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The Plant Sulfolipid: A Crystallographic Study

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The molecular configuration of the plant sulfolipid was studied *via* a three-dimensional structure analysis of the anhydrous rubidium salt of the deacylated sulfolipid acid, $RbC_9H_{17}O_{10}S$. The crystallographic constants of this compound are: $a=10\cdot87_6$, $b=7\cdot19_3$, $c=9\cdot31_1$ (all $\pm0\cdot005$ Å), $\beta=99\cdot7\pm0\cdot3°$, space group $P2_1$. The analysis established the sulfolipid as a derivative of 6-sulfo-6-deoxy-α-D-glucopyranosyl-(1-1′)-D-glycerol, thus confirming the results of chemical studies. An anomalous enzyme reaction involving β-galactosidase is discussed in terms of the steric configuration.

The molecular ions are connected with each other through a network of O–H · · · O hydrogen bonds and also through ionic interaction between the rubidium ions and the sulfonate groups. All interatomic distances and bond angles exhibit normal values.

Introduction

A sulfonic acid lipid was isolated from plants and photosynthetic microorganisms by Benson, Daniel & Weiser (1959). The sulfolipid, a fatty acid ester of a glycerol sulfoglycoside, is the only sugar sulfonic acid derivative yet known in nature. In many cases, the sulfolipid and the free sulfoglycoside derived from it occur in concentrations exceeding those of the sugar phosphate esters, the intermediates in sucrose synthesis in plants. The ubiquity and abundance of the sulfolipid within green plants and its relationship to active metabolism imply that its sulfosugar plays a role in plant physiology as important as that of the sugar phosphates.

In the course of subsequent studies (Shibuya & Benson, 1961), it was discovered that β-galactosidase can hydrolyze certain glycosides of this sulfonic acid. Since the enzyme had been considered to be completely specific for β-galactosides, confusion arose when other chemical evidence suggested the existence of a glucose ring in the sulfolipid. It was then postulated that the sulfonate group and the glycerol portion of the molecule were arranged on one side of the ring plane in such a way as to form an island of oxygen atoms which is similar to, or identical with, the island of oxygen atoms found in a β-galactoside, the normal substrate for the enzyme. The problem of steric configuration of the sulfoglycoside becomes a keypoint in understanding the function of the sulfolipid in photosynthesis and the possible enzyme reactions in its metabolism (Benson, 1961; Daniel, Miyano, Mumma, Yagi, Lepage, Shibuya & Benson, 1961).

The present study was therefore undertaken with the object of elucidating the steric configuration of the molecule and confirming and correlating various chemical observations, especially those on the ring structure. For this purpose, the two fatty acid residues of the molecule were removed and the rubidium salt of this deacylated sulfolipid acid, 6-sulfo-6-deoxy-α-D-glucopyranosyl-(1-1′)-D-glycerol, was studied. The crystal, with the presence of a rubidium ion and a sulfur atom in the chemical unit, is quite amenable to a full three-dimensional X-ray analysis.

The molecular formula of the compound and the atomic numbering based on the standard notation for the pyranosyl ring system which will be followed hereafter are shown in Fig. 1(*a*) and (*b*) respectively.

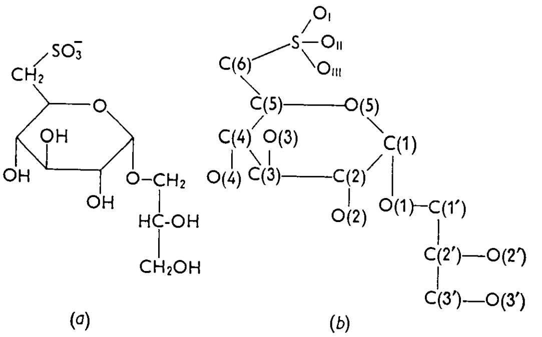

Fig. 1. (*a*) Molecular configuration. (*b*) Numbering of atoms.

The existence of rather heavy atoms in the crystal did not encourage the determination of hydrogen positions in the present study; no numbers are assigned to hydrogen atoms.

Experimental

The free acid of the compound in Fig. 1(*a*) was caused to react with a stoichiometric amount of Rb_2CO_3 in an ethanol–water solution from which well-developed crystals were obtained. These crystals were then studied by X-rays to determine their crystallographic constants. They belong to the monoclinic system with $a=10\cdot87_6\pm0\cdot005$, $b=7\cdot19_3\pm0\cdot005$,

$c=9{\cdot}31_1\pm0{\cdot}005$ Å, $\beta=99{\cdot}7\pm0{\cdot}3°$. The only observed systematic absences are for $0k0$ with k odd; this leads to $P2_1$ or $P2_1/m$ as the space group, the latter being excluded by the optical activity of the molecule. The above cell dimensions and the observed density of $1{\cdot}850_8$ g.cm^{-3} revealed the existence of two chemical units with a molecular weight of 400·0, which agrees well with the expected value of 402·6 for $RbC_9H_{17}O_{10}S$, based on the chemical formula for the anhydrous rubidium salt as suggested by a chemical analysis. The calculated molecular weight proves that the molecule does not take a sulfate ester structure such as C(5)–C(6)–O–SO_3. Another possible sulfate ester C(5)–O–SO_3 was later ruled out by the observed bond distances.

The intensities of the reflections were collected on a Weissenberg camera by using the multiple-film technique (Cu $K\alpha$). The crystals were rotated around the b axis ($k=0$ to 6), the a axis ($h=0$ to 5) and the c axis ($l=0$ to 5); the intensity data were then corrected for the Lorentz and polarization factors as well as for the absorption and the α_1–α_2 splitting effect using a program written for an IBM 7090 computer. The data obtained from different axial settings were then correlated on the 7090 and a set of three-dimensional observed structure factors was obtained; in all, 1662 independent reflections were obtained. Since there is no doubt about the occurrence of D-isomers for sugar compounds in nature, no effort was made to study the absolute configuration of the molecule; the absolute configuration of the glycerol residue can easily be determined once the relative relationship between the residue and the ring is established.

Structure determination

Since ready access to an IBM 7090 computer is possible at the Research Center, every step of the structure analysis was done with three-dimensional data. The work represents a typical example of the classical heavy-atom method coupled with the extensive use of the high-speed computer. As is customary in such a structure analysis, the first step towards the elucidation of the structure was made through a study of the three-dimensional Patterson function. A 7090 program was written which enabled us to obtain the approximate scale factor and the crystal temperature factor by Wilson's method (Wilson, 1942). The over-all temperature factor thus obtained was $4{\cdot}7\times10^{-16}$ cm^2; the scale factor was later used as a starting scale factor for the least-squares refinement of the structure. The same program also made it possible to prepare the coefficients for an origin-removed Patterson function; they were written on a tape in binary and used as the input for a Patterson summation on the 7090. The result was studied systematically and the positions of the rubidium ion and the sulfur atom were easily obtained; they are (0·119, 0·250, 0·194) for Rb^+ and (0·230, 0·220, 0·080) for S. The phases of the structure factors based on these two atoms were then calculated and the observed amplitudes were divided into their real and imaginary parts using the 7090; the results were again written on a tape and used as the input for an approximate three-dimensional electron-density computation. Because the y coordinate of S is rather close to $\frac{1}{4}$ (that of Rb is set as $\frac{1}{4}$, since one of the y coordinates can be arbitrarily assigned a value in $P2_1$), a provisional mirror plane exists in the approximate Fourier summation, and each correct light atom peak in the map was accompanied by a ghost mirror image peak of a small but considerable height; however, fifteen out of the nineteen light atoms were located without ambiguity. The second electron-density calculation was made by taking into account the contributions of Rb, S and these fifteen light atoms; all the light atoms were then located without difficulty in the resultant map. At this point the validity of the structure was studied by evaluating all possible *intra-* and *inter*-molecular distances less than 4·5 Å. Since no unusual surrounding was found for any of the atoms, the third electron-density calculation was made in the same way as described above. The map

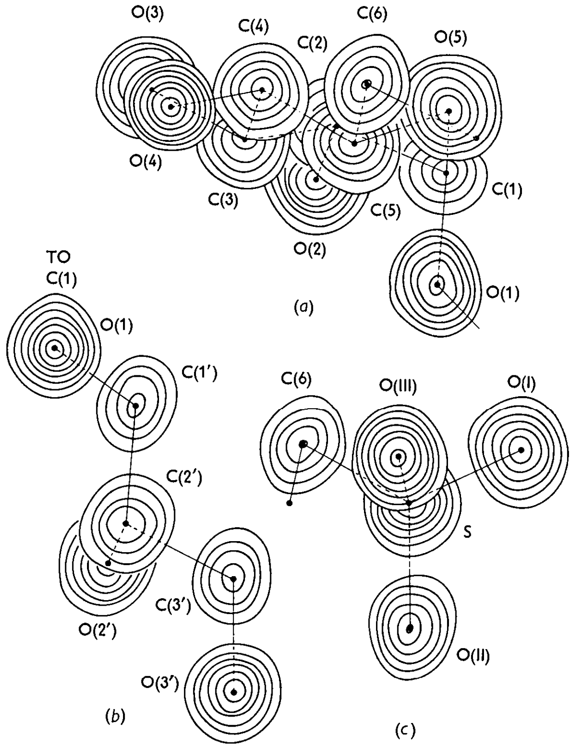

Fig. 2. Composite diagrams of the three-dimensional electron-density function. Contours are drawn at intervals of 1·5 e.Å^{-3} starting with 2 e.Å^{-3} (for S at 5 e.Å^{-3} intervals). (*a*) The glucose ring and O(1), (*b*) the glycerol residue and (*c*) the sulfonate group.

clearly showed those atoms put into the calculation of phases with very little background noise peaks.

Refinement of the structure

Although all the atoms were well discernible in the last electron-density map, the atomic coordinates thus determined and the over-all temperature factor gave a rather poor but encouraging disagreement index, $\Sigma||F_o|-|F_c||/\Sigma|F_o|$, of 0·27. Therefore, these parameters were then subjected to least-squares refinements on the 7090. Since the rubidium ion and the sulfur atom are heavier than the rest, the thermal motions of these two atoms were taken into account by assigning anisotropic temperature factors; the light atoms were treated as undergoing isotropic thermal motions.

The refinement proceeded rather smoothly and after 6 cycles of refinement the disagreement index was reduced to 0·123 including the non-observed reflections. The least-squares program used in this study is based on the full-matrix program written by Trueblood, Gantzel & Sparks (1961), modified by the present author. The structure-factor calculation of the last cycle was recorded on a binary tape and used for the final three-dimensional electron-density calculation. In Fig. 2, composite diagrams of the density function are shown.

In Table 1, the final values of the atomic parameters together with the standard deviations of the coordinates are given. The anisotropic temperature factors of Rb and S are decoded into the principal axes of their thermal vibration ellipsoids and listed in Table 1 in the same unit as other isotropic temperature factors. Interatomic distances and angles were evaluated using those atomic coordinates on the 7090. The results are the basis for the subsequent discussions.

The comparison between the observed and calculated structure factors for all the three-dimensional data is listed in Appendix I.*

Discussion

(a) *The general molecular configuration*

The result of the present analysis substantiates the molecular configuration deduced from the chemical studies. The sugar ring is, as predicted, a glucopyranose ring, α-linked to a glycerol residue through O(1). The relative configuration of the ring and the glycerol residues shows that if the former has the D configuration (which is most consistent with its chemical and biological properties), the latter also possesses the D configuration in conformity with the result of the radiochromatographic study (Miyano & Benson, 1962).

The orientation of the sulfonate group poses an interesting problem. In order to account for the reactivity of β-galactosidase on this molecule, it was

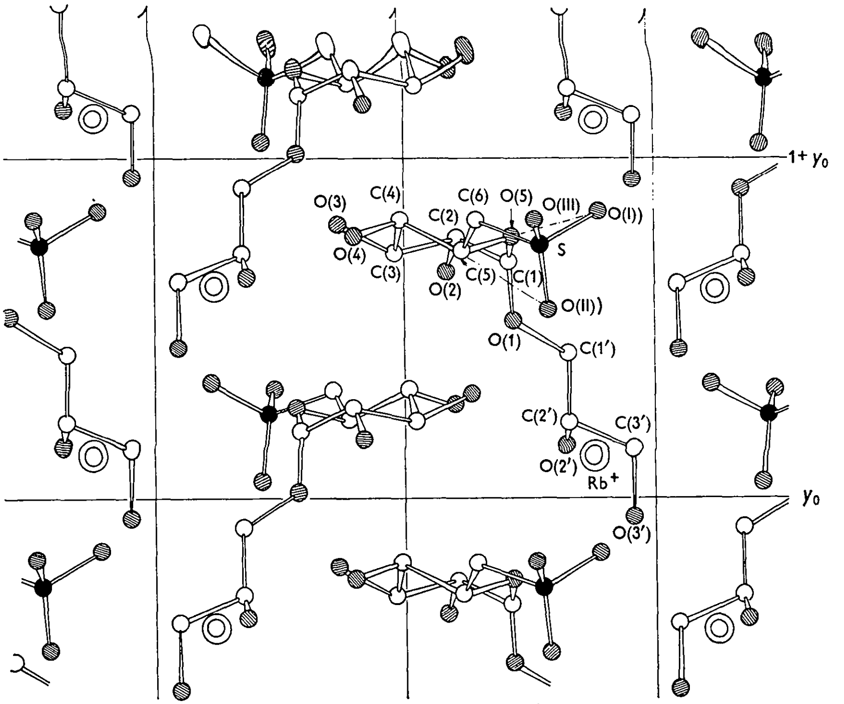

Fig. 3. The projection of the structure along the *c* axis. The short contacts (see text) are shown by chain lines.

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* Appendix I, consisting of 34 pages of 1401 print-out, has been withdrawn from publication. A copy of this material will be furnished by the author upon request.

Table 1. *Atomic coordinates (in fractions of cell edges), their standard deviations (Å) and individual temperature factors*

For Rb and S, the three principal axes of their thermal vibration ellipsoids are listed in the same units as isotropic temperature factors

Atoms	x	$\sigma(x)$	y^*	$\sigma(y)$	z^*	$\sigma(z)$	$B(10^{-16}\ \text{cm}^2)$	
Rb^+	0·1181	0·0012	1·2500	0·0009	1·1904	0·0010	3·72, 2·85, 2·11	
S	0·2247	0·0028	1·8038	0·0021	1·0742	0·0022	4·28, 3·98, 3·38	
O(I)	0·1182	0·011	1·9013	0·008	0·9872	0·009	3·95	Sulfonate
O(II)	0·2164	0·013	1·6045	0·011	1·0590	0·011	4·81	
O(III)	0·2414	0·009	1·8693	0·007	1·2261	0·008	3·65	
O(1)	0·2874	0·009	1·5552	0·008	0·6291	0·009	3·69	Ring
O(2)	0·4159	0·010	1·7174	0·008	0·4325	0·009	4·17	
O(3)	0·6293	0·009	1·8424	0·007	0·6286	0·007	3·35	
O(4)	0·6109	0·008	1·8214	0·006	0·9330	0·007	2·92	
O(5)	0·2833	0·009	1·8257	0·007	0·7565	0·008	3·36	
C(1)	0·2903	0·012	1·7474	0·010	0·6177	0·010	3·38	
C(2)	0·4068	0·012	1·8059	0·009	0·5605	0·010	3·28	
C(3)	0·5239	0·011	1·7627	0·009	0·6745	0·010	3·09	
C(4)	0·5027	0·013	1·8585	0·010	0·8219	0·011	3·40	
C(5)	0·3848	0·011	1·7841	0·009	0·8685	0·010	3·10	
C(6)	0·3641	0·014	1·8680	0·011	1·0116	0·012	3·75	
O(2′)	0·1887	0·012	1·2015	0·010	0·5159	0·011	4·96	Glycerol residue
O(3′)	0·0418	0·011	0·9984	0·009	0·6819	0·010	4·29	
C(1′)	0·1734	0·013	1·4719	0·010	0·6617	0·011	3·43	
C(2′)	0·1708	0·016	1·2685	0·013	0·6568	0·014	4·47	
C(3′)	0·0492	0·015	1·1970	0·012	0·6931	0·013	3·96	

* The y and z parameters are chosen in such a way as to show one chemical unit near the twofold screw axis at $a=0$ and $c=1$.

suggested that there exists in the molecule an island made of an identically spaced group of four oxygen atoms including one from the sulfonate group (Shibuya & Benson, 1961). This condition can only be achieved by the orientation of the sulfonate group as found in the crystalline state. Here, the sulfonate group is rotated around the C(6)–C(5) bond towards O(6) in the ring; this places the glycerol residue and the major portion of the sulfonate group on the same side of the sugar ring with short approaches of 3·07 and 3·04 Å for O(I)–O(5) and O(II)–C(5), respectively. To illustrate this configuration, the projection of the structure along the c axis is shown in Fig. 3. Although it is not unreasonable to suggest that the molecule assumes a conformation in solution similar to that in the solid state, the shape of the molecule during an enzyme reaction might differ from the normal conformation as found in the crystal. The interpretation of the anomalous enzyme specificity towards the sulfoglycoside should be more reliable as a result of this crystallographic analysis.

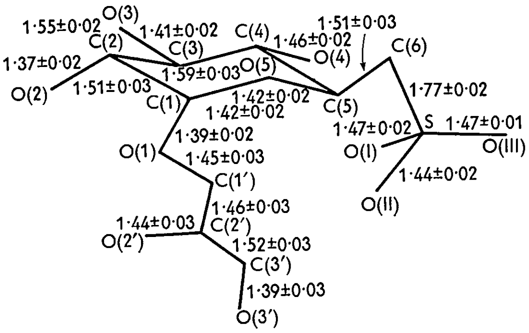

Fig. 4. Bond distances in Å.

(*b*) *Bond distances and angles*

Bond distances and angles and their standard deviations were calculated from the atomic coordinates in Table 1 and listed in Figs. 4 and 5; the angles around S are listed separately in Fig. 5(*b*). It is apparent from these figures that the bond distances and angles are all quite normal and no unusual distances or angles are found among these values.

The configuration of the sulfonate group can be compared to similar data in various sulfonates as well as sulfones. The S–O distances ranging from 1·44 to 1·47 Å in the present structure are comparable to those in other aromatic sulfonates, *e.g.* 1·41–1·47 Å in magnesium and zinc benzene- and toluene-sulfonates (Broomhead & Nicol, 1948; Hargreaves, 1960), 1·43–1·45 Å in sulfamic acid (Osaki, Tadokoro & Nitta, 1955; Sass, 1962) and more recently 1·44–1·46 Å in the molecule of 2-diazonium-4-sulfophenol (Greenberg & Okaya, 1964). The observed C(6)–S distance of 1·77 Å is rather short compared with the theoretical single S–C bond distance but is closer to aromatic bond distances as observed in the above-mentioned sulfonates. However, as found in the two C–S distances (1·669 and 1·718 Å) in the thiazolium group of thiamine hydrochloride, C–S distances with partial

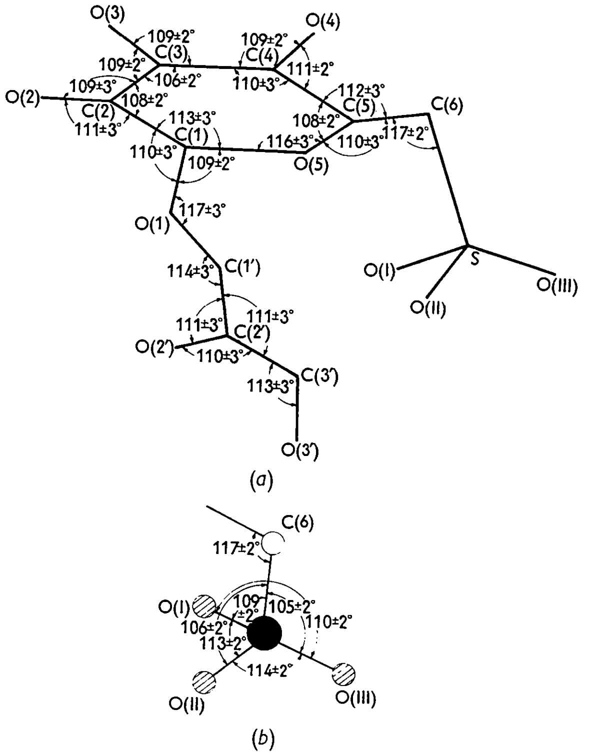

Fig. 5. (*a*) Bond angles. (*b*) Bond angles around S.

double-bond characters exhibit much shorter separations (Kraut & Reed, 1962). As usual, the angles around S are all normal tetrahedral angles. The C(6)–S and C(5)–C(6) distances definitely rule out a sulfate ester with an oxygen attached directly to C(5) for the molecule.

The glucose ring takes the Sachse's *trans* form, a real chair form; the bond distances and angles within the ring are all normal and comparable to the experimental values found in the structures of α-glucose (McDonald & Beevers, 1952), β-glucose (Ferrier, 1960), sucrose (Beevers, McDonald, Robertson & Stern, 1952), α-rhamnose monohydrate (McGeachin & Beevers, 1957), cellobiose (Jacobson, Wunderlich & Lipscomb, 1961), α-glucose monohydrate (Killean, Ferrier & Young, 1962) and others. The sulfonate group is attached to the ring through C(6) which is connected to C(5) of the ring. The proximity of the group to the glycerol residue results in a somewhat large angle of 117° around C(6). The angle of 116° around the ring oxygen atom O(5) is again slightly larger than the tetrahedral angle; however, such an angle can be found in some of the above-mentioned structures, *e.g.* cellobiose (Jacobson *et al.*, 1961).

The glycerol residue is attached to the ring in the axial configuration through O(1) as a bridge oxygen atom, around which an angle of 117° is found. This angle is similar to that around the bridge oxygen atom between the two β-glucose residues in the structure of cellobiose (Jacobson *et al.*, 1961). The bond distances and angles in the glycerol residue are again normal except the rather short C(1′)–C(2′) distance of 1·46 Å. The C(2′)–O(2′) and C(2′)–C(3′) bonds are rotated around the C(1′)–C(2′) bond in such a way as to separate O(1) from O(2′) and O(3′) as far as possible. Those two bonds and the C(1′)–O(1) bond are in *trans–gauche* configurations around the middle C(1′)–C(2′) bond.

(*c*) *The rubidium ion*

The rubidium ion is surrounded by eight oxygen atoms, four oxygen atoms from three sulfonate groups and four hydroxyl oxygen atoms. The observed Rb–O distances range from 2·89 to 3·38 Å; although these Rb–O distances are rather similar to those found in various other rubidium salts, the coordination found in the present crystal is different from some other examples. One can find examples of different coordination numbers for rubidium ions; ninefold coordination with Rb–O distances ranging from 2·90 to 3·25 Å for rubidium dihydrogen citrate (Nordman, Weldon & Patterson, 1960), eightfold coordination in rubidium hydrogen D-tartrate (van Bommel & Bijvoet, 1958), and the present crystal, sevenfold in rubidium hydrogen fumarate (Gupta, 1956), and sixfold in rubidium benzylpenicillin (Crowfoot, Bunn, Rogers-Low & Turner-Jones, 1949). The coordination around the ion in the present crystal is shown in Fig. 6. As is evident from the figure, each ion is connected with O(I) and O(III) of a sulfonate group and with

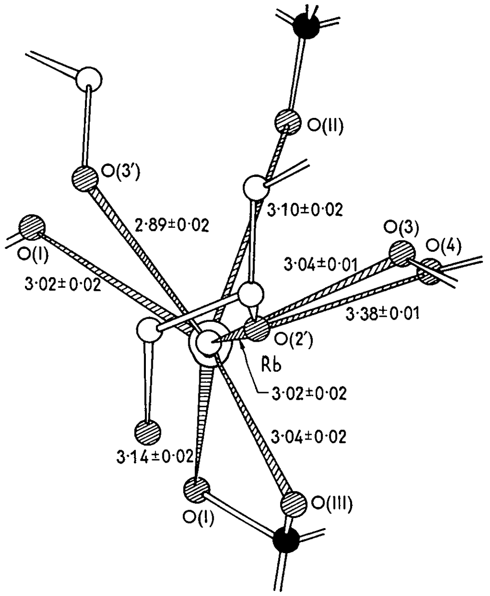

Fig. 6. Coordination around the rubidium ion.

O(II) of the sulfonate group which is one unit cell above along the *b* axis; the ion is then linked to again O(I) of the sulfonate group related to the first by the twofold screw axis at $a=0$ and $c=\frac{1}{2}$ with a translation of plus one-half along the *b* direction. The rubidium ion acts as a binding medium of sulfonate groups by forming SO_3^-–Rb^+–SO_3^- channels parallel to the *b* axis around the twofold screw axes. The four hydroxyl oxygen atoms are supplied from three molecular ions as follows: from the molecule whose O(3) and O(4) possess *y* coordinates close to that of the rubidium, O(2′) from the molecule related to the first by the twofold screw axis at $a=\frac{1}{2}$ and $c=0$, and O(3′) from the molecule related to the second by the twofold screw axis at $a=0$ and $c=1$.

(*d*) *Short intermolecular contacts and a suggested hydrogen-bond scheme*

In Fig. 7, some of the short *inter*molecular approaches which might be considered as hydrogen bonds are shown by dotted lines. Since no effort has been taken to locate hydrogen positions, the assignment of hydrogen bonds to these short contacts must be made by an indirect criterion. It is quite natural to assume that, since the sulfonate group does not possess a proton, the hydrogen involved in any hydrogen bond between a sulfonate oxygen atom and a hydroxyl group is supplied by the hydroxyl group. Therefore, O(4) and O(2) make hydrogen bonds to O(II) and O(III), bonds I and II, respectively. Since a hydroxyl group has one hydrogen atom only, the other two hydrogen bonds are easily assigned; from O(3) to O(2), bond III and then from O(2′) to O(3′), bond IV. For each bond, the relation between the molecules to which the oxygen atoms belong is listed in Table 2; the so-called Donohue angles (Donohue, 1952) are also listed in Table 2 as further evidence towards the criteria. Around O(3′), there are two rather short O–O distances; one to O(5) and the other to O(I), both of the molecule one unit cell below along the *b* axis. Although the second seems to be more promising because of the stronger negativity of O(I), the related Donohue angle is a disappointing one of 99° whereas the angle related to the contact between O(3′) and O(5) has a normal value of 111°, and the fifth hydrogen bond is assigned to this contact, the hydrogen, without doubt, being supplied by O(3′). Existence of a hydrogen bond made to the ether oxygen in a glucose ring is also witnessed in the various structures cited in the previous section. The

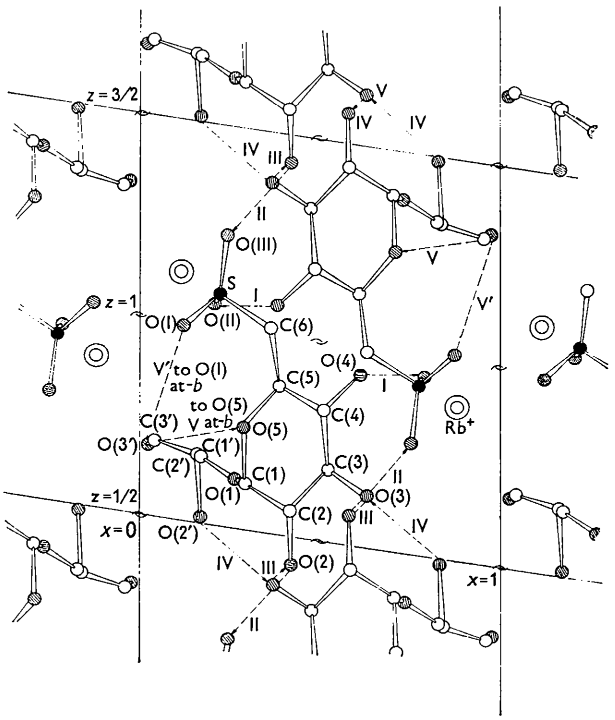

Fig. 7. Projection of the structure along the *b* axis. Short O–O approaches are indicated by chain lines (see text).

Table 2. *Short intermolecular* O–O *approaches and related data*

The coordinates of atoms in square brackets are derived from the corresponding values in Table 1 by the relations ($1-x$, $\frac{1}{2}+y$, $1-z$). The required cell translations are self explanatory

Approaches	Between	And	Distances	Related Donohue angles	
I	O(4)	[O(II)] at $+c$	2·76 Å	C(4)–O(4) · · · O(II) O(4) · · · O(II)–S	110° 134
II	O(2)	O(III) at $-c$	2·70	C(2)–O(2) · · · O(III) O(2) · · · O(III)–S	106 121
III	O(3)	[O(2)]	2·78	C(3)–O(3) · · · O(2) O(3) · · · O(2)–C(2)	108 127
IV	O(2′)	[O(3)] at $-b$	2·77	C(2′)–O(2′) · · · O(3) O(2′) · · · O(3)–C(3)	123 135
V	O(3′)	O(5) at $-b$	2·88	C(3′)–O(3′) · · · O(5)	111
V′	O(3′)	O(I) at $-b$	2·91	C(3′)–O(3′) · · · O(I) O(3′) · · · O(I)–S	99 136

hydrogen bonds found in the present crystal are all of normal distances as compared with those in other sugar structures, with average strength (Wallwork, 1962). It is interesting to note here that O(I), which is not involved in a hydrogen bond, is coordinated to two rubidium ions, whereas each of the hydrogen-bonded O(II) and O(III) deals only with one rubidium ion.

The molecules are held together tightly in the structure by these hydrogen bonds and also through the ionic interaction around the rubidium ion. It is noteworthy that the structure consists of two different types of layer, one through $a=0$, where only the ionic force around the ion exists and the other through $a=\frac{1}{2}$ where the hydrogen bonds connect molecules around the twofold screw axis.

The author is indebted to Dr A. A. Benson for the samples of the rubidium salt and many interesting discussions on the biological significance of the compound.

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A Versatile Cooling Technique for X-ray Diffraction by Single Crystals at Temperatures below 90 °K

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The customary technique for maintaining a crystal at low temperatures by a cold gas stream has been modified by the introduction of a light Dewar vessel attached to the X-ray goniometer, its contents of liquid nitrogen being controlled continuously by a constant level device.

The temperature of the cold gas stream was measured as a function both of the consumption of liquid nitrogen and of the distance of the specimen from the delivery tube. With standard Weissenberg diffraction techniques a complete sequence of equi-inclination photographs can be taken at 87 °K.

Introduction

The advantages of employing low temperatures in X-ray diffraction studies are now widely recognized. Especially in structure analyses of organic crystals, where the often large thermal motions have an adverse effect on the reliability of the determination of interatomic distances, employment of efficient cooling techniques results in a striking increase in accuracy (Cruickshank, 1960); moreover, low-tem-