Infra-red Spectra of Carbohydrates. Part I. Some Derivatives of D-*Glucopyranose.*

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It is confirmed that determinations of infra-red spectra offer powerful means for the comparison of supposedly identical samples of carbohydrates. Moreover, spectra over the frequency range 730—960 cm.$^{-1}$ enable derivatives of D-glucopyranose to be assigned to either the α- or the β-series, no matter whether they are reducing sugars, methyl glucosides, or polysaccharides; thus, α-anomers absorb at 844 ± 8 cm.$^{-1}$, and β-anomers at 891 ± 7 cm.$^{-1}$. In addition, an indication may be obtained of the positions of the glycosidic linkages in a polyglucosan. The forms of the vibrations responsible for absorption in this region are discussed briefly.

Previous work has shown that infra-red absorption spectra can be used to establish the identity of, or to distinguish between, two carbohydrate samples (Kuhn, *Analyt. Chem.*, 1950, **22**, 276; Fletcher and Diehl, *J. Amer. Chem. Soc.*, 1952, **74**, 5774; Stevenson and Levine, *Science*, 1952, **116**, 705). Also the main spectral range has been sub-divided into more restricted regions, related to absorption by –OH, C=O, C–H stretching and bending vibrations, etc. (Thompson, Nicholson, and Short, *Discuss. Faraday Soc.*, 1950, **9**, 222; Forziati and Rowen, *J. Res. Nat. Bur. Stand.*, 1951, **46**, 38). More detailed study has been made of the 3300 cm.$^{-1}$ region of the cellulose spectrum in relation to hydrogen bonding and crystallinity (Brown, Holliday, and Trotter, *J.*, 1951, 1532), and of the C=O stretching region, and other bands, in the spectra of chitin, hyaluronic acid, and similar substances (Orr, Harris, and Sylvén, *Nature*, 1952, **169**, 544; Darmon and Rudall, *Discuss. Faraday Soc.*, 1950, **9**, 251). Knowledge of the lower frequencies, at which ring C–C and C–O stretching vibrations are likely to be active, has been obtained by Burket and Melvin (*Science*, 1952, **115**, 516), who correlated the increased intensity of absorption at 794 cm.$^{-1}$ by some dextrans with the proportion of periodate-resistant units, probably involving glucose units linked through position 3 (see also Abdel-Akher, Hamilton, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 4970; Lohmar, *ibid.*, p. 4974; Jeanes and Wilham, *ibid.*, p. 5339; Barker, Bourne, Bruce, and Stacey, *Chem. and Ind.*, 1952, 1156). The present work (for a preliminary report see Barker, Bourne, Stacey, and Whiffen, *Chem. and Ind.*, 1953, 196), which is confined to the frequency range 730—960 cm.$^{-1}$, confirms this assignment, and extends the use of this region for the characterisation of glucose polymers. It also affords a method for differentiating between α- and β-anomers in the glucose series.

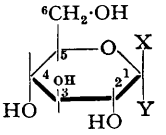
Experimental

The spectra were measured with a Grubb–Parsons single-beam spectrometer, with a sodium chloride prism, the "Nujol" mull technique being used. Most of the mono- and di-saccharides were crystalline, but some of the oligosaccharides were obtained by freeze-drying aqueous solutions, and consisted presumably of mixtures of the α- and β-anomeric forms. The polysaccharides were usually freeze-dried, ground in ether to give a more suitable physical state, and dried again. The Tables show the frequencies (cm.$^{-1}$) of the absorption bands, together with indications of their relative intensities, and comments if the bands were especially broad. A few specimen absorption curves are shown in Fig. 1.

Discussion

Identification of Anomers.—The most striking feature of the set of spectra is the absorption peak (type 2*a*) at *ca.* 844 cm.$^{-1}$, of moderate or strong intensity, which can be correlated with the presence of the α-D-glucopyranose structure, as opposed to its β-anomer. This peak, which is displayed by α-D-glucopyranose and its reducing methyl ethers, by methyl α-D-glucopyranoside and its methyl ethers, by derivatives of α-D-glucosamine (Table 1), and by oligo- and poly-glucosans containing α-linkages (Table 3), but not by those compounds

TABLE 1. *Monosaccharides derived from* α-D-*glucopyranose.*



α-D-Glucopyranose : X = H; Y = OH.
β-D-Glucopyranose : X = OH; Y = H.
Methyl α-D-glucopyranoside : X = H; Y = OMe.
Methyl β-D-glucopyranoside : X = OMe; Y = H.

Key : br = broad; f.d. = freeze-dried; m = moderate strength; s = strong; v = very; w = weak.
Means are immediately followed by their standard deviations.

Compound	Frequencies (cm.$^{-1}$) of absorption peaks: OMe, etc.	Type 1	Other peaks	Type 2*a*	Type 3
α-D-Glucopyranose	—	914 s	—	837 s	774 s
3-*O*-methyl-	951 m	916 s	—	838 m	749 m
6-*O*-methyl-	947 m	923 s	—	846 m	772 m
3 : 4-di-*O*-methyl-	947 m; 935 m	919 m	890 vw (2*b* ?)	843 m	763 m
2 : 3 : 6-tri-*O*-methyl-	945 s	919 w	—	854 s	762 s
2 : 4 : 6-tri-*O*-methyl-	961 s; 945 m; 928 w	919 w; 909 w	—	850 s	769 vs
2 : 3 : 4 : 6-tetra-*O*-methyl-	961 s; 951 m; 928 vw	919 w	903 vw; 888 w (2*b* ?)	850 s	767 s
2-amino-2-deoxy-, hydrochloride	(933) w	909 m	884 m	850 m	770 m
2-acetamido-2-deoxy-	(923) w	909 m	886 w	855 s	781 m; 768 w
2-amino-2-deoxy-3-*O*-methyl-, hydrochloride	—	911 w	901 m	850 m	765 w
Mean	—	915 ± 5	—	847 ± 6	767 ± 8
Methyl α-D-glucopyranoside	—	896 s	—	840 s	745 s
2-*O*-methyl-	951 m	901 s	—	833 s	748 s
2 : 3-di-*O*-methyl-	954 s br	901 s; 892 m	—	840 s	739 s
2-acetamido-2-deoxy- *	951 s; 925 w	896 s	857 w	840 m	758 m
2-acetamido-2-deoxy-3-*O*-methyl-	951 m	914 m; 901 m	—	854 m	758 w
2-acetamido-2-deoxy-3 : 4 : 6-tri-*O*-methyl-	951 m; 945 m	907 m; 896 m	—	843 m	758 m
Mean	—	900 ± 8	—	842 ± 7	751 ± 8

* Shows peaks also at 778 (w) and 765 (w) cm.$^{-1}$.

TABLE 2. *Monosaccharides derived from* β-D-*glucopyranose.*

Compound	Frequencies (cm.$^{-1}$) of absorption peaks: OMe, etc.	Type 1	Type 2*b*	Other peaks	Type 3
β-D-Glucopyranose	—	909 m	896 vs	856 w (2*a* ?)	—
2-*O*-methyl-	949 s	912 w	901 s	859 vw (2*a* ?)	—
2 : 3-di-*O*-methyl-	961 s; 945 s	914 m	888 m	872 w	789 m br; 768 m br
2-amino-2-deoxy-3 : 4 : 6-tri-*O*-methyl-, hydrochloride	956 w; 940 s	923 w	901 m	—	—
Mean	—	914 ± 6	896 ± 6	—	—
Methyl β-D-glucopyranoside	961 s	—	884 s	—	783 m
4-*O*-methyl-	959 m; 938 m	914 vw	896 w	872 m	765 vw
6-*O*-methyl-	958 m	914 s	886 m	—	768 vw
3 : 4-di-*O*-methyl-	949 m; 938 s	921 m	880 s	—	—
4 : 6-di-*O*-methyl-	966 s; 951 m; 947 w	—	905 m	—	—
2 : 3 : 4-tri-*O*-methyl-	936 s br	—	892 m	—	—
2 : 3 : 4 : 6-tetra-*O*-methyl-	955 m; 935 m	—	905 m	—	—
2-acetamido-2-deoxy-	935 m	—	896 m; 890 m	—	—
2-acetamido-2-deoxy-4 : 6-di-*O*-methyl-	947 m	923 m	888 m	—	—
2-acetamido-2-deoxy-3 : 4 : 6-tri-*O*-methyl-	949 m; 938 w; 928 w	—	884 m	—	—
Mean	—	918 ± 5	891 ± 8	—	772 ± 9

TABLE 3. *Higher saccharides derived from* α-D-*glucopyranose.*

Compound	Principal type of glucosidic linkage	Frequencies ($cm.^{-1}$) of absorption peaks: Type 1	Other peaks	Type 2*a*	Other peaks	Type 3
β-Maltose	α-1 : 4	907 s	894 s (2*b*)	846 s	—	778 s
Maltotriose (f.d.)	,,	919 m vbr	—	834 m	—	767 w
Maltotetraose (f.d.)	,,	927 m br	—	861 w; 840 m	—	763 m
Amylose (potato)	,,	938 s vbr	—	857 m; 838 m	—	756 m
Amylopectin (potato)	α-1 : 4; α-1 : 6-branches (trace)	931 s br	—	840 m br	—	756 m
Glycogen (*Mytilis*)	,, ,,	925 s br	—	840 m br	—	758 m
Glycogen (rabbit liver)	,, ,,	928 m br	—	835 m br	—	762 w
Glycogen (*Neisseria perflava*)	,, ,,	928 s br	—	840 m br	—	760 w
β-Dextrin (potato)	,, ,,	928 s br	—	840 m br	—	758 w
*cyclo*Maltohexaose	α-1 : 4 (cyclic)	949 m; 938 s	896 w; 872 vw	859 w; 840 m	797 vw	760 m; 746 w
*cyclo*Maltoheptaose	,,	938 s br	890 w	855 s; 841 s; 826 vw	792 w	773 m; 763 m; 752 m
Mean		930 ± 9		843 ± 10		761 ± 8
α-Panose	α-1 : 4; α-1 : 6	919 vs	—	862 w; 846 s	790 m	775 s; 756 s
*iso*Maltose (f.d.)	α-1 : 6	919 m br	—	838 w	—	768 m
*iso*Maltotetraose (f.d.)	,,	919 m br	—	840 w	—	770 m
Dextran (B-512)	α-1 : 6; α-1 : 4-branches	919 m br	—	840 m	—	768 m
Dextran (*Acetobacter capsulatum*)	,, ,,	914 m br	—	837 m	—	766 m
Dextran (*Betacoccus arabinosaceous*)	α-1 : 6; α-1 : 3-branches	917 s br	—	841 m	794 w (1 : 3-type 3)	768 m
Dextran (NRRL-B-742)	α-1 : 6; α-1 : 3- and α-1 : 4-branches	914 s br	—	840 m	793 w (1 : 3-type 3)	770 m
Mean	—	917 ± 2	—	839 ± 1	—	768 ± 1
Nigerose (f.d.)	α-1 : 3	919 s br	—	840 w	—	783 m
Trisacc. fraction from nigeran (f.d.)	α-1 : 3; α-1 : 4	919 s br	—	837 m	794 m (1 : 3-type 3)	766 m
Oligosacc. fraction from nigeran (f.d.)	,,	928 s br	—	840 m	794 m (1 : 3-type 3)	768 m
Nigeran (mycodextran)	,,	921 s; 905 w	—	860 m; 840 s	789 s (1 : 3-type 3)	—
αα-Trehalose *	αα-1 : 1	924 vw; 909 s	865 w	850 m; 840 m	—	802 m

* Shows a peak also at 955 $cm.^{-1}$ (s).

TABLE 4. *Higher saccharides derived from* β-D-*glucopyranose.*

Compound	Principal type of glucosidic linkage	Frequencies ($cm.^{-1}$) of absorption peaks: Type 1	Type 2*b*	Other peaks	Type 3
β-Cellobiose	β-1 : 4	925 w br	892 s	—	773 m
α-Cellotriose	,,	931 m	892 m; 884 m	843 m (2*a*)	773 m
β-Cellotetraose	,,	925 w vbr	896 s	—	768 vw
Cellulose (*Acetobacter aceti-genum*)	,,	914 w br; 933 w br	894 w	—	766 vw
Cellulose (cotton)	,,	914 w br; 933 w br	894 w	—	766 vw
Methyl β-cellobioside *	,,	919 w	901 s	—	806 wv vbr
Gentiobiose (f.d.)	β-1 : 6	917 m vbr	894 w	—	770 w
Luteose	,,	919 w br	888 m; 880 w	—	—
α-Laminaribiose	β-1 : 3	919 m	888 m	872 m; 837 vw (2*a*)	778 vw
Laminaritetraose (f.d.)	,,	920 m br	891 s	—	771 vw
Laminarin	,,	917 w br	890 s	—	—
Yeast glucan	,,	919 m br	890 s	—	—
α-Sophorose	β-1 : 2	919 m br	892 m	843 m (2*a*)	765 m
Crown-gall polysaccharide	β-1 : 2	919 w br	888 m; 880 m	—	—
ββ-Trehalose	ββ-1 : 1	919 w	896 s br	—	—
αβ-Trehalose	αβ-1 : 1	923 m br	884 m	843 m (2*a*)	780 m
Mean	—	921 ± 4	890 ± 5	—	774 ± 9

* Shows a peak also at 966 $cm.^{-1}$ (m.).

examined which were devoid of α-D-glucopyranose units (Tables 2 and 4), is remarkably constant in frequency, as is indicated by the small overall standard deviation of ±8 cm.$^{-1}$. It is, however, double in αα-trehalose, as might be expected since interactions are more important when the two reducing groups are joined directly together. Two peaks appear also in this region with maltotetraose, amylose, *cyclo*maltohexaose, *cyclo*maltoheptaose, (*i.e.*, α- and β-Schardinger dextrins), panose, and nigeran; they may arise through coupling of the vibrations of physically adjacent groups in different glucose residues, *e.g.*, between adjacent units in neighbouring turns of the amylose helix. The weak peaks of type 2*a* shown by β-D-glucopyranose and 2-*O*-methyl-β-D-glucopyranose were quite possibly caused by traces of the α-anomers in these samples.

The conclusion is that the appearance of a type 2*a* peak seems to offer a good indication of the presence of an α-unit in a derivative of D-glucopyranose. It is interesting that Spedding and Stamm (*J. Chem. Phys.*, 1942, **10**, 176) found a strong Raman line in α-D-glucose at 842 cm.$^{-1}$, which has no direct counterpart in β-D-glucose, although there is a medium band at 901 cm.$^{-1}$ which might be correlated with the infra-red band at 896 cm.$^{-1}$. The α-anomers invariably displayed two other infra-red absorption peaks in the region under examination (type 1 at 917 ± 13 cm.$^{-1}$ and type 3 at 766 ± 10 cm.$^{-1}$), but, as will be apparent later, these are less useful for diagnosing anomeric character.

All the derivatives of β-D-glucopyranose examined, including reducing monosaccharide methyl ethers, methyl β-D-glucopyranoside and its methyl ethers, derivatives of β-D-glucosamine (Table 2), and oligo- and poly-glucosans containing β-linkages (Table 4), gave an absorption band (type 2*b*) of moderate or strong intensity, which appeared to correspond to type 2*a* but occurred at a different frequency, namely, at 891 ± 7 cm.$^{-1}$. Unfortunately some of the type 1 absorptions of the α-compounds lie in the same range as the type 2*b* absorptions of the β-compounds, and so the presence of a band at *ca.* 891 cm.$^{-1}$ is not conclusive evidence for a β-glucose unit. For this reason, it would be unwise to conclude that absorption at 896 and 890 cm.$^{-1}$ by *cyclo*maltohexaose and *cyclo*maltoheptaose, respectively, is indicative of traces of β-linkages. Some of the β-compounds show absorption of types 1 and 3, with a marked tendency for these bands to be weaker than those in the α-series; in some cases the former band may be overlaid by the stronger type 2*b* band.

Three crystalline disaccharides (α-laminaribiose, α-sophorose, β-maltose) and one crystalline trisaccharide (α-cellotriose), in which the anomeric characters of the reducing groups differed from those of the glucosidic linkages, gave, as would be expected, absorption bands of both types 2*a* and 2*b*, as also did αβ-trehalose.

Polymer Identification.—Although infra-red spectra of all carbohydrates are useful for establishing the identities of samples of doubtful authenticity, they are likely to be particularly useful in providing rapid indications regarding the structures of new polyglucosans, before the final confirmation of such structures by the more classical methods. It has been shown already that the absorption peaks of types 2*a* and 2*b* form a basis for the classification of the principal glucosidic linkages in a polyglucosan as α or β. In addition, it is possible, from a consideration of the bands of types 1 and 3, to obtain information about the positions of such linkages in α-polyglucosans (see Fig. 1); unfortunately, no such correlation is possible with the β-anomers.

The absorptions of types 1 and 3 displayed by α-polyglucosans of the two classes most commonly encountered are: starch class (1 : 4-linkages), 930 ± 4, 758 ± 2; dextran class (1 : 6-linkages), 917 ± 2, 768 ± 1 cm.$^{-1}$. The peak at 793 ± 3 cm.$^{-1}$ given by certain dextrans, and by the nigeran (mycodextran) series of compounds, seems to arise from α-1 : 3-linkages. Polyglucosans containing α-1 : 2-linkages were not available. It is interesting that, whereas in the dextran series there is no systematic movement of the frequencies of absorption bands of the types 1 and 3 in passing from the disaccharide through the oligosaccharides to the polysaccharides, in the starch series there are gradual transitions in frequency from 907 to 930 ± 4, and from 778 to 758 ± 2 cm.$^{-1}$

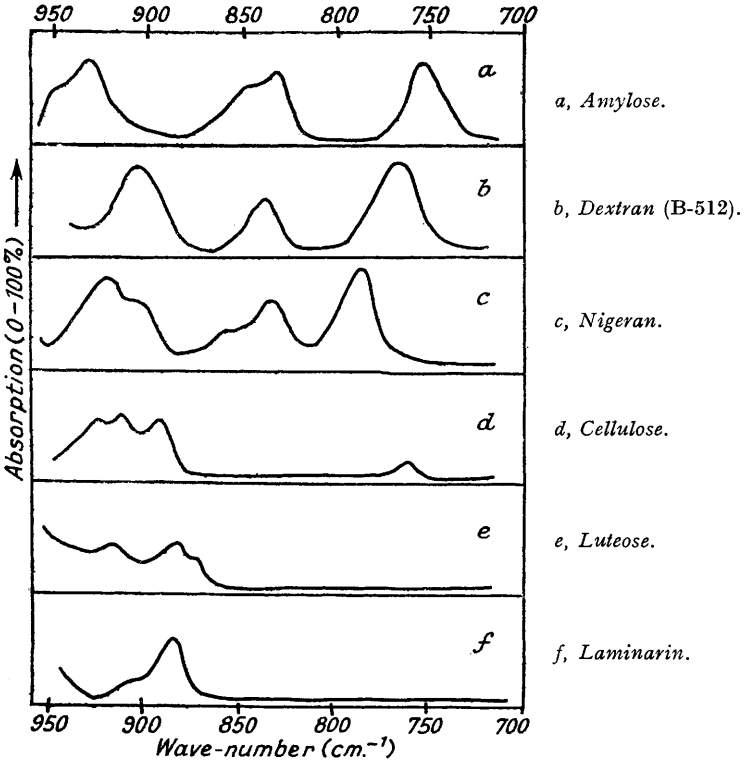
Assignment.—With such complex molecules as the sugars it is hardly to be expected that assignment of many of the observed bands to particular vibrational modes will be possible. The range examined (730—960 cm.$^{-1}$) is likely to include the in-phase C–O stretching mode of methyl ethers, comparable with that of dimethyl ether at 918 cm.$^{-1}$

(Herzberg, " Infra-red and Raman Spectra," D. Van Nostrand Co. Inc., New York, 1945, p. 354), and indeed a number of bands appear in the 930—960 cm.$^{-1}$ range for methylated glucoses, whereas the majority of the unmethylated glucoses are relatively transparent in this region. There remain three principal sets of bands, for which the average values (cm.$^{-1}$) and standard deviations are :

Type 1 : α-anomers, 917 ± 13 ; β-anomers, 920 ± 5.
Type 2 : α-anomers, 844 ± 8 ; β-anomers, 891 ± 7.
Type 3 : α-anomers, 766 ± 10 ; β-anomers, 774 ± 9.

Burket and Badger (*J. Amer. Chem. Soc.*, 1950, **72**, 4397) have interpreted the spectra of tetrahydropyran, which has the same ring structure as pyranose sugars, and have attri-

FIG. 1.



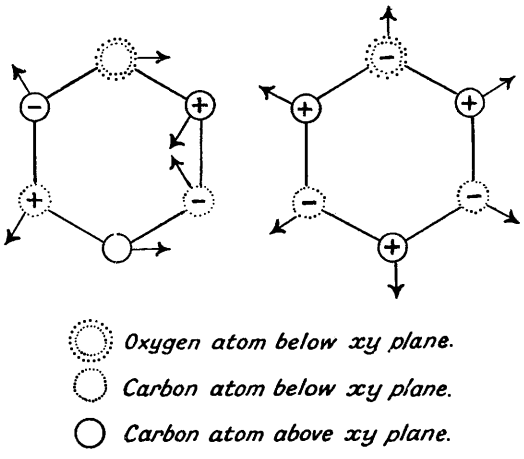
buted the strong infra-red active band at 875 cm.$^{-1}$ to a ring vibration, pictured essentially as shown in Fig. 2. It can be seen that this vibration includes a considerable contribution from the ring C–O–C antisymmetrical stretching, and is therefore likely to be of moderate intensity in the infra-red absorption spectrum. Its frequency will be not very dependent on the configuration at $C_{(1)}$ and type 1 bands are quite possibly due to this mode.

The 856 cm.$^{-1}$ vibration of tetrahydropyran is essentially a CH_2 rocking vibration which can have no counterpart in the substituted rings, but the ring breathing frequency appearing at 813 cm.$^{-1}$, and pictured as in Fig. 3 (Burket and Badger, *loc. cit.*), may plausibly be correlated with type 3 vibrations. Since the stretching of the four ring C–C links contributes to the frequency, but scarcely to the intensity, of the band, and since the small amount of C–OH stretching involved scarcely affects the frequency, but may alter the intensity by virtue of the more dipolar nature of the C–O bonds, it is to be expected that the intensity is much more sensitive to the sugar configuration and to the introduction

of substituents at the hydroxyl groups than is the frequency; this was found experimentally to be so.

Concerning type 2 absorption, it is difficult to believe that frequencies with standard deviations of only ± 8 and ± 7, over sets of compounds in which the groups attached at position 1 vary from hydrogen atoms to polysaccharide chains, are connected closely with a mode involving much motion of the external oxygen atom attached to $C_{(1)}$. This suggests that the motion is one of the C–H deformation modes in which the hydrogen on $C_{(1)}$ is involved intimately. The observed frequencies are not unreasonable for such a case, because a force constant $0{\cdot}46r_{CH}^2 \times 10^5$ dynes/cm. (cf. Herzberg, *op. cit.*, p. 193) leads to a

FIG. 2. FIG. 3.



frequency of 880 cm.$^{-1}$, if the mass and moment of inertia of the glucose structure are taken as infinite. The difference between the α- and the β-glucose frequency would in this case be related to the fact that the hydrogen atom in the α-anomer lies in the equatorial belt of the chair form of the six-membered ring, whereas in the β-anomer it is in the "polar" position. This statement is based on the presumption that the particular chair configuration adopted by the ring is that in which as many non-hydrogen substituents as possible are in the equatorial belt (cf. Reeves, *J. Amer. Chem. Soc.*, 1950, **72**, 1499). An objection to this view is that the hydrogen atoms at positions 2, 3, 4, and 5 do not appear to absorb here, but $C_{(1)}$ is the only carbon atom which carries two oxygen atoms as well as the hydrogen, and this may cause a marked change in force constant or intensity; the spectrum of 1-deutero-D-glucose should settle this point. Further information regarding the validity of these tentative assignments, as well as useful data for characterisations, should be gained from studies of the infra-red spectra of other sugars, and this is now in hand.

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