was prepared by a modification of the published procedure [34] as follows. L-*glycero*-Tetrulose, obtained by oxidation of erythritol (6) by *Acetobacter suboxydans*, was reduced with sodium borohydride in water to give a mixture of erythritol (6) and L-threitol (7). This mixture was selectively oxidized by *Acetobacter suboxydans*, and the contaminating L-*glycero*-tetrulose was removed using an anion-exchange resin in the bisulphite form.[35]

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