X-ray Crystal Analysis of the Substrates of Aconitase. IV. The Configuration of the Naturally Occurring Isocitric Acid as Determined from Potassium and Rubidium Salts of its Lactone*

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The relative configuration of the two asymmetric carbon atoms in the naturally occurring isomer of isocitric acid which is synthesized by aconitase from citric acid has been determined by an X-ray crystallographic analysis of the potassium and rubidium salts of the lactone. The space group for the salts, which are isomorphous, is $P2_12_12_1$. The structure was solved with the use of data from the three projections and refined by least-squares methods using complete matrices. It was shown that the carboxylic acid groups in the lactone have the *cis* configuration.

There are four isomers of 1-hydroxy-1,2,3-propane-tricarboxylic acid (Fig. 1, I*a*–IV*a*). One of these is the naturally occurring (+)-isocitric acid, synthesized by aconitase from the symmetric citric acid (*cf. e.g.* Ochoa, 1951). The configuration of this isocitric acid is therefore of considerable interest in determining the stereospecific nature of this reaction. The present investigation was undertaken in order to determine first the relative and then the absolute configuration of (+)-isocitric acid. The lactone was used because, at that time, crystals of the acid itself or of any of its salts were not available.

While this work was in progress Gawron & Glaid (1955) and Gawron, Glaid, LoMonte & Gary (1958) showed that the lactone from (+)-isocitric acid is either I*b* or II*b* (Fig. 1). This result was confirmed by the present work (Glusker, Patterson, Love & Dornberg, 1958). Since Greenstein and his co-workers

(Greenstein, Izumiya, Winitz & Birnbaum, 1955; Winitz, Birnbaum & Greenstein, 1955) had obtained indications that the α-carbon had the configuration L*s*, it was inferred that (+)-isocitric acid had the configuration II*a*. More recently, however, Kaneko & Katsura and their co-workers (Kaneko, Katsura, Asano & Wakabayashi, 1960; Kaneko & Katsura, 1960; Katsura, 1961*a*, *b*, *c*) have shown by classical chemical methods that (+)-isocitric acid has the configuration I*a*. The last result has been confirmed by anomalous dispersion measurements, which have shown that the structures derived in the present paper have the absolute configuration I*b* (Patterson, Johnson, van der Helm & Minkin, 1962).

Experimental

Dr H. B. Vickery and Dr D. G. Wilson of the Connecticut Agricultural Experiment Station provided us with excellent crystals of the monopotassium and monorubidium salts of the lactone of (+)-isocitric acid. These were prepared from the acid extracted from the leaves of the succulent plant *Bryophyllum calycinum*.

The crystals were well-formed compact polyhedra with a marked tendency to cleave parallel to the *b* face. The space group and cell dimensions of the isomorphous rubidium and potassium salts were determined with a Buerger precession camera, the methods described by Patterson & Love (1960) being used. The space group is $P2_12_12_1$, with four molecules in the unit cell. The measured cell dimensions and the densities (determined by flotation) are given in Table 1.

Since the purpose of this investigation was to determine the relative and absolute configuration of the ion it was decided that the analysis should be attempted by two-dimensional methods. Photographs of the three principal zones were taken. An *hkh* photograph was also taken for the potassium salt.
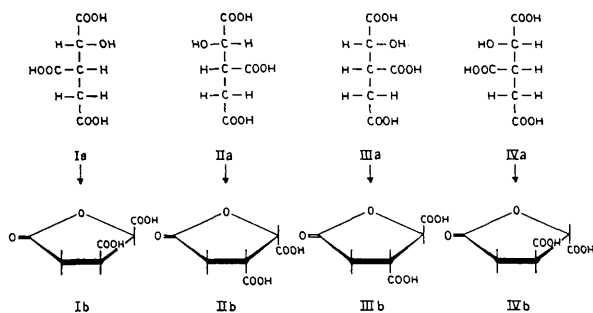

Fig. 1. Isomers of (*a*) 1-hydroxy-1,2,3-propanetricarboxylic acid and (*b*) the corresponding butyrolactone-4,5-dicarboxylic acid.

Table 1. *Cell dimensions and densities*

	a	b	c	Cell volume	Density (obs.)	Density (calc.)
K salt	9·059 Å	12·681 Å	6·640 Å	762·8 Å³	1·838 g.cm⁻³	1·848 g.cm⁻³
Rb salt	9·190 Å	12·639 Å	6·825 Å	792·7 Å³	2·142 g.cm⁻³	2·166 g.cm⁻³

Table 2. *Some data on photographs taken*

		$hk0$	$0kl$	$h0l$	hkh
Number of reflections					
K salt:	Observed	137	95	73	104
	Total	178	113	79	109
Rb salt:	Observed	124	93	76	
	Total	178	112	81	
Camera		Integrated precession	Integrated Weissenberg	Non-integrated Weissenberg	Integrated precession
Radiation		Mo $K\alpha$	Cu $K\alpha$	Cu $K\alpha$	Mo $K\alpha$
Method of measurement of intensities		Photometered	Photometered	Eye-estimated	Eye-estimated
Temperature factor B					
Potassium ion		3·06	1·73	1·99	3·58
Lactone ion		3·14	1·90	2·26	3·58

Some data on the photographic methods are shown in Table 2.

The data for each zone were corrected for the Lorentz and polarization factors with the aid of a 602A IBM computer and then scaled by Wilson's method.

Structure determination

The position of the heavy atom was determined from unsharpened $|F|^2$ syntheses for the $hk0$ and $0kl$ projections of the rubidium salt. For the rubidium salt 113 $hk0$ and 78 $0kl$ signs could be determined with little ambiguity by both isomorphous replacement and the heavy atom approach. For the potassium salt there was considerable ambiguity in the isomorphous sign determination because the y parameter of the heavy atom was close to ¼.

The Fourier maps which were calculated for the rubidium salt are shown in Figs. 2(a) and 3(a). The interpretation, which was quite difficult, made use of the expected coordination radius of oxygen around the metal ion (Nordman, Weldon & Patterson, 1960a, b). Scale models were constructed of two molecules, one with the two carboxyl groups cis with respect to the ring and the other with them trans. The $hk0$ map could be fitted by the cis model only, to give an R value for the potassium salt of 0·38, which refined by difference Fourier syntheses to 0·28 after one stage, and then to 0·11 after several stages. However, two models consistent with the $hk0$ map fitted the $0kl$ map equally well at this point (cf. Fig. 3(a)). One model gave an R value of 0·43 for the potassium salt and a Fourier and difference Fourier synthesis which indicated refinement toward the
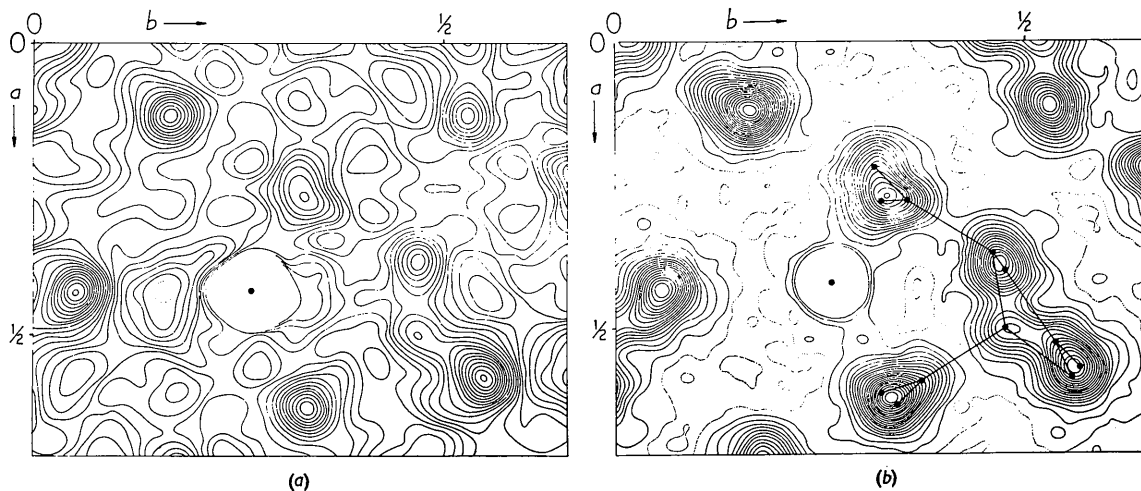

Fig. 2. $hk0$ projection: (a) Rubidium isocitric lactone. Phases of rubidium atom. (b) Potassium isocitric lactone. Phases of final structure. Contour interval 1 e.Å⁻². 0 and 1 e.Å⁻² dotted. Higher contours for metal omitted.
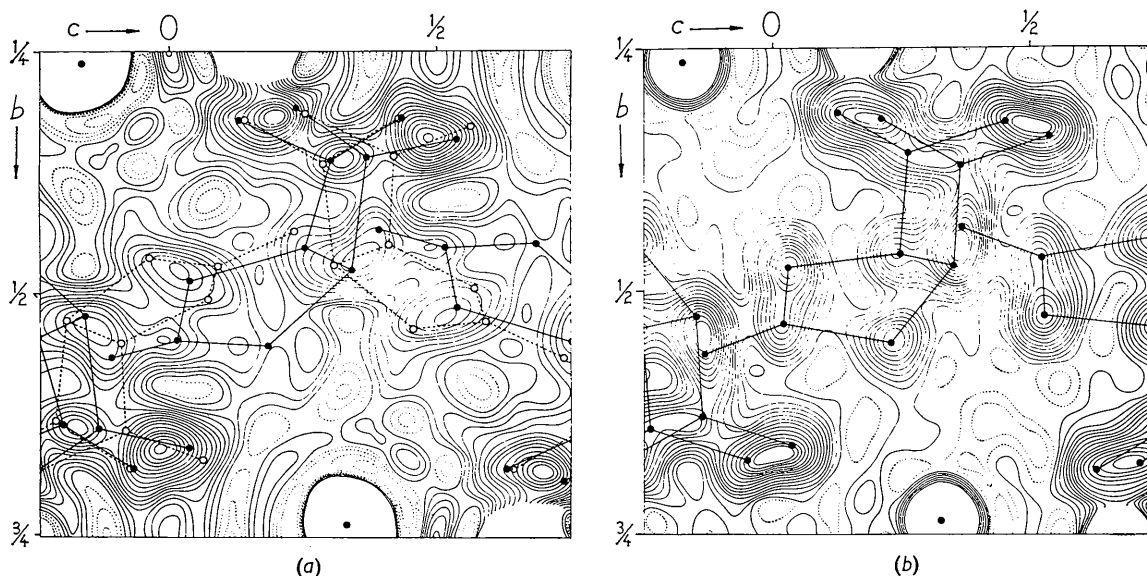
Fig. 3. 0kl projection: (a) Rubidium isocitric lactone. Phases from isomorphous replacement. Trial structures: Filled circles and dark lines, $R = 0.34$. Open circles and broken lines, $R = 0.43$ (K salt). (b) Potassium isocitric lactone. Phases of final structure. Contours as for Fig. 2, except 0, 1, 2, 3 e.Å^{-2} dotted in (a).

alternative model. The latter gave an R value of 0.34 which refined to 0.14.

It was essential to differentiate between the methylene carbon atom and the oxygen atom in the ring. Therefore the scattering factors for the two atoms in question were interchanged, and R values and difference Fourier syntheses were calculated. These calculations agreed in demonstrating that the model chosen was indeed correct. This conclusion was supported by the values of distances of closest approach of these atoms to a metal ion which were 3.00 Å for the oxygen and 3.95 Å for the methylene carbon atom.

Utilizing x and z parameters from the $hk0$ and $0kl$ projections four possible models for the $h0l$ projection resulted owing to the close similarity of the y values for the two oxygen atoms in each carboxyl group (Table 3). Two models could be eliminated by examining a Fourier synthesis for the rubidium salt, using heavy atom signs. The two remaining had positional parameters so close together that the choice between them was left to the subsequent least-squares calculation.

Refinement of the structure of the potassium salt

Initially, as mentioned above, the structures were refined in projection, shifts indicated by difference Fourier syntheses being used. When the R value was 0.11 for the $hk0$ projection and 0.14 for the $0kl$ projection refinement was continued by the method of least squares using an IBM 602A computer to calculate the 26×26 matrices for the positional parameters for each projection. In calculating the matrix terms each reflection was weighted according to the reciprocal

Table 3

(a) Coordinates of atoms in one molecule of potassium isocitric lactone

In a right-handed system these coordinates give the absolute configuration determined by Patterson *et al.* (1962)
(Values as fractions of cell edges)

	x	y	z	σx	σy	σz
K	0.9185	0.2357	0.1713	0.0003	0.0003	0.0007
O(1)	0.2794	0.3248	0.4525	0.0012	0.0008	0.0024
O(2)	0.2182	0.3168	0.1265	0.0012	0.0008	0.0024
O(3)	0.5672	0.5647	−0.1306	0.0015	0.0011	0.0027
O(4)	0.6090	0.3228	0.2125	0.0014	0.0009	0.0028
O(5)	0.6306	0.3407	0.5402	0.0013	0.0011	0.0023
O(6)	0.3985	0.4753	0.0308	0.0012	0.0009	0.0023
C(1)	0.5005	0.4744	0.3545	0.0016	0.0012	0.0037
C(2)	0.5252	0.5352	0.0241	0.0017	0.0014	0.0033
C(3)	0.5882	0.3706	0.3646	0.0018	0.0012	0.0038
C(4)	0.2763	0.3573	0.2629	0.0018	0.0012	0.0040
C(5)	0.3668	0.4604	0.2485	0.0019	0.0013	0.0037
C(6)	0.5864	0.5539	0.2319	0.0023	0.0015	0.0038

(b) Average values of standard deviations for each type of atom

	σx	σy	σz
K	0.0027 Å	0.0033 Å	0.0045 Å
O	0.012	0.012	0.017
C	0.017	0.017	0.025

(c) Standard deviations for bond lengths

K – O	0.025 Å
O – O	0.034
C – O	0.042
C – C	0.049

of the square of the standard deviation of the measurements of F from the films. The average values of the relative standard deviations in intensity ($\sigma I/I$), were 0.09 for $hk0$, 0.13 for $0kl$, 0.12 for $h0l$. Unobserved

terms were not included in the least squares calculation.

An 'educated' Gauss–Seidel method, described by Greenberger & Ward (1953), was used to solve the equations for coordinate shifts. In all projections many of the off-diagonal terms in the matrices were very large and certainly could not have been ignored. With each cycle of refinement of the coordinates the scale factor and two isotropic temperature factors, one for the metal and the other for the lactone ion, were calculated.

In one cycle of refinement the R value dropped from 0·11 to 0·08 for the $hk0$ projection and from 0·14 to 0·12 for the $0kl$ projection. The final R values, with independently determined parameters for each projection, were 0·07 for $hk0$, 0·12 for $0kl$ and 0·13 for $h0l$. The four hydrogen atoms attached to carbon atoms were included at tetrahedral positions (C–H 0·95 Å) in the later structure factor calculations but were not included in the matrices. It was not possible to locate the hydrogen atom in the hydrogen bond from Fourier or difference Fourier maps. The final values for the temperature factors (which include an absorption effect) are given in Table 2.

In order to estimate the accuracy of positional parameters for the three projections the three final 26×26 matrices were inverted.* This made possible the determination of the standard deviations of the two coordinates of any atom in each projection directly from the standard deviations of the observations. The final parameters, given in Table 3a, were in each case obtained by forming a weighted aver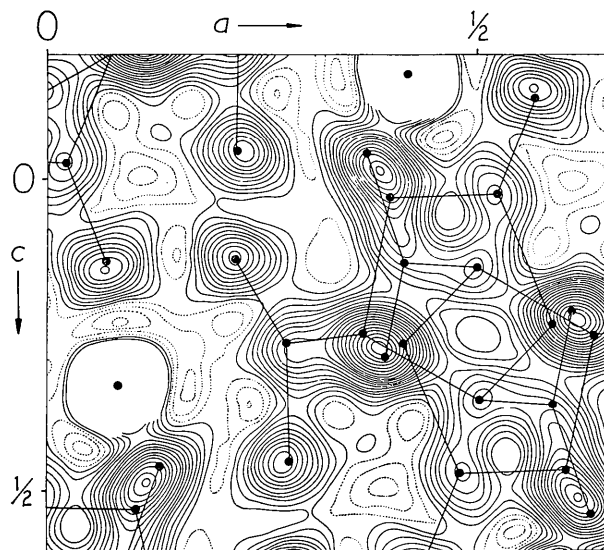age of the two values obtained for each parameter from the projections. The standard deviations of each of these averages are included in Table 3a and were used to determine the average value of the standard deviations for each type of atom given in Table 3b. From these the standard deviations for bond lengths were calculated (Table 3c).

As a check on the correctness of this structure, the final parameters were used in a structure factor calculation for the previously unused hkh data. The R value obtained was 0·10.

At the end of the refinement electron density maps for the three projections were calculated (Figs. 2(b), 3(b), 4).

Discussion of the structure

This study has shown that the lactone derived from (+)-isocitric acid has the cis configuration (Fig. 5).
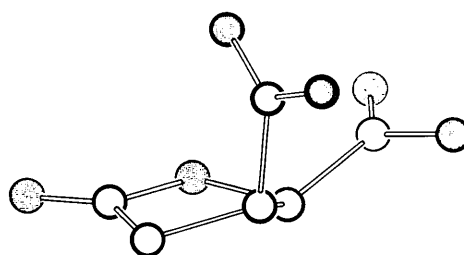

Fig. 5. View of isocitric lactone ion showing the cis configuration. Oxygen atoms stippled.
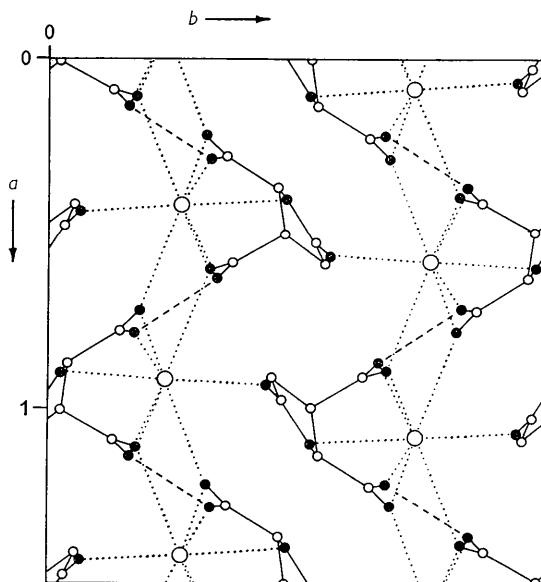

Fig. 6. Overall packing of potassium isocitric lactone. Large open circles: potassium. Small open circles: carbon. Filled circles: oxygen. Dashed lines: hydrogen bonds. Dotted lines: K–O contacts.

Fig. 4. $h0l$ projection. Potassium isocitric lactone. Phases of final structure. Contours as for Fig. 3(a).

* We are indebted to the University of Pennsylvania Computer Center and the National Science Foundation for their support in carrying out these calculations on UNIVAC I.

Thus, in the light of the determination of absolute configuration (Patterson et al., 1962), the correct configurational formula is (4S:5R)-cis-butyrolactone-

4,5-dicarboxylic acid (Cahn & Ingold, 1951; Cahn, Ingold & Prelog, 1956).

The overall packing in the crystal is shown in Fig. 6. The lactone ions are linked by hydrogen bonds (2·49 Å), to form a spiral chain about the screw axes in the a direction. These chains are bound together by their participation in the coordination polyhedra of the potassium ions to form layers perpendicular to the b axis. Between two such layers the only bonding is a single coordination link between the ketonic oxygen O(3) of the ring and the potassium ion (cf. Fig. 6). Thus the very striking cleavage parallel to (010) is explained.
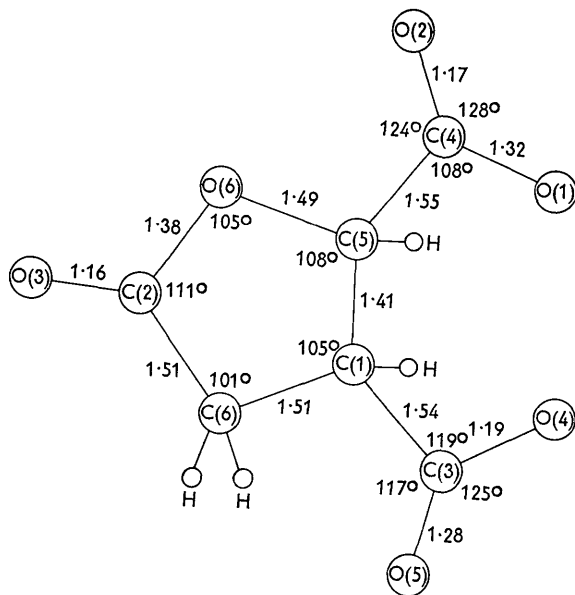

Fig. 7. Bond lengths and angles in the lactone ion.

The covalent bond lengths and bond angles are shown in Fig. 7. It should be emphasized here that the standard deviations of bond lengths given in Table 3(c) are probably somewhat low, owing to systematic errors such as our failure to correct for absorption. We therefore believe that differences of the order of 0·1 to 0·15 Å in bond lengths or departure of bond lengths from expected values are probably not significant.

Within these reservations, we can point out that there is no apparent difference between the carboxyls. Both show the characteristic long and short C–O distances of an unionized carboxyl group. The hydrogen bond is between the two oxygens with the longer C–O distances. The carboxyl groups are coplanar with the ring carbons to which they are attached. The ring oxygen O(6) is close to the plane of its α carboxyl group (in this case 0·4 Å out of the plane) as was the hydroxy group in rubidium dihydrogen citrate (Nordman et al., 1960a), citric acid (Nordman et al., 1960b), and sodium dihydrogen citrate (Glusker, van der Helm, Love, Dornberg & Patterson, 1960).

The distance between C(2) and O(6) is shorter than that between C(5) and O(6), as has been noted in other lactones (Mathieson & Taylor, 1961; Fridrichsons & Mathieson, 1962).
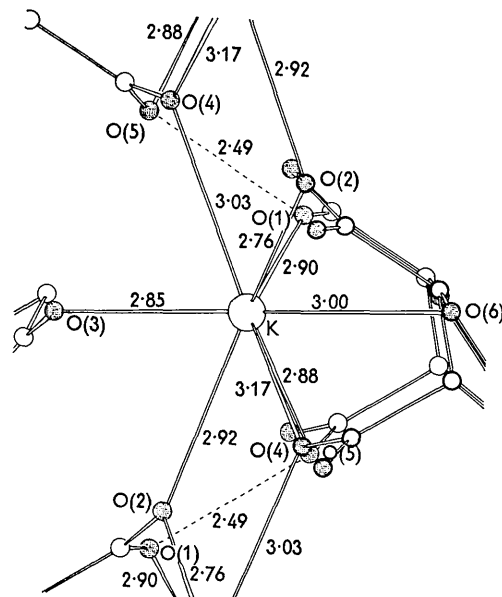

Fig. 8. Coordination of oxygen atoms around the potassium ion.

The coordination sphere about the metal is illustrated in Fig. 8. Eight oxygen atoms pack around each potassium ion. Each lactone ion forms two bidentate chelate rings with different potassium ions (separated by a c translation). One of the rings is five-membered (KO(2)C(4)C(5)O(6)) while the other is seven-membered (KO(1')C(4')C(5')C(1')C(3')O(5')). The coordination polyhedra occur in chains along the a axis sharing one edge defined by O(2) and O(4) atoms from different lactone ions.

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A Second Comparison of Various Commercially Available X-ray Films*

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Various properties of commercially available X-ray films were tested for the second time, with an improved technique. (For the first tests, see Acta Cryst. (1956), 9, 520.) The tested films were of forty-three types, made by eighteen manufacturers. The tested properties were: speeds for Cu and Mo radiation; fog density; granularity; absorption of Ag, Mo, Br, Cu, Fe and Cr $K\alpha$ radiation; D–E characteristics; film homogeneity; sensitivity for safety lights; aging effect, etc. The results are tabulated and the properties of the films are briefly discussed.

1. Introduction

Some years ago, a comparison of 41 commercially available X-ray films was carried out under the sponsorship of the Commission on Crystallographic Apparatus of the International Union of Crystallography (1956) at the University of Groningen by Dr D. W. Smits and Prof. E. H. Wiebenga. The aim of the investigation was to obtain reliable information about the relative merits of various types of X-ray film. As since the time of this comparison new types of X-ray film had appeared and the properties of the investigated types might have been changed, the Commission decided at its Conference in Stockholm (1959) that the investigation be repeated. In addition it was felt desirable that some improvements in the experimental technique be made and some additional properties be included in the new comparison.

Two of the more important improvements made here were the following:

1. Fluorescent Cu and Mo radiations, which are almost pure $K\alpha$ lines, were used instead of filtered radiations from Cu and Mo targets.

2. The film speeds were measured in an absolute scale (in μ^2/photon) instead of a relative one.

Forty-three different types of X-ray film made by eighteen manufacturers in ten countries were collected.‡ All the films were received between September 1960 and May 1961, and were tested during the period March 1961 to February 1962.

2. Experimental

(a) Experimental arrangement

The experimental arrangement is schematically shown in Fig. 1. It was constructed by the Rigaku-Denki Co. Ltd.§ to a special order.

The input to the X-ray generator was first stabilized by a 3 kVA electronic stabilizer St. The high voltage was of full-wave rectified and smoothed by a condenser C of 0·2 μF, the tube current usually being

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* Reprints of this article can be obtained from the authors, or from the General Secretary of the International Union of Crystallography, Dr D. W. Smits, Mathematisch Instituut, University of Groningen, Reitdiepskade 4, Groningen, The Netherlands.

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‡ Films made in the U.S.S.R. were not available in the present investigation. Some of their data are given by Богомолов et al. (1961).

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