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# The Crystal Structure of a Trisaccharide, Raffinose Pentahydrate

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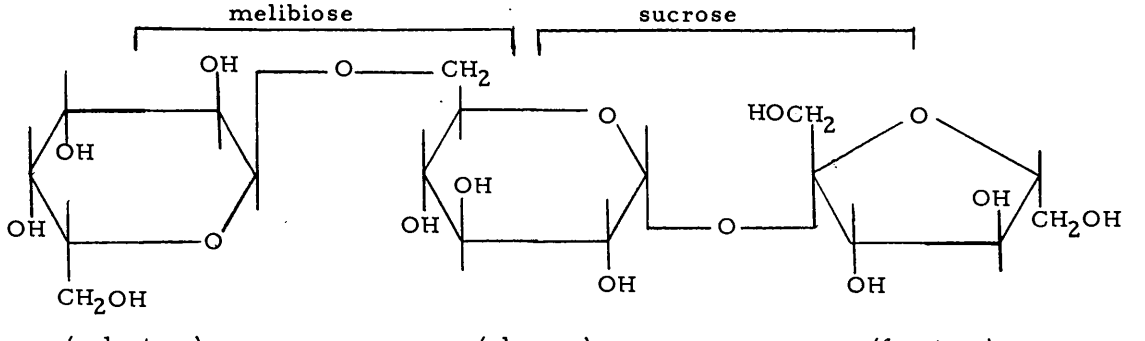
The crystal structure of raffinose pentahydrate has been solved by a non-centrosymmetric direct method and refined to an $R$ index of 0·060. The space group is $P2_12_12_1$ with four formula units of $C_{18}H_{32}O_{16}.5H_2O$ per unit cell, and the lattice parameters are $a=8{\cdot}966$ (10), $b=12{\cdot}327$ (15) and $c=23{\cdot}837$ (24) Å, measured with Cu $K\alpha$ radiation at room temperature. Raffinose is galactosyl-glucosyl-fructose, an oligosaccharide occurring naturally in great abundance. The molecules are coiled into segments of hydrogen-bonded helices with one turn per unit cell around the screw axes along **a**. There are no intramolecular hydrogen bonds. The conformation of the glycosidic link between glucose and fructose, which comprise the sucrose moiety of raffinose, is different from that previously found in crystal structures containing the sucrose molecule.

## Introduction

Raffinose is a naturally occurring trisaccharide isolated from a variety of plants including beet sugar molasses, cottonseed meal, and the seeds of various food legumes. It was first crystallized from *Eucalyptus* manna (Johnston, 1843). It belongs to a family of oligosaccharides, the chemistry and structural relationships of which have been reviewed by French (1954). The Haworth formulation is given in (I) and the chemical name is α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl (1 → 2)-β-D-fructofuranoside.




(I)

The sucrose moiety has the α-C(1)–O(1) glycosidic bond, the characteristics of which have been the subject of some discussion (Sundaralingam, 1968; Berman, Chu & Jeffrey, 1967) as well as the 1 → 6 glucose–galactose linkage, the conformation of which has not previously been studied crystallographically. Neither the galactose and melibiose (α-D-galactopyranosyl-(1 → 6)-α-D-glucose) moieties (see Fig. 1 for numbering convention), nor the related higher oligosaccharides such as stachyose, verbascose and the sucrogalactans, have been studied crystallographically; the structures of raffinose and sucrose may be useful in predicting their conformations. Since raffinose crystallizes with an unusually high number of water molecules of hydration, it is of some interest to determine the extent of the influence of these water molecules on the conformation of the trisaccharide molecule.

### *Crystal data*

Orthorhombic, $C_{18}H_{32}O_{16}.5H_2O$, m.p. 118°.

Space group $P2_12_12_1$, from systematic extinctions $h00$, $0k0$, $00l=2n+1$.

$Z=4$.

$a=8{\cdot}966(10)$, $b=12{\cdot}327(15)$, $c=23{\cdot}837(24)$ Å measured at room temperature.

$d_m=1{\cdot}479$ g.cm$^{-3}$, $d_x=1{\cdot}496$ g.cm$^{-3}$, $\mu_{Cu\ K\alpha}=12{\cdot}2$ cm$^{-1}$, $\lambda=1{\cdot}5418$ Å, $F(000)=1272$.

## Experimental

Large prismatic colorless crystals were obtained commercially from K and K Laboratories, Inc. They had

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etch marks and were frequently cracked. A crystal of dimensions $0{\cdot}4 \times 0{\cdot}2 \times 0{\cdot}2$ mm was mounted on a glass fiber along the $a$ axis, and data were collected on a Picker four-angle card-controlled automatic diffractometer using Cu $K\alpha$ radiation and a maximum $2\theta$ of 130°. Constant 2° $\theta/2\theta$ scans were used and the 2532 data were reduced to structure amplitudes, with no corrections for absorption, using programs written by Shiono (1966) and McGandy (1967). Of these, 435 were below $0{\cdot}5\sigma$ and were considered unobserved.

### Solution of the structure

The structure of raffinose pentahydrate was solved by a combination of direct method techniques and $E$ map refinements. Three origin and one enantiomorph defining phases, as well as one phase determined by symbolic addition as shown below, were used to extend and refine phases using the approach programmed by Hall (1967).

| | Reflection | Parity | Phase | $E$ |
|---|---|---|---|---|
| Origin | 6 7 0 | $g\ u\ 0$ | 0 | 3·45 |
| | 0 12 1 | $0\ g\ u$ | $\pi$ | 2·70 |
| | 5 0 14 | $u\ 0\ g$ | 0 | 3·11 |
| Enantiomorph | 7 0 15 | $u\ 0\ u$ | $\pi/2$ | 2·36 |
| Starting | 0 2 4 | $g\ g\ g$ | $\pi$ | 2·48 |

The initial E map using 200 $E$'s showed part of the structure from which 14 trial peaks were selected to calculate the phases for structure factors which met the criterion $|F_c| \geq \frac{1}{3}|F_o|$ (Karle, 1968) and had $E > 1{\cdot}9$. These were used as input for another 10 cycles consisting of five iterations each of tangent refinement followed by an $E$ synthesis. The phases were recalculated from the highest 30 peaks and applied to those amplitudes which showed good agreement and for which $E > 1{\cdot}5$ for the final $E$ map which revealed the structure. It is clear from the results that the course of the structure analysis might have been simplified had the highest peaks on each $E$ map been chosen as a basis for

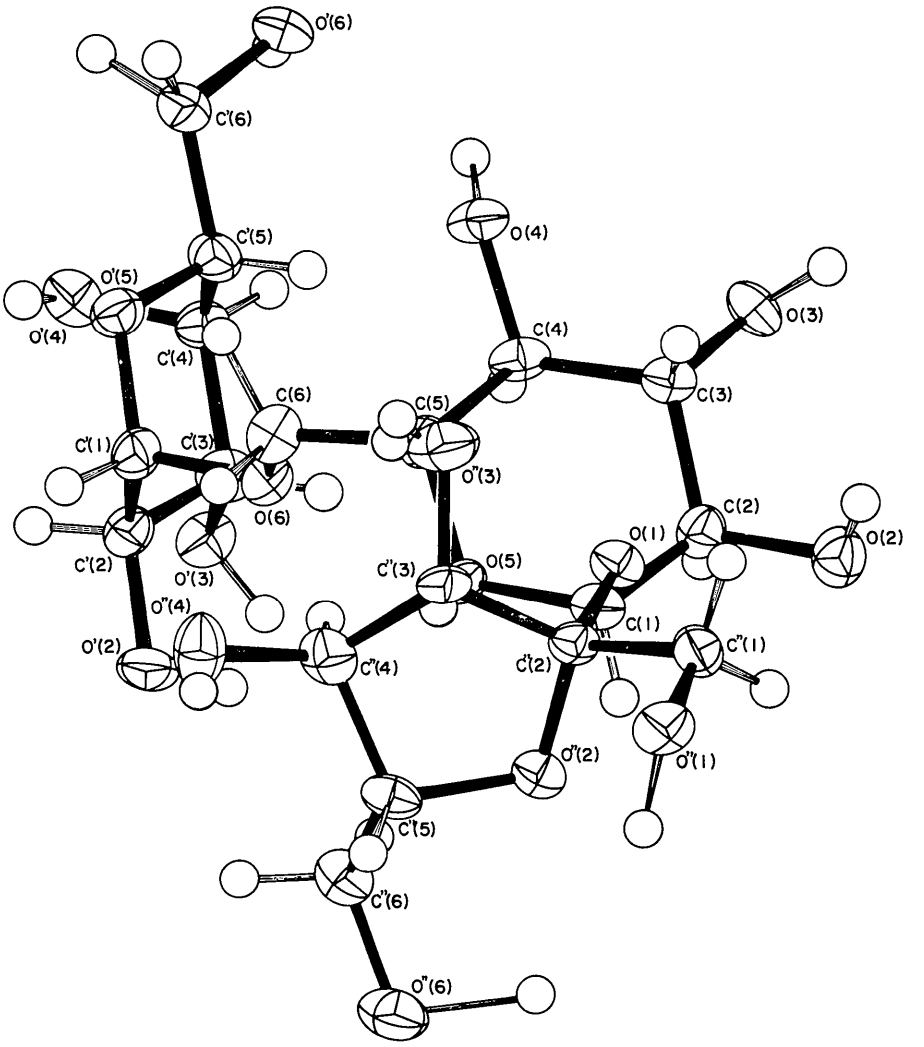



Fig. 1. Identification and numbering of the atoms in the raffinose molecule.

further tangent refinement, without seeking a chemically significant model. This may be a special characteristic of carbohydrates, as compared with hydrocarbons or alkaloids, in the difficulty of recognizing connected sequences of atoms in a partial structure. Carbohydrates contain approximately equal numbers of carbon and oxygen atoms, with rings formed of four or five carbon atoms to one oxygen atom, with the substituent hydroxyl groups directed outwards. In addition to this dispersal of oxygen atoms in the molecule, there may also be water molecules of hydration, as in raffinose. Since at the beginning of a determination the highest maxima on $E$ syntheses are usually predominantly oxygen atom positions, a seemingly meaningless assortment of peaks may be essentially correct. Another special feature of raffinose, which may account for some of the difficulty in starting the phase determination, was noticed after the structure was solved. Several of the atomic parameters were found to be related by near pseudo symmetry, *e.g.* C(3) is related to O(3′) by $\frac{1}{2}+x, y, \bar{z}$, O(5) to O(4) by $x, \bar{y}, z$.

## Refinement

The first structure factor calculation with all atoms except hydrogens gave an $R$ of 0·27, which was reduced to 0·12 by full-matrix isotropic least squares. Three cycles of anisotropic block-diagonal least squares were calculated and the hydrogen atoms located by interpretation of successive difference maps (Shiono, 1966; Stewart, 1964). They were assigned the isotropic temperature factors of the atoms to which they are bonded. Refinement of the carbon and oxygen parameters was completed by using the Hughes (1941) weighting scheme and the final $R$ value for 2097 observed reflections is 0·060. The final parameters are given in Table 1 and the structure factor listing in Table 2.

## Description of the structure

Figs. 1 and 2 illustrate the configuration and conformation of the molecule of raffinose with the standard numbering for carbohydrates. The two pyranose rings

Table 1. *Fractional atomic coordinates and thermal parameters in raffinose pentahydrate*

Key to atomic numbering is given in Fig. 1. The temperature factor expression used was
$\exp[-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+2hk\beta_{12}+2hl\beta_{13}+2kl\beta_{23})]$.

Numbers in parentheses refer to standard deviations of the last place.

| | $x$ | $y$ | $z$ | $\beta_{11}$ | $\beta_{22}$ | $\beta_{33}$ | $\beta_{12}$ | $\beta_{13}$ | $\beta_{23}$ |
|---|---|---|---|---|---|---|---|---|---|
| C(1) | 0·2773 (7) | 0·3201 (5) | 0·1426 (2) | 0·0070 (7) | 0·0033 (3) | 0·0008 (1) | 0·0001 (4) | 0·0000 (2) | 0·0000 (2) |
| C(2) | 0·3911 | 0·3948 | 0·1161 | 0·0063 | 0·0036 | 0·0010 | 0·0002 | −0·0000 | 0·0001 |
| C(3) | 0·3587 | 0·5137 | 0·1311 | 0·0083 | 0·0031 | 0·0007 | −0·0002 | −0·0002 | 0·0000 |
| C(4) | 0·1989 | 0·5418 | 0·1161 | 0·0088 | 0·0023 | 0·0007 | 0·0007 | −0·0002 | 0·0001 |
| C(5) | 0·0921 | 0·4606 | 0·1419 | 0·0063 | 0·0032 | 0·0008 | −0·0000 | 0·0006 | 0·0004 |
| C(6) | −0·0696 | 0·4781 | 0·1252 | 0·0076 | 0·0047 | 0·0008 | 0·0010 | 0·0002 | 0·0001 |
| C(1′) | −0·2216 | 0·5064 | 0·0451 | 0·0061 | 0·0032 | 0·0011 | 0·0003 | −0·0004 | 0·0004 |
| C(2′) | −0·2320 | 0·4731 | −0·0160 | 0·0071 | 0·0031 | 0·0013 | 0·0002 | −0·0006 | 0·0001 |
| C(3′) | −0·1234 | 0·5364 | −0·0519 | 0·0091 | 0·0042 | 0·0007 | 0·0000 | −0·0005 | −0·0000 |
| C(4′) | −0·1460 | 0·6583 | −0·0428 | 0·0064 | 0·0033 | 0·0012 | 0·0001 | 0·0002 | 0·0003 |
| C(5′) | −0·1297 | 0·6811 | 0·0192 | 0·0027 | 0·0036 | 0·0010 | −0·0006 | 0·0004 | −0·0001 |
| C(6′) | −0·1592 | 0·7988 | 0·0349 | 0·0067 | 0·0035 | 0·0013 | −0·0001 | 0·0003 | 0·0000 |
| C(1″) | 0·3850 | 0·2125 | 0·2742 | 0·0068 | 0·0040 | 0·0013 | −0·0002 | −0·0007 | 0·0005 |
| C(2″) | 0·2510 | 0·2399 | 0·2380 | 0·0074 | 0·0024 | 0·0008 | 0·0004 | −0·0003 | 0·0003 |
| C(3″) | 0·1113 | 0·2749 | 0·2706 | 0·0081 | 0·0022 | 0·0010 | −0·0002 | −0·0001 | 0·0002 |
| C(4″) | −0·0176 | 0·2366 | 0·2315 | 0·0090 | 0·0043 | 0·0010 | −0·0005 | 0·0005 | 0·0003 |
| C(5″) | 0·0480 | 0·1285 | 0·2114 | 0·0091 | 0·0029 | 0·0009 | −0·0012 | 0·0002 | 0·0003 |
| C(6″) | −0·0054 | 0·0920 | 0·1538 | 0·0108 | 0·0055 | 0·0011 | −0·0009 | −0·0000 | 0·0001 |
| O(1) | 0·2993 (5) | 0·3253 (3) | 0·2008 (2) | 0·0077 (5) | 0·0029 (3) | 0·0008 (1) | −0·0005 (3) | 0·0005 (2) | 0·0003 (1) |
| O(2) | 0·5397 | 0·3639 | 0·1299 | 0·0071 | 0·0045 | 0·0013 | −0·0000 | 0·0004 | 0·0003 |
| O(3) | 0·4539 | 0·5863 | 0·1000 | 0·0085 | 0·0047 | 0·0009 | −0·0022 | 0·0002 | 0·0003 |
| O(4) | 0·1569 | 0·6462 | 0·1369 | 0·0108 | 0·0025 | 0·0011 | 0·0006 | 0·0005 | −0·0001 |
| O(5) | 0·1314 | 0·3520 | 0·1264 | 0·0062 | 0·0026 | 0·0010 | 0·0003 | −0·0004 | 0·0002 |
| O(6) | −0·0790 | 0·4717 | 0·0649 | 0·0078 | 0·0038 | 0·0008 | 0·0008 | 0·0001 | 0·0001 |
| O(2′) | −0·2156 | 0·3589 | −0·0228 | 0·0103 | 0·0030 | 0·0019 | −0·0002 | −0·0017 | −0·0001 |
| O(3′) | −0·1483 | 0·5131 | −0·1105 | 0·0123 | 0·0056 | 0·0009 | 0·0017 | −0·0006 | −0·0004 |
| O(4′) | −0·2859 | 0·6947 | −0·0642 | 0·0104 | 0·0042 | 0·0010 | −0·0006 | −0·0002 | 0·0004 |
| O(5′) | −0·2384 | 0·6207 | 0·0505 | 0·0072 | 0·0031 | 0·0013 | 0·0007 | 0·0002 | 0·0001 |
| O(6′) | −0·0494 | 0·8713 | 0·0134 | 0·0114 | 0·0036 | 0·0012 | −0·0012 | −0·0000 | −0·0000 |
| O(1″) | 0·3468 | 0·1279 | 0·3116 | 0·0104 | 0·0040 | 0·0011 | 0·0001 | −0·0003 | 0·0005 |
| O(2″) | 0·2082 | 0·1459 | 0·2082 | 0·0077 | 0·0036 | 0·0008 | −0·0002 | −0·0002 | 0·0001 |
| O(3″) | 0·1123 | 0·3860 | 0·2811 | 0·0126 | 0·0032 | 0·0014 | 0·0000 | 0·0013 | −0·0001 |
| O(4″) | −0·1583 | 0·2302 | 0·2559 | 0·0060 | 0·0070 | 0·0014 | 0·0002 | 0·0005 | 0·0010 |
| O(6″) | 0·0463 | −0·0138 | 0·1395 | 0·0127 | 0·0048 | 0·0015 | −0·0018 | 0·0004 | −0·0007 |
| O(*W*1) | 0·7985 (7) | 0·0846 (5) | 0·0353 (2) | 0·0128 (7) | 0·0066 (3) | 0·0020 (1) | 0·0004 (5) | 0·0000 (2) | −0·0004 (1) |
| O(*W*2) | 0·5905 | 0·1372 | 0·1193 | 0·0125 | 0·0050 | 0·0017 | 0·0005 | −0·0003 | 0·0000 |
| O(*W*3) | 0·6492 | 0·4159 | 0·2355 | 0·0119 | 0·0056 | 0·0016 | −0·0005 | 0·0001 | 0·0001 |
| O(*W*4) | 0·0218 | 0·2516 | 0·0252 | 0·0104 | 0·0053 | 0·0015 | 0·0012 | −0·0003 | −0·0001 |
| O(*W*5) | 0·6006 | 0·0008 | 0·2163 | 0·0120 | 0·0075 | 0·0025 | 0·0012 | 0·0007 | 0·0012 |

Table 1 (*cont.*)

| | *x* | *y* | *z* |
|---|---|---|---|
| HC(1) | 0·300 | 0·240 | 0·130 |
| HC(2) | 0·390 | 0·390 | 0·070 |
| HC(3) | 0·375 | 0·525 | 0·174 |
| HC(4) | 0·184 | 0·540 | 0·073 |
| HC(5) | 0·058 | 0·461 | 0·185 |
| HC(6) | −0·130 | 0·560 | 0·140 |
| H′C(6) | −0·140 | 0·420 | 0·150 |
| HC(1′) | −0·308 | 0·467 | 0·067 |
| HC(2′) | −0·342 | 0·488 | −0·030 |
| HC(3′) | −0·014 | 0·515 | −0·040 |
| HC(4′) | −0·064 | 0·700 | −0·065 |
| HC(5′) | −0·022 | 0·661 | 0·034 |
| HC(6′) | −0·185 | 0·820 | 0·080 |
| H′C(6′) | −0·259 | 0·850 | 0·030 |
| HC(1″) | 0·470 | 0·190 | 0·250 |
| HC(2″) | 0·420 | 0·280 | 0·300 |
| HC(3″) | 0·103 | 0·231 | 0·308 |
| HC(4″) | −0·020 | 0·290 | 0·200 |
| HC(5″) | 0·020 | 0·067 | 0·240 |
| HC(6″) | 0·034 | 0·147 | 0·123 |
| H′C(6″) | −0·121 | 0·093 | 0·153 |

Table 1 (*cont.*)

| | *x* | *y* | *z* |
|---|---|---|---|
| HO(2) | 0·570 | 0·400 | 0·160 |
| HO(3) | 0·540 | 0·610 | 0·130 |
| HO(4) | 0·150 | 0·720 | 0·110 |
| HO(2′) | −0·120 | 0·340 | −0·021 |
| HO(3′) | −0·080 | 0·450 | −0·120 |
| HO(4′) | −0·350 | 0·680 | −0·040 |
| HO(6′) | −0·060 | 0·880 | −0·030 |
| HO(1″) | 0·320 | 0·060 | 0·280 |
| HO(3″) | 0·070 | 0·380 | 0·310 |
| HO(4″) | −0·160 | 0·180 | 0·290 |
| HO(6″) | 0·200 | 0·000 | 0·140 |
| H(*W*1) | 0·880 | 0·030 | 0·020 |
| | 0·890 | 0·140 | 0·030 |
| H(*W*2) | 0·590 | 0·210 | 0·110 |
| | 0·660 | 0·110 | 0·080 |
| H(*W*3) | 0·590 | 0·420 | 0·270 |
| | 0·700 | 0·350 | 0·240 |
| H(*W*4) | 0·110 | 0·220 | 0·007 |
| | 0·000 | 0·280 | 0·000 |
| H(*W*5) | 0·610 | 0·060 | 0·180 |
| | 0·600 | −0·050 | 0·190 |

Table 2. *Observed and calculated structure factors*

Columns are: $|F_{obs}|$, $|F_{cal}|$, $A_{cal}$, $B_{cal}$. * denotes unobserved reflections.

Table 2 (*cont.*)

are chair form and the furanose ring is puckered; they do not deviate markedly from the conformation of known monosaccharides. The bond angles and distances for the pyranosides shown in Fig.2 show good agreement with previously reported values (*cf.* Berman, Chu & Jeffrey, 1967; Jeffrey & Rosenstein, 1964). The average C–C and C–OH bond lengths are 1·520 and 1·430 Å and their average estimated standard deviations are 0·008 and 0·007 Å respectively. The glycosidic bond length in the galactosyl C(1′)–O(6) does not deviate from the average C–OH bond length but the glucosyl C(1)–O(1) is slightly shortened by $3\sigma$, similar to the anomeric bond in methyl glucoside (Berman & Kim, 1968). The ring C–O–C bond lengths differ by $2\sigma$ in both the glucoside and galactoside. These results are, in general, consistent with those tabulated for other glycosidic sugars (Sundaralingam, 1968; Berman, Chu & Jeffrey, 1967). The internal C–C–C ring angles* are close to tetrahedral (110·3°) and the C–C–O angles range from 104·4 to 112·7°; those of the ring oxygen atoms average 113·8°; the C(1)–O(1)–C(2″) angle is 122°, which is significantly larger than the comparable angle (114°) in sucrose (Brown & Levy, 1963). The decrease of this angle in sucrose which Sundaralingam (1968) ascribed in part to internal hydrogen bonding does not apply in this case. The C(1′)–O(6)–C(6) angle is quite small (111°), in fact it is the smallest reported thus far for a glycosidic link. The conformation angles of the pyranose rings range from 52·7 to 59·5° and 53·8 to 62·2° for the glucose and galactose units respectively, in comparison with 54·8 to 56·0° for sucrose, using the convention of Brown & Levy (1963).

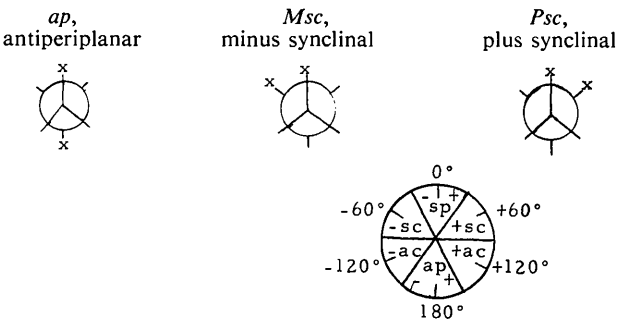
* The average estimated standard deviations of all the angles is 0·50°.

The furanose ring has a geometry similar to that of the fructose moiety of sucrose and of ethyl-1-thio-α-D-glucofuranoside (Parthasarathy & Davies, 1967). In this particular structure, the C(4″) is 'primarily' out of the plane and C(3″) is 'secondarily puckered' (Sundaralingam, 1965). As shown in Table 3, the conformation angle of the directed bond C(2″) → O(2″) which is opposite the C(4″) atom is smallest, in agreement with Sundaralingam's (1965) observation. The internal C–C–C angles are smaller than in the pyranose rings, especially those associated with the out of plane atoms. The C–O bonds at these atoms, C(3″)–O(3″) and C(4″)–O(4″) are 6σ shorter than the average C–OH. The relation of the puckering of furanose rings to the bond lengths and angles has been discussed by Sundaralingam (1965).

Table 3. *Conformation angles*

| | Sucrose | Raffinose |
|---|---|---|
| (*a*) Linkage and mobile directed bonds* | | |
| C(5)—C(6) | *Msc* | *Msc* ( −64·8°) |
| C(6)—O(6) | | *ap* (−169·5) |
| C(1′)–O(6) | | *Psc* ( +71·8) |
| C(1)—O(1) | *Pac* | *Psc* ( +81·7) |
| O(1)—C(2″) | *Msc* | *Psp* ( +11·4) |
| C(2″)–C(1″) (with respect to the ring oxygen) | *ap* | *Msc* ( −59·2) |
| C(5″)–C(6″) | *Msc* | *Psc* ( +68·9) |
| C(5′)–C(6′) | | *ap* (+172·4) |
| (*b*) Furanose ring (convention of Brown & Levy, 1963). | | |
| C(4″)–C(3″) | −34·9° | −38·6° |
| C(5″)–C(4″) | +7·4 | +33·5 |
| O(2″)–C(5″) | −8·3 | −16·2 |
| C(2″)–O(2″) | −14·5 | −9·4 |
| C(3″)–O(2″) | +31·0 | +30·6 |

* Conformation nomenclature is that of Klyne & Prelog (1960).




The overall conformation of the trisaccharide may be described in terms of the sucrose and melibiose

Table 4. *Hydrogen bonding distances and angles*

(*a*) Raffinose

| *i* | *j* | *k* | $D_{ijk}$ | ∠(*ijk*) | Symmetry operation* |
|---|---|---|---|---|---|
| C(2) | O(2) | *W*(3) | 2·776 Å | 118·7° | 5551 |
| C(3) | O(3) | O(1″) | 2·806 | 96·05 | 6553 |
| C(4) | O(4) | O(4′) | 2·664 | 112·7 | 5652 |
| C(2′) | O(2′) | *W*(4) | 2·720 | 114·09 | 4552 |
| C(3′) | O(3′) | *W*(2) | 2·840 | 94·9 | 4552 |
| C(4′) | O(4′) | O(6′) | 2·776 | 119·9 | 4652 |
| C(6′) | O(6′) | O(3) | 2·755 | 118·7 | 4652 |
| C(1″) | O(1″) | *W*(3) | 2·845 | 115·1 | 6453 |
| C(3″) | O(3″) | O(6″) | 2·670 | 125·3 | 5553 |
| C(4″) | O(4″) | O(4) | 2·755 | 113·8 | 5453 |
| C(6″) | O(6″) | O(3′) | 2·823 | 111·8 | 5552 |

(*b*) $H_2O$

| | | | |
|---|---|---|---|
| *W*(1) | *W*(4) | 2·882 | 6551 |
| | O(6′) | 3·000 | 6451 |
| *W*(2) | O(2) | 2·842 | 5551 |
| | *W*(1) | 2·812 | 5551 |
| *W*(3) | O(4″) | 2·906 | 6551 |
| | *W*(5) | 2·725 | 6553 |
| *W*(4) | O(5) | 2·884 | 5551 |
| | O(2′) | 2·755 | 5551 |
| *W*(5) | *W*(2) | 2·860 | 5551 |
| | O(3″) | 2·937 | 6453 |

(*c*) Intermolecular oxygen–oxygen distances less than 3·2 Å.

| | | | |
|---|---|---|---|
| O(1) | *W*(5) | 3·06 | 6553 |
| O(3) | O(5′) | 3·03 | 6551 |
| O(6) | *W*(4) | 3·01 | 5551 |
| O(6′) | O(5′) | 3·18 | 5652 |

* The first three digits code a lattice translation. The last specifies one of the following operations:

1: $x, y, z$, 2: $\frac{1}{2}+x, \frac{1}{2}-y, -z$,
3: $-x, \frac{1}{2}+y, \frac{1}{2}-z$, 4: $\frac{1}{2}-x, -y, \frac{1}{2}+z$.

moieties. In sucrose (Brown & Levy, 1963) the fructoside O(1′) and O(6′) are hydrogen-bond donors to the glucosidic oxygen atoms O(2) and O(5) whereas in raffinose the sucrose section contains no intramolecular hydrogen bonding. The O(1″) and O(6″) atoms are hydrogen bonded to hydroxyl groups of adjacent molecules and atoms O(2) and O(5) are in close contact or hydrogen bonded to water molecules. As shown in Table 3, the conformation angles of the bonds C(2″)-C(1″) and C(5″)-C(6″) are entirely different

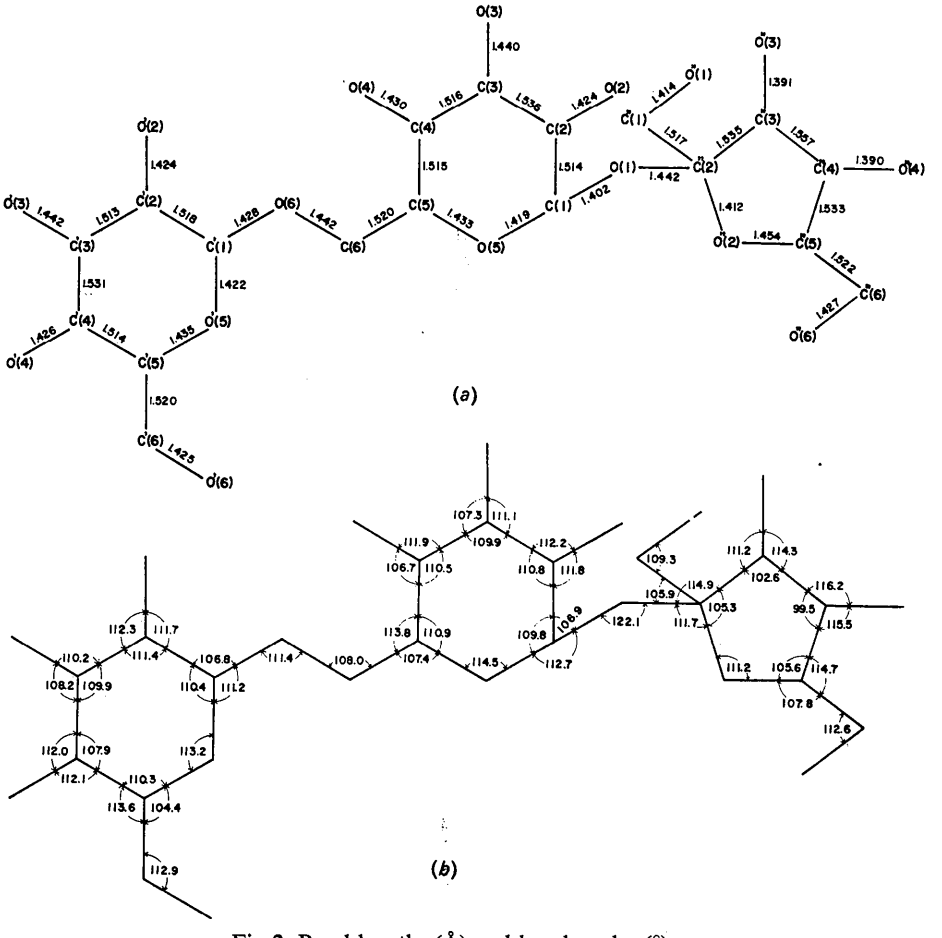



Fig. 2. Bond lengths (Å) and bond angles (°).

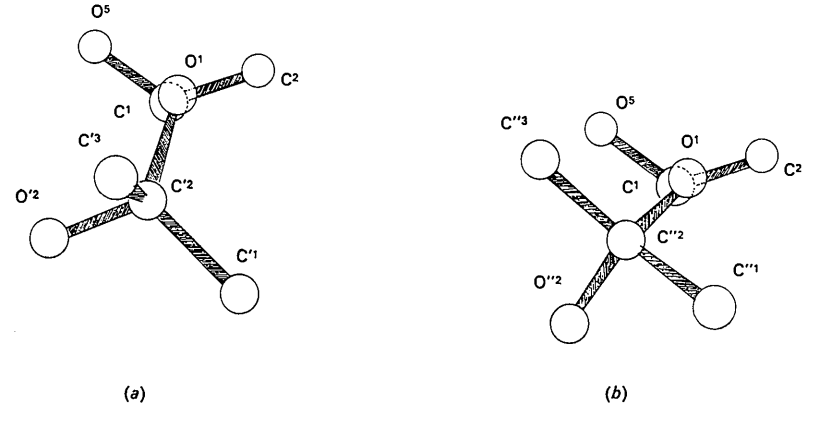



Fig. 3. The conformation of the 1 → 2 link in (*a*) sucrose and (*b*) raffinose.

from those in sucrose and therefore direct the fructose oxygen atoms away from the glucose residue. As shown in Fig. 3 and Table 3, the 1 → 2 linkage is also different for the two structures; the relative orientation of the two rings differs by about 25°. This difference could possibly be caused by differences in hydrogen bonding interactions.

The melibiose residue of raffinose also has no intramolecular hydrogen bonding. The conformation of the C(5′)–C(6′) terminal of the galactose is *ap* and directs the oxygen atom toward the hydroxyl group of another molecule. An *ap* conformation for a galactosidic C(5)–C(6) should be preferred over the *Msc* conformation since with the former there is no potentially unfavorable H(O6′)–H(O4′) interaction as in glucosides, and with the latter there would be a short O(6′)–O(4′) contact. From the intramolecular interactions alone, however, the *Psc* conformation would seem to be equally favorable. The 1 → 6 linkage connecting the glucose and galactose to form melibiose involves atoms C(5), C(6), O(6) and C(1) which form a zigzag chain, the plane of which perpendicularly bisects the mean planes of the two pyranose rings (Fig. 4). The angle between the planes of the rings is 111°. The conformation of the C(1′)–O(6) directed bond is *Psc* as expected (Sundaralingam, 1968) and the C(5)–C(6) conformation is *Msc*. While the *Psc* conformation is just as likely (in fact it predominates in other carbohydrate structures), the *Msc* conformation allows the structure to be relatively open; *i.e.* the galactose residue is directed away from fructose (see Fig. 5) leaving both ends of the molecule free, in this case to hydrogen bond with other molecules. Conceivably, however, these could form covalent bonds with other sugar residues. The overall conformation of the raffinose molecule found in this structure, therefore could be used as a basis for the construction of higher polysaccharides such as the sucrogalactans.

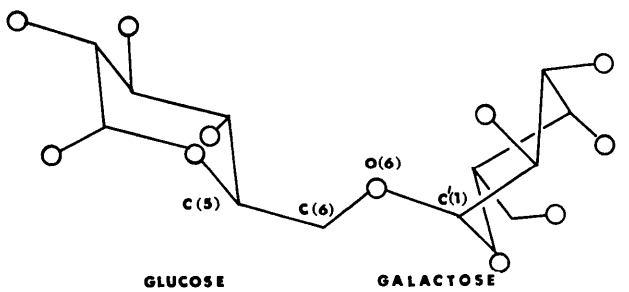



Fig. 4. The conformation of the 1→6 linkage.

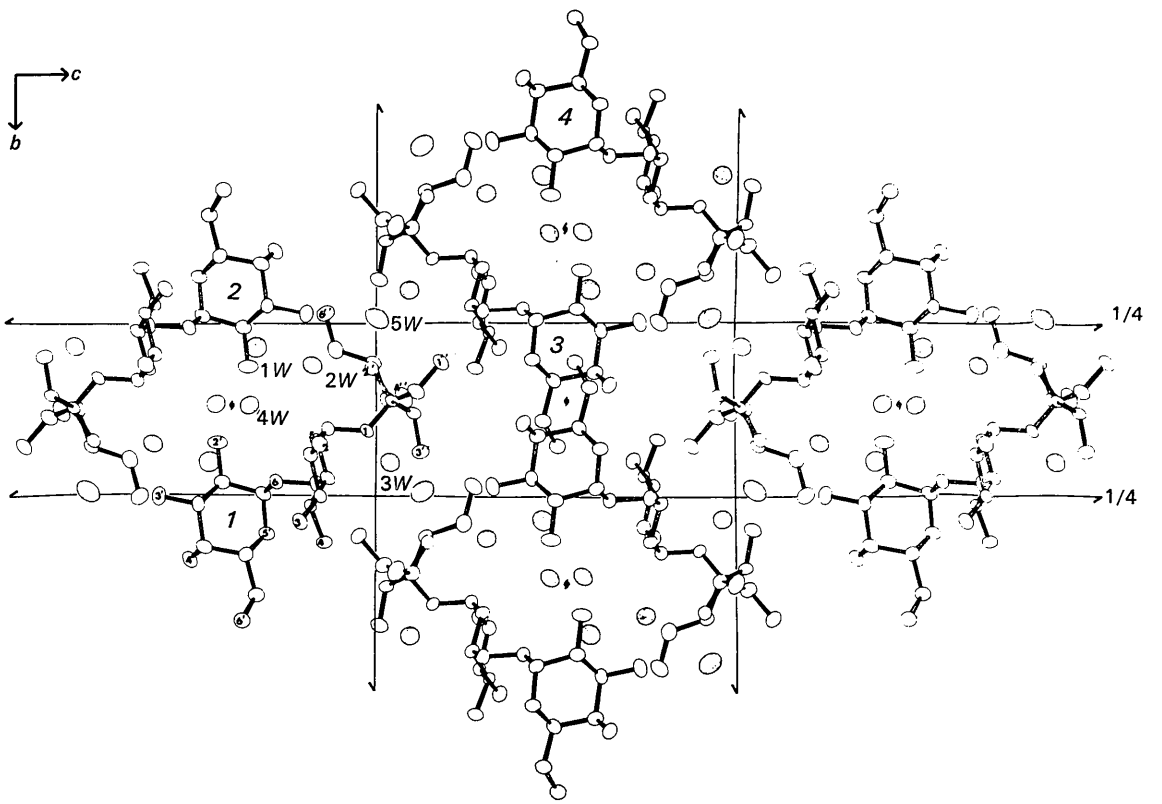



Fig. 5. *a*-axis projection of the raffinose crystal structure. Shaded positions are water molecules. The hydroxyl groups of one molecule are numbered. Italicized numbers represent the symmetry operations given in Table 4.

### Hydrogen bonding

All of the hydroxyl groups in raffinose act as both donors and acceptors in the hydrogen bonding scheme. Neither bridge oxygen atom of the glycosidic links accepts a hydrogen bond. The ring O(5) of the glucoside is a hydrogen bond acceptor whereas the O(5′) ring of the galactoside is not involved in hydrogen bonding. There may be, as suggested by Sundaralingam (1968), an inverse correlation between the hydrogen bonding capability of the ring oxygen atom and the angle of the adjacent glycosidic link.

The molecules of raffinose which alone show some degree of helicity are arranged in two hydrogen bonded helices per unit cell with the O(3′) atom of one molecule donating a hydrogen atom to the O(6″) atom of a molecule related by the two-screw axis in the **a** direction (Fig. 5). Contained within each helix are six water molecules *W*(1), *W*(2) and *W*(4) and their symmetry related atoms. Each bind respectively to 3, 2 and 4 atoms in the helix. In this particular structure the water molecules appear to fill in the otherwise open structure. Fig. 6 illustrates the details of the binding helix. The helices are connected by chains of hydrogen bonds with *W*(3) and *W*(5) participating in these chains. The schematic for the hydrogen bonding is shown in (II) and the appropriate distances and angles are given in Table 4.

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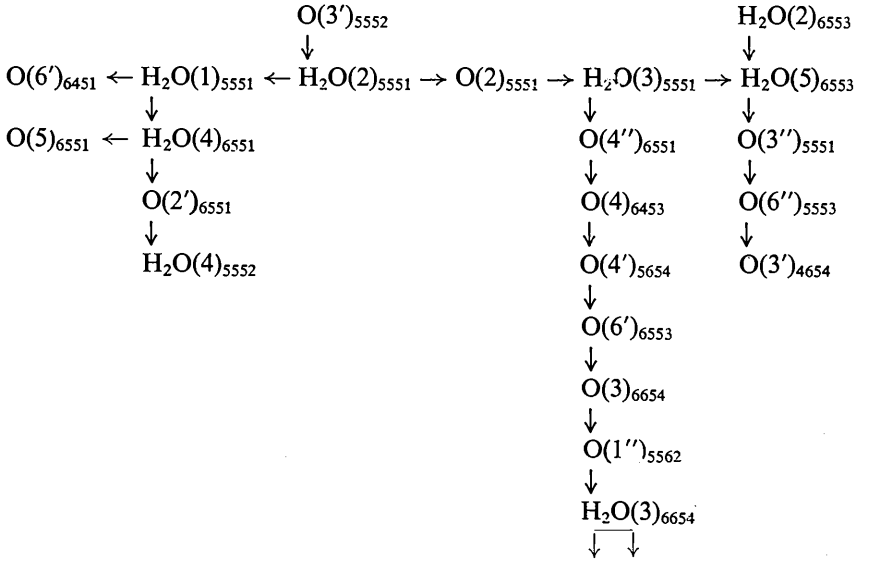


(II) The hydrogen bonding scheme

Since the hydrogen atoms were not located with a high degree of certainty, a neutron diffraction study would be necessary to confirm this scheme. The four close contacts shown in Table 4 are probably not hydrogen bonds, since the geometry is restrictive and all of the hydroxyl groups and water molecules are already involved as donors to other atoms.

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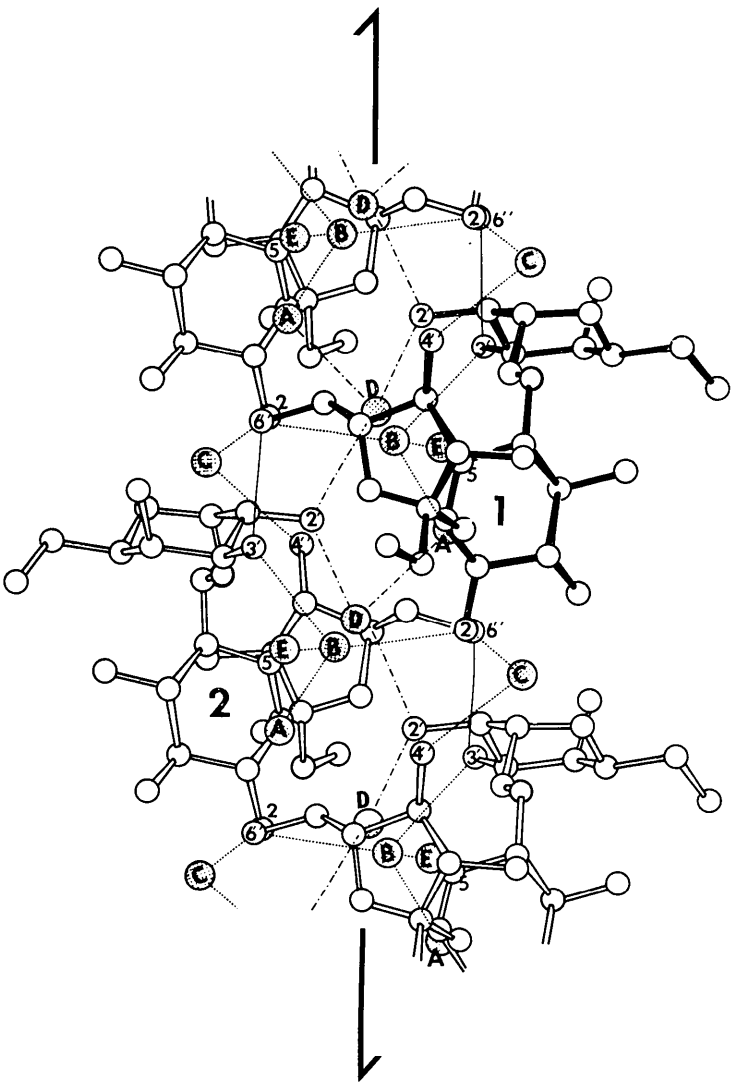


Fig. 6. The crystal structure viewed perpendicular to the *a* screw axis. The hydroxyl groups are numbered. The water molecules 1, 2, 3, 4, 5 are designated by *A*, *B*, *C*, *D*, *E*. Dotted lines represent hydrogen bonds.

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