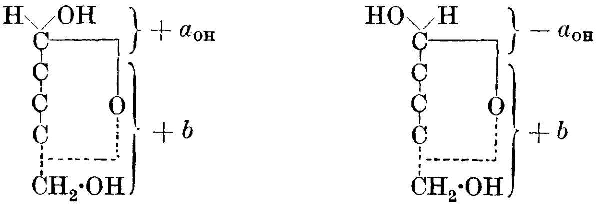
CLVII.—*Ring Structure and Optical Relationships in the Mannose–Rhamnose–Lyxose Series of Sugars. Isolation of a New Form of Lyxose.*

By Walter Norman Haworth and Edmund Langley Hirst.

C. S. Hudson's application to the sugar group of the "principle of optical superposition" suggested by van 't Hoff has been of great service in the study of the complex problems of optical isomerism in the series glucose–galactose–arabinose–xylose. The calculations of molecular-rotation values based on the consideration of two parts of the sugar molecule have led to strikingly concordant results in that series for the estimation of the rotational effect of the groups attached to the reducing carbon atom 1. In an aldose, these groups, $+a_{OH}$ and $-a_{OH}$, correspond to the spatial distribution in the α- and β-isomerides of any one sugar, the remaining portion of the molecule being regarded as b.

(1)

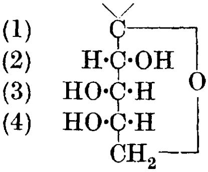


In the methylglucosides, the a value becomes $\pm a_{OMe}$ and in the isomeric acetates $\pm a_{OAc}$, etc. The molecular-rotational increment can be obtained by difference: $(+a_{OH} + b) - (-a_{OH} + b)$, and such differences approximate very closely in the glucose–galactose–arabinose–xylose series to the constant values $a_{OH} = 8{,}500$; $a_{OMe} = 18{,}500$; $a_{OAc} = 19{,}100$, and so on for other types of groupings.

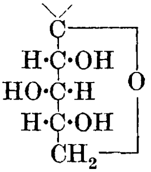
Using these values in those cases where one stereochemical form is unknown, Hudson has been successful in calculating the rotation of the missing form, and in several cases the values so derived have been verified by the subsequent discovery of the new form.

Similarly the magnitude $2b$ can be calculated by addition of the molecular rotations of two α- and β-forms, thus : $(+a_{OH} + b) + (-a_{OH} + b)$. Here the considerations are more complex, and any departures from the regularity of the additive principle are more likely to become manifest. An example is that of the $b_{\text{arabinose}}$ value. According as this is calculated from the observed molecular rotation of (1) the sugar or (2) the methylarabinoside, $b_{\text{arabinose}} = 18{,}700$ or 21,800. A difference of 3,100 (increased to over 4,000 by taking alternative standards) may not be considered serious when the total rotation is large, but the percentage error is likely to be very marked when the total rotation is small. This percentage error is liable to be much exaggerated when the rotational values are dissected to give the individual contributions of the several carbon atoms in the b portion.

A recent attempt has been made by Hudson (*J. Amer. Chem. Soc.*, 1926, **48**, 1424, 1434) to correlate the values for an intermediate grouping in the sugar chain. Thus methylarabinoside (I) and methylxyloside (II) differ only in the orientation of the groups at the fourth carbon atom. Similarly also methylgalactoside (III) and methylglucoside (IV) differ only at C_4 if the ring structure of each be considered identical.



(I.)



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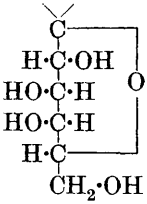

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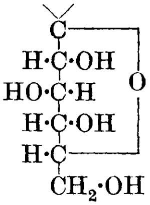


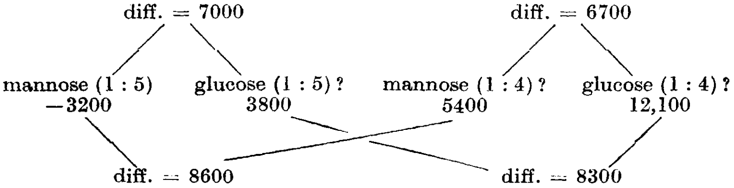
(IV.)

On the basis of the additive principle of optical superposition, Hudson assigns a constant value in each sugar derivative to C_4 which is + or − according as the OH at this carbon atom is directed to the left or to the right. If the rest of the molecule other than C_4 be called p for the pentosides and h for the hexosides, both these factors are eliminated by subtraction. Thus for (I) and (II) we have $+C_4 + p = 40{,}300$ and $-C_4 + p = 25{,}200$, which gives $C_4 = 7{,}550$. Also for (III) and (IV) we have $+C_4 + h$ and $-C_4 + h$. On the chemical evidence of determinations of structure Hudson accepts the six-atom ring for arabinoside (I), xyloside (II), and galactoside (III). He uses the present calculations, however, to test the ring structure of the known α-methylglucoside. Now for (III) $+C_4 + h = 37{,}380$, and from these data the magnitude of (IV), $+C_4 + h = x$, can be ascertained by adopting the above value for C_4, and it is thus found that $x = 22{,}280$. This falls short of the observed molecular rotation of α-methylglucoside (30,630) by 8,350, and corresponds to a specific rotation of +115° for the latter instead of the observed $[\alpha]_D = +\ 158°$.

On these grounds Hudson rejects the six-atom ring structure for α-methylglucoside, which he considers has a five-atom ring. Such a conclusion is at variance with other and direct chemical evidence which we have contributed (Charlton, Haworth, and Peat, J., 1926, 89; Hirst, *ibid.*, p. 350; Haworth, Hirst, and Miller, J., 1927, 2436). Moreover this latter evidence is as convincing as that which Hudson had already accepted for the pentosides and hexoside, (I), (II), and (III), as the basis of these calculations.

The clear inference to be drawn is that C_4 in the pentoside series has a different value from C_4 in the hexoside series. We hold that even on the principle of optical superposition this could only be expected (compare Drew and Haworth, J., 1926, 2303). Hudson prefers, however, the contrary view.

Now it appears that the hypothetical value of $[M]_D$ $(22{,}280) = x$, reached by Hudson, corresponds to a magnitude for b_{glucose} of +3,780, which differs from the observed value of +12,100 by +8,320. This happens also to be about the numerical difference (8,600) between $b_{\text{mannose}\ (\alpha\text{-})} = -3{,}200$ (calculated from α-mannose) and $b_{\text{mannose}\ (\beta\text{-})} = +5{,}400$ (calculated from β-mannose). Hudson here makes the assumption that the agreement between these numerical differences is ascribable to one and the same cause, namely, the differences contributed by a 1 : 5- and a 1 : 4-oxide ring, and assigns, therefore, the six-atom ring (1 : 5) structure to α-mannose on these grounds, and the five-atom ring (1 : 4) structure to β-mannose. He is led to suggest that there should exist not only an α-mannose (1 : 4) and a β-mannose (1 : 5) which are at present unknown, but also two new forms of methylglucoside (1 : 5), α- and β-, as well as two new forms of glucose corresponding to these. The numerical features of the following system would then, approximately, be made to balance :



On the assumption of the possibility of the existence of these unknown sugar forms, the following table of " epimeric differences " can be constructed :

1 : 4	α-methylglucoside (known), $[\alpha]_D$	+158°	diff. = 33°.
	α-methylmannoside (unknown)	+125°	
1 : 5	α-methylglucoside (unknown)	+115°	diff. = 36°.
	α-methylmannoside (known)	+ 79°	
1 : 4	α-methylglucoside tetra-acetate (known)	+130°	diff. = 28°.
	α-methylmannoside tetra-acetate (unknown)	+102°	
1 : 5	α-methylglucoside tetra-acetate (unknown)	+83°	diff. = 34°.
	α-methylmannoside tetra-acetate (known)	+49°	

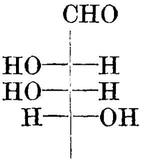
These hypothetical " epimeric differences " are now made the basis, in Hudson's paper, of a calculation of the expected specific rotations of ten compounds related to α- and β-methylglucosides regarded as 1 : 4 (butylene) oxides.

A table of agreement with the observed values for these compounds is then given. The scheme of correlation is dependent upon the fortuitous agreement between the value of b_{glucose} calculated by the above methods and that value of the coefficient (3,500) which is required to make $b_{\text{mannose (1 : 4)?}}$ minus $b_{\text{mannose (1 : 5)}}$ equal to $b_{\text{glucose (1 : 4)?}}$ minus $b_{\text{glucose (1 : 5)?}}$. It will necessarily follow then that the " epimeric differences " $b_{\text{mannose (1 : 5)}}$ minus $b_{\text{glucose (1 : 5)}}$ and $b_{\text{mannose (1 : 4)}}$ minus $b_{\text{glucose (1 : 4)}}$ are also equal to one another.

This is the relationship which lies behind the impressive agreement of the observed and calculated rotations of glucose derivatives given by Hudson in support of his contention that α-methylglucoside is a 1 : 4 oxide. *The only inference that can be drawn from his table is that the rotational value of* C_2, *calculated from appropriately selected standards, is approximately constant.* Support for this view may be found in the rotations of known substances, without introducing any assumptions either of ring structure or of the existence of hypothetical compounds. For example, the real or observed epimeric differences between several of the α-mannose and α-glucose derivatives and between several of the β-mannose and β-glucose derivatives are regular. This particular statistical method of reaching a conclusion on ring structure may therefore be set aside.

Let us consider the optical relationships in the mannose–rhamnose–lyxose series from another point of view. Calculations of the coefficients expressed as a_{OH}, a_{OMe}, b_{mannose}, b_{rhamnose}, b_{lyxose} based solely on the known rotations of compounds in this series, *without reference to glucose and galactose,* have revealed the interesting fact that the rotations of the different forms of the three sugars and of their glucosides may be accommodated with consistency within a single scheme similar to that worked out by Hudson for the glucose–galactose series. Had these sugars initially been more readily available, the possibility would have arisen of their being accepted as standards, and an anomalous behaviour would then have been attributed to the present "standard" series comprising glucose and galactose. It seems, therefore, that the hypothesis of different ring structures in α- and β-mannoses affords little or no assistance in interpreting the experimental observations. In the absence of definite chemical evidence to the contrary we consider it would be most helpful to the future study of the problem to regard α- and β-mannose as being not necessarily dissimilar in ring structure, and to seek the reason for divergences in optical and other physical properties occasioned by the special arrangement of hydroxyl groups in mannose and the related sugars rhamnose and lyxose.

It will be apparent from the foregoing account that further observations on the rotational behaviour of these systems containing the grouping (V) would be of interest. For these reasons we have commenced a detailed study of lyxose and its derivatives, which has resulted in the first place in the discovery of a new crystalline form of lyxose differing markedly from that previously described.



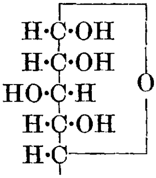
(V.)

At the outset no crystalline lyxose was available, and a specimen was prepared by Weerman's method (*Rec. trav. chim.*, 1917, **37**, 16). This material was recrystallised from alcohol, and the pure lyxose obtained was not the α-variety described by other workers. For this α-form Hudson and Yanowsky (*J. Amer. Chem. Soc.*, 1917, **39**, 1034) found $[\alpha]_D$ +5·5° in water, decreasing to a constant value −14°, and Weerman, who appears to have obtained the same substance, gives m. p. 106—107°. The new variety showed, however, a higher m. p., 117—118°, and was strongly lævorotatory, giving in aqueous solution $[\alpha]_D$ −70°, decreasing in less than 30 minutes to the same constant value as the α-form, −14°. The inter-relationship between the new form and the other form of lyxose was established by the following observations: (1) the equilibrium value of the specific rotation was $[\alpha]_D$ −14°; (2) the *p*-bromophenylhydrazone, m. p. 155—157°, and the osazone, m. p. 157—160°, were identical with the corresponding derivatives prepared from α-lyxose; (3) the new variety was converted in good yield into crystalline α-methyl-lyxoside, m. p. 108°, $[\alpha]_D$ +59°, which has been described by Phelps and Hudson (*J. Amer. Chem. Soc.*, 1926, **48**, 503).

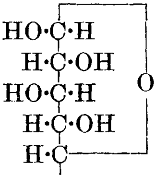
The molecular-rotation difference between α-lyxose and the new variety is 11,000, comparable with 8,500 and 10,000, respectively, for mannose and rhamnose. The analogy with mannose was further extended by measuring the velocity coefficient for the mutarotation of the new form of lyxose : the course of the change followed the unimolecular law and the velocity coefficient was approximately equal in value to that found by Hudson and Yanowsky for α-lyxose. Riiber and Minsaas (*Ber.*, 1927, **60**, 2402) have observed similar regularities in the mutarotation of α- and β-mannose.

The simplest and most obvious interpretation of these results is to regard the new substance as the β-form of lyxose, corresponding to the α-form in the same way as does β-mannose to α-mannose, inasmuch as similar and anomalous optical behaviour is shown by lyxose, mannose and rhamnose. This is the explanation favoured by the present authors. The situation is complicated, however, by the fact that the specific rotation of the hitherto unknown β-lyxose has been calculated by Hudson and Yanowsky (*loc. cit.*), whose estimated value, obtained by their solubility method, is $[\alpha]_D$ −36° instead of our observed value −70°. The discrepancy may be viewed from another angle by considering the proportion in which α- and β-lyxose are considered by Hudson to be present in the equilibrium mixture in 90% alcohol. This is given as 68% α- to 32% β- and it is significant that the proportion of α- is markedly less than is the case for either mannose or rhamnose. There appeared to be no particular reason for such a divergence and we therefore decided to repeat the solubility experiments, using the properties of the new β-form to calculate the rotation of the α-form. We obtained for α-lyxose the calculated value of $[\alpha]_D$ +5°, which is very close to the observed value +5·5°. This corresponds to an equilibrium mixture (in 90% alcohol) containing 83% of the α-form.

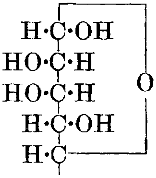
When the α- and β-forms of a sugar are considered from the point of view of the *cis*- and *trans*-relationships of the hydroxyl groups on the first and second carbon atoms, a striking regularity is manifested. This may be expressed by the statement that in the equilibrium mixture of the two isomerides the tendency is for the *trans*-form to predominate.



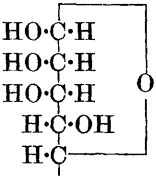
α-glucose (*cis*)



β-glucose (*trans*)



α-mannose (and rhamnose and lyxose) (*trans*)



β-mannose (and rhamnose and lyxose) (*cis*)

When there are two *cis*-hydroxyl groups as in mannose, rhamnose, and lyxose, the preponderance of the *trans*-form at equilibrium tends to be greater in alcohol than in water, whereas the reverse is the case when the hydroxyl groups on carbon atoms 2 and 3 are *trans* to one another. Detailed figures are given in the accompanying table,* where column (B) shows the percentage of one form in the equilibrium mixture, as obtained by direct measurement in Hudson and Yanowsky's solubility experiments. The figures for lyxose are, however, those obtained during the present investigation. In column (A) is given the percentage of the *trans*-isomeride in the equilibrium mixture in aqueous solution, the figure being calculated from the known rotation values for the pure forms on the assumption that the equilibrium mixture contains only the α- and β-forms of the sugar. (The evidence at present available shows that there is little reason for doubting the essential truth of this assumption.) In addition, the fact that α-glucose and α-mannose are respectively *cis*- and *trans*-compounds in this sense may perhaps serve to explain the various small differences in certain physical properties noted by Riiber and Minsaas (*loc. cit.*) when comparing glucose and mannose.

Sugar.	*trans*- Variety.	(A) Proportion of *trans*-form at equilibrium in water (%).	(B) Proportion of *cis*-form present in aqueous alcohol (%).
Glucose	β-	66	44·7 α-
Galactose	β-	69	41·8 α
Xylose	β-	65	44 α-
Arabinose	α-	58	38 β-
Lyxose	α-	75	17 β-
Mannose	α-	62	18 β-
Rhamnose	α-	73	15 β-
α-Glucoheptose	β-	89	12 α-

Since the proportion of the α- and β-forms at equilibrium is intimately connected with the relative solubilities of the two varieties, it is apparent from the above table that there is a relationship between the *cis*- and *trans*-nature of the hydroxyl groups and the relative solubilities of the α- and β-forms. This is most pronounced in those cases which afford a possibility for one isomeride to have three adjacent *cis*-hydroxyl groups (mannose, lyxose, rhamnose), and an accumulation of two or three such groupings gives precisely the conditions appropriate for the formation of co-ordinate linkages. The question whether such linkages are formed is obviously a matter for further inquiry. We are endeavouring to test this idea as a possible means of explaining both the solubility relationships amongst the α- and β-forms of sugar derivatives and the optical anomalies displayed in the mannose series when compared with the glucose–galactose series.

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* A similar table can be constructed for the disaccharides melibiose, maltose, lactose, and cellobiose, where in every case the *trans*-form is present in excess at equilibrium.

EXPERIMENTAL.

Preparation of Lyxose.—A pure specimen of lyxose was obtained from crystalline galactonolactone by following, with slight modifications, the directions of Weerman (*loc. cit.*). Under the conditions described, difficulty was experienced in separating the lyxose, in the form of its insoluble *p*-bromophenylhydrazone, from the accompanying mineral salts. In order to carry out this operation successfully the lyxose solution must be concentrated and the *p*-bromophenylhydrazine must be dissolved in the minimum quantity of alcohol. If necessary, the solution containing lyxose *p*-bromophenylhydrazone is evaporated to dryness and the mineral salts are removed by solution in water. Lyxose *p*-bromophenylhydrazone is most easily purified by recrystallisation from hot water.

The lyxose regenerated from the *p*-bromophenylhydrazone was in the form of a syrup. This was exposed to the air from time to time and rubbed with a glass rod, until at the end of 14 days rapid crystallisation commenced. Recrystallisation from alcohol gave needles, m. p. 117—118°, which were slightly hygroscopic (compare Ruff and Ollendorf, *Ber.*, **1900**, **33**, 1798).

After several preliminary experiments by Weerman's and by Ruff and Ollendorf's method the following procedure was found to be the most convenient for the preparation of larger quantities of lyxose. Calcium galactonate (Clowes and Tollens, *Annalen*, 1900, **310**, 166), which did not require to be separated completely from calcium bromide, was treated with hydrogen peroxide and ferric acetate according to the method of Ruff and Ollendorf (*loc. cit.*). After removal of the greater portion of the calcium salts in the way described by these authors, the pentose content of an aliquot portion of the resulting alcoholic solution of lyxose was estimated by the furfural method. The remainder of the alcoholic solution was then concentrated to small bulk and the calculated quantity of *p*-bromophenylhydrazine, dissolved in the minimum quantity of alcohol, was added. Lyxose *p*-bromophenylhydrazone soon crystallised and a further amount was obtained by addition of water to the mother-liquor. Recrystallisation from water gave a pure product from which lyxose was regenerated by treatment with benzaldehyde in the usual manner. The syrupy lyxose was finally dissolved in 3—4 volumes of absolute alcohol and on addition of a nucleus rapid crystallisation ensued. The yield of crystalline lyxose from the *p*-bromophenylhydrazone averaged 80—90% of the theoretical. The overall yield from galactose was low (10%), owing mainly to the small proportion of calcium galactonate which is degraded, even under the most favourable conditions.

The crude β-lyxose thus obtained had m. p. 114—115° and recrystallisation from alcohol yielded the pure product in needles, m. p. 117—118°. These were very soluble in water but only slightly soluble in absolute alcohol. Measurements in 90% and 85% alcohol (by weight) at 18° gave respectively solubilities of 0·8 and 1·7 g. per 100 c.c. The new compound was strongly lævorotatory in aqueous solution and showed rapid mutarotation to a final equilibrium value $[\alpha]_D$ −14°. The mutarotation followed a unimolecular course, the constant $k_1 + k_2$ being 0·082 at 21° and 0·056 at 17°. The initial value of $[\alpha]_D$ in water was calculated to be −70°, and the observed values in 90% ethyl alcohol were $[\alpha]_{5461}$ −80° (initial value) and −9° (equilibrium value). Detailed figures for the mutarotation are given in the following tables, the value of $k_1 + k_2$ being calculated from the formula $k_1 + k_2 = 1/t\,.\log\{(r_0 - r_\infty)/(r - r_\infty)\}$. The first column gives in minutes the time elapsed after solution of the lyxose. The dissolution in water was complete in 15 seconds. For α-lyxose Hudson and Yanowsky found $k_1 + k_2 = 0{\cdot}065$ at 20°.

Solvent—water. Temp. 21°.
$c = 3{\cdot}83$.

Time (mins.).	$[\alpha]_D$.	$k_1 + k_2$.
1	−60°	—
2	52·5	0·078
4	40	0·083
6	32·5	0·079
8	26·5	0·081
10	22	0·084
12	19·5	0·084
14	17	0·091
18	15	—
∞	14	—

For $t = 0$, $[\alpha]_D = -70°$
by calculation.

Solvent—water. Temp. 17°.
$c = 1{\cdot}1$.

Time (mins.).	$[\alpha]_D$.	$k_1 + k_2$.
4	−48°	—
7	37	0·057
12	27	0·052
14	23·5	0·055
16	21	0·057
18	19	0·059
20	18	0·058
22	17	0·059
∞	14	—

For $t = 0$, $[\alpha]_D = -71°$
by calculation.

The β-lyxose remained unchanged after prolonged contact with 90% alcohol and up to the present we have been unable to induce the α-form to separate from solution.

Calculation of the Rotation of α-Lyxose by the Method of Hudson and Yanowsky.—Crystalline β-lyxose (m. p. 117—118°) was finely powdered and shaken for 6 minutes with 90% ethyl alcohol. After filtration the solution was allowed to reach equilibrium; the reading α_{5461} −0·07° was then recorded, corresponding to a concentration of 0·8 g. per 100 c.c. Another sample was shaken for several hours with 90% alcohol; after filtration, the reading α_{5461} −0·43° was obtained, or $c = 4{\cdot}8$. The equilibrium mixture therefore contained 0·8 g. of β-lyxose and 4·0 g. of α-lyxose per 100 c.c. Since in 90% alcohol $[\alpha]_{5461}$ for β-lyxose was found to be −80°, and the equilibrium value −9°, the calculated value of $[\alpha]_{5461}$ for the α-lyxose was found to be +5°. A repetition of the experiment with

85% alcohol gave 1·7 as the initial solubility of β-lyxose in this solvent, and as the final solubility of the equilibrium mixture was 10·5, the calculated value was $[\alpha]_{5461}$ +5° for α-lyxose. The observed value corresponding to $[\alpha]_D$ +5·5° was $[\alpha]_{5461}$ 6·5°.

Conversion of β-*Lyxose into Known Derivatives.*—The *p*-bromophenylhydrazone (needles, m. p. 155—157°) and the characteristic osazone, identical with xylosazone, m. p. 157—160°, were prepared and found to be identical with the known compounds. A further proof of identity was obtained by the preparation of α-methyl-lyxoside from β-lyxose. A solution of the sugar in methyl alcohol containing 1% of hydrogen chloride was boiled for 2 hours as described by Phelps and Hudson (*loc. cit.*). The acid was neutralised with lead carbonate and the resulting colourless solution was evaporated to a syrup, which soon crystallised. The solubility of α-methyl-lyxoside in ethyl acetate was found to be so great as to render the method of recrystallisation recommended by Phelps and Hudson difficult to carry out satisfactorily with small quantities of material. The following method works well with quantities of a centigram upwards. The substance was dissolved in a very little absolute ethyl alcohol, and petroleum (b. p. 60—80°; light petroleum, b. p. 40—60°, is useless) was added carefully until a faint permanent cloudiness appeared. This was removed by addition of a drop of alcohol and thereafter α-methyl-lyxoside crystallised rapidly. Two crystallisations were sufficient to give the pure substance, m. p. 108—109°, $[\alpha]_D$ +59° in water ($c = 1$). Yield, 70%.

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UNIVERSITY OF BIRMINGHAM,
EDGBASTON.

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