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X-ray Crystal Analysis of the Substrates of Aconitase.* II. Anhydrous Citric Acid

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Anhydrous citric acid crystallizes in the space group $P2_1/a$ with four molecules in a cell of dimensions

$$a = 12{\cdot}821,\quad b = 5{\cdot}622,\quad c = 11{\cdot}545\ \text{Å},\quad \beta = 111{\cdot}17°.$$

The structure was analyzed by means of the $h0l$ and $hk0$ projections but was refined by least squares using three-dimensional data measured photometrically on integrated films. The main carbon chain is an approximately planar zig-zag with one of the terminal carboxyl groups in the same plane and the other at right angles to it. The central carboxyl group is approximately co-planar with its α-hydroxyl group. In the crystal all available hydroxyl hydrogens form hydrogen bonds.

The present study of citric acid was undertaken as part of a study of the structural chemistry of the substrates of the enzyme aconitase (cf. Nordman, Weldon & Patterson, 1960).

Experimental

The usual form of citric acid is the monohydrate which is grown by slow evaporation of a solution saturated at room temperature. It is orthorhombic (Groth, 1910) and has been shown (Burns & Iball, 1954) to belong to the space group $P2_12_12_1$ with four molecules in a cell of dimensions

$$a = 6{\cdot}290,\quad b = 9{\cdot}318,\quad c = 15{\cdot}39\ \text{Å}.$$

We have found that the anhydrate, which is obtained by cooling a hot saturated solution, crystallizes in the space group $P2_1/a$ with four molecules in a cell of dimensions

$$a = 12{\cdot}821,\quad b = 5{\cdot}622,\quad c = 11{\cdot}545\ \text{Å};\quad \beta = 111{\cdot}17°.$$

The standard deviation of the cell dimensions is $\pm 0{\cdot}2\%$ and that of the angle $\pm 0{\cdot}10°$. The centrosymmetrical space group and the short b-axis suggested that the anhydrate was more suitable than the hydrate for X-ray analysis.

Three-dimensional diffraction data were collected using an integrating precession camera (Nordman, Patterson, Weldon & Supper, 1955) and an integrating Weissenberg camera (Wiebenga & Smits, 1950). Layers parallel to $h0l$ with $k \leq 3$ and layers parallel to $0kl$ with $h \leq 7$ were recorded on the precession camera using Mo $K\alpha$ radiation. A number of diagonal nets were also photographed with this arrangement. The $h4l$ and $h5l$ nets were photographed on the Wiebenga camera using Cu $K\alpha$ radiation. The precession camera was also used to photograph with Cu $K\alpha$ the portion of the reciprocal lattice inside the 1 r.l.u. sphere for this radiation.

All films were photometered with an automatic scanning and recording microphotometer. A calibrated scale was prepared correlating relative X-ray exposure with photographic density. Measurements at the center of each integrated peak and of the background were made for each diffraction spot, and the difference between these measurements was taken as the diffraction intensity.

With Mo $K\alpha$ radiation filtered in the usual manner with Zr foil, the long wavelength white radiation streak from a strong reflection may overlap the next higher order of the same plane. This difficulty may be avoided by photometering along the streak, i.e. radially from the center of the photograph. Since this detracts from one of the main advantages of the integrating precession camera, additional Zr filters were inserted alone or in combination with a 0·6 mm sheet of aluminum directly in front of the casette. In this manner cleaner photographs were obtained in return for a very much longer exposure time.

Of the 2237 non-equivalent reflections inside the 1 r.l.u. sphere for Mo $K\alpha$, 2092 were photographed. For the most part, those which were omitted lay outside the limits of the Weissenberg camera using Cu $K\alpha$ and occurred on the $h4l$ and $h5l$ layers. Among the reflections photographed, 1246 (59·6%) were within measurable range and 846 were below this range.

Different exposures of the same net were put on a common scale, averaged, and corrected graphically for the Lorentz-polarization factor (Waser, 1951; Gren-

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ville-Wells & Abrahams, 1952). The stacks parallel to $0kl$ and $h0l$ were then brought to a common scale by an averaging procedure in which the ratios established by all intersecting rows were taken into account. After the two stacks were put on a common scale, the diagonal nets were adjusted to this scale by taking the average ratio for the planes in common between the diagonal net and the set already standardized.

The complete set of three-dimensional data was placed on an absolute basis by means of a Wilson plot. It is interesting to note that the value $B = 1{\cdot}30$ Å^2 obtained from the Wilson plot was in close agreement with the final value $B = 1{\cdot}292$ Å^2 obtained from the least-squares procedure, and that the final value of the scale factor k was 0·975 with respect to the Wilson figure.

Structure determination

The early stages of the investigation consisted of a series of unsuccessful attempts to analyze the structure. An $h0l$ Patterson projection showed one large peak whose position and size seemed to indicate two hydrogen bonded carboxyl groups either in the dimer form or in a chain about a screw axis. No other features of this map could be interpreted either directly or with the aid of shift methods, and attempts to refine the shift maps were unsuccessful.

As soon as three-dimensional data were available, a three-dimensional Patterson function was computed on XRAC.* The origin peak was not removed, but a moderate sharpening factor was applied. From this series we obtained confirmation of the configuration of the carboxyls, but could not obtain a decision between the possibilities of dimer and screw axis chain. We could also observe the interatomic vectors associated with the carboxyl groups among the peaks near the origin, but could not identify with any certainty any other vectors within the molecule.

An attempt was then made to apply 'direct' methods to the problem. Since the largest unitary structure factor was 0·48, no direct use of inequalities (Harker & Kasper, 1948) could be made.

The procedure originally suggested by Gillis (1948) of applying an extra temperature factor (equal to the original) was attempted. This yielded a few signs and a large number of relationships between signs. The method of Zachariasen (1952) was then used to give tentative allocation to a larger number of signs. In this work three-dimensional data were used but the main effort was concentrated on the signs of the $h0l$ zone. Several $h0l$ Fourier projections were calculated from the signs obtained in this way differing in choice of doubtful strong signs, and in many cases unsuccessful attempts at Fourier refinement were made.

Although it did not prove possible to fit a molecule to any one of the $h0l$ Fourier projections referred to above, a number of features repeated themselves in many of them and in the Harker section. There seemed to be an indication that the one carboxyl group originally postulated was approximately perpendicular to the ac plane and pointed across the center at $0, \frac{1}{2}$. This group seemed to be joined by a ridge of high density to a second carboxyl, approximately parallel to the ac plane pointing toward the screw axis at $\frac{1}{4}, 0$. At this point, a model consisting of the two carboxyls as described above with their α carbons was set up as a starting point for a refinement procedure which is essentially the 'error synthesis' of Crowfoot, Bunn, Rogers-Low & Turner-Jones (1949).

In this refinement process only those reflections having $\sin^2\theta/\lambda^2 \leq 0{\cdot}200$ were considered, but no special modification function was used to cut off at this point. Using the eight atom model (two carbons and three oxygens omitted) a set of structure factors was calculated. A Fourier series was then computed using only those terms for which both F_o and F_c were so large that it was impossible for the sign to change without the combined effect of all the omitted atoms coupled with a considerable shift of the assumed atoms. A difference Fourier was also computed using these terms and also the terms for which F_o was extremely small. The Fourier and the difference Fourier were then considered together. If the two series agreed in suggesting a shift or the addition or removal of an atom or a change in its atomic number this suggestion was followed for the next model. If the two did not agree action was not taken. Four atoms were added after the first cycle giving an R factor of about 0·38. From then on the improvement was very slow for about six cycles, during which atoms were removed and added and some atoms moved steadily while others moved more or less at random. The R factor then began to drop slowly to about 0·30 and then more rapidly to about 0·18 (all $h0l$ reflections) after thirteen cycles of refinement.

Table 1. *Coordinates for oxygen and carbon atoms for a single molecule*

(Multiple of cell edges)

	X	Y	Z
O_1	0·0790	0·6724	0·4358
O_2	0·0447	0·2813	0·4268
O_3	0·3777	−0·0773	0·1885
O_4	0·1989	−0·1773	0·0905
O_5	0·0142	0·2722	0·0682
O_6	−0·0113	−0·0249	0·1849
O_7	0·1994	0·0132	0·3520
C_1	0·0898	0·4568	0·3998
C_2	0·1598	0·4382	0·3195
C_3	0·1627	0·1955	0·2616
C_4	0·2489	0·2084	0·1960
C_5	0·2692	−0·0304	0·1515
C_6	0·0458	0·1332	0·1684

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Table 2. *Comparison of observed and calculated structure factors*

All atoms included at coordinates of Tables 1 and 5. Unobserved reflections in the range of the photographs are marked with an asterisk and the F_o value given is an upper limit. H, K, L are used for h, k, l

L	OBS	CALC
0,0,L		
1	6.6	4.9
2	41.2	-43.8
3	52.4	-58.7
4	55.0	-59.3
5	39.6	47.9
6	8.7	-5.8
7	*5.8	-7.3
8	42.8	-54.2
9	8.6	-7.1
10	7.6	1.1
11	13.8	20.2
12	20.0	21.8
13	*6.0	-11.6
14	*9.6	0.6
2,0,L		
-15	*6.2	-0.2
-14	*6.2	3.5
-13	6.6	3.0
-12	9.2	7.1
-11	17.3	-20.6
-10	8.3	11.4
-9	19.7	-17.3
-8	36.8	-41.5
-7	*5.5	0.2
-6	40.4	-46.9
-5	61.8	72.5
-4	31.6	39.8
-3	29.4	32.0
-2	29.4	-25.8
-1	49.1	51.4
0	16.0	18.0
1	79.1	-79.8
2	18.1	25.2
3	80.0	-93.4
4	10.8	-6.1
5	11.9	13.8
6	*5.8	-0.4
7	27.5	32.4
8	27.1	-29.7
9	9.1	-9.1
10	*6.4	-6.3
11	11.2	12.6
12	*6.3	3.3
13	*6.0	-5.8
4,0,L		
-15	*06.0	-0.6
-14	12.9	15.2
-13	4.8	-5.6
-12	4.6	-4.9
-11	*6.2	-4.2
-10	*6.2	6.7
-9	26.3	-22.8
-8	20.5	23.5
-7	12.4	-12.3
-6	*5.0	-7.2
-5	39.4	35.9
-4	99.0	-13.3
-3	12.9	12.5
-2	4.8	-1.7
-1	35.3	35.3
0	42.8	43.7
1	65.5	63.2
2	22.6	26.3
3	53.5	-57.9
4	37.6	40.7
5	19.4	-24.0
6	15.5	14.2
7	18.2	-16.8
8	38.0	-36.7
9	*6.4	0.8
10	7.6	-11.8
11	9.5	12.3
12	10.1	8.3
13	5.1	7.3
6,0,L		
-15	10.7	10.4
-14	*5.6	-5.5
-13	*6.0	1.3
-12	21.7	27.3
-11	8.0	-5.2
-10	8.3	-21.5
-9	24.3	-29.7
-8	14.3	-19.9
-7	25.2	-26.3
-6	14.4	15.7
-5	41.0	-39.0
-4	5.8	5.1
-3	74.0	82.3
-2	18.2	-17.4
-1	73.9	79.5
0	12.9	11.5
1	12.3	-9.5
2	29.3	-22.4
3	9.4	-12.6
4	13.0	11.8
5	27.7	-29.9
6	*6.3	-5.0
7	21.6	-22.3
8	8.3	9.1
9	4.8	8.3
10	*6.0	2.6
11	13.7	13.8
12	*5.2	-6.4
8,0,L		
-16	6.7	12.9
-15	8.8	14.0
-14	*5.6	-5.9
-13	*6.0	0.3
-12	19.4	-22.9
-11	14.7	-12.6
-10	10.8	13.5
-9	15.7	-19.4
-8	*5.8	-3.0
-7	*5.8	-8.8
-6	13.3	15.1
-5	25.5	28.1
-4	23.7	24.9
-3	51.1	-48.4
-2	13.0	-14.5
-1	45.6	45.4
0	*5.5	-2.3
1	22.0	21.8
2	33.1	-31.5
3	*6.2	-12.8
4	*6.3	3.7
5	12.1	11.0
6	19.4	22.1
7	12.6	-17.6
8	15.9	-17.6
9	10.7	-13.3
10	7.8	8.0
10,0,L		
-14	*6.4	4.7
-13	*6.4	-3.9
-12	7.4	-11.0
-11	10.2	11.2
-10	*6.4	3.6
-9	*6.3	5.7
-8	*6.2	-5.7
-7	36.1	-41.4
-6	*6.0	-5.8
-5	*5.9	0.9
-4	*5.8	11.0
-3	18.3	16.9
-2	15.9	14.1
-1	20.0	-14.8
0	7.9	7.1
1	28.4	31.1
2	10.9	9.0
3	*6.4	3.4
4	30.0	-28.4
5	7.8	-9.5
6	11.3	-8.4
7	9.4	-9.5
8	*6.0	8.3
9	*6.0	-2.6
12,0,L		
-15	19.5	25.9
-14	6.2	-2.3
-13	*6.4	-1.3
-12	13.7	-15.6
-11	8.6	-14.5
-10	*6.5	-3.1
-9	*5.8	2.5
-8	*6.5	-8.7
-7	*6.4	-5.0
-6	*6.4	-5.0
-5	*6.2	-0.1
-4	27.5	29.5
-3	21.5	21.3
-2	*6.4	1.7
-1	10.3	-5.7
0	*6.5	4.5
1	8.9	-4.7
2	7.9	-8.9
3	9.4	-9.2
4	*6.4	-2.9
5	*6.2	1.8
6	8.7	-6.4
7	5.1	9.0
14,0,L		
-14	6.0	-7.8
-13	7.1	3.5
-12	8.2	-8.7
-11	*6.1	6.3
-10	*6.0	-2.7
-9	*6.3	-4.2
-8	*6.4	4.3
-7	*6.5	-4.3
-6	*6.6	-0.7
-5	12.3	10.1
-4	*6.3	3.2
-3	*6.3	-0.6
-2	*6.2	-3.4
-1	*6.2	-0.4
0	*6.0	-0.6
1	*6.1	-0.5
2	12.7	12.5
3	8.8	8.4
4.	*5.6	-5.9
16,0,L		
-12	9.0	-9.4
-11	9.2	7.9
-10	5.6	4.5
-9	5.8	3.7
-8	13.9	-18.0
-7	16.3	-18.2
-6	7.3	7.7
-5	9.2	7.7
-4	9.2	10.8
-3	6.1	4.9
-2	*6.3	-1.5
-1	9.0	9.4
0	*2.4	0.7
1	4.4	6.7
0,1,L		
1	44.1	-44.9
2	11.3	10.8
3	10.3	9.3
4	6.5	-9.8
5	14.6	-14.5
6	15.8	-13.5
7	*5.5	-1.6
8	13.4	-15.1
9	*9.1	0.8
10	8.4	2.8
11	8.0	8.7
12	*9.9	6.0
13	*9.9	-
14	*10.0	10.0
1,1,L		
-15	*10.2	3.5
-14	*1.0	-1.9
-13	8.5	-5.1
-12	9.6	12.6
-11	6.6	6.1
-10	17.2	-18.4
-9	6.5	2.9
-8	*8.5	-4.1
-7	*7.7	-4.9
-6	37.9	37.3
-5	29.5	-32.1
-4	5.4	-7.3
-3	16.3	-15.8
-2	17.6	18.0
-1	67.8	83.9
0	15.0	-12.3
1	9.8	-8.8
2	58.9	-67.9
3	16.5	-14.6
4	6.4	5.9
5	27.3	25.2
6	10.9	7.4
7	21.2	-15.8
8	8.8	11.8
9	17.5	-14.1
10	7.3	7.9
11	20.4	17.8
12	*10.2	-8.3
13	*1.0	-0.3
14	*10.2	-0.7
2,1,L		
-15	*01.0	1.7
-14	10.3	11.6
-13	*1.0	7.0
-12	14.8	-12.6
-11	11.5	-12.3
-10	*9.1	-5.7
-9	*8.7	2.0
-8	*8.2	4.7
-7	9.1	-12.2
-6	12.4	-13.2
-5	*6.0	-0.7
-4	23.2	25.7
-3	58.3	74.6
-2	66.3	70.1
-1	3.8	1.7
0	28.5	-31.9
1	4.3	-3.0
2	8.8	11.1
3	50.1	-55.3
4	32.6	-29.2
5	24.1	-26.3
6	*8.0	-8.0
7	*8.7	6.1
8	*9.1	0.9
9	18.9	19.6
10	10.0	5.5
11	10.9	12.4
12	*10.2	3.5
13	*10.2	1.4
14	*10.2	-2.0
3,1,L		
-15	*10.2	-4.9
-14	*10.2	-13.2
-13	*10.2	3.3
-12	*9.9	4.5
-11	*9.5	4.9
-10	*9.1	6.7
-9	9.9	-12.9
-8	28.0	-28.4
-7	22.4	21.5
-6	39.3	40.9
-5	27.2	29.9
-4	11.2	10.2
-3	88.4	-96.4
-2	12.0	-10.5
-1	9.8	-8.2
0	26.1	26.0
1	49.5	49.6
2	60.1	-62.2
3	6.1	-7.2
4	*6.7	4.1
5	18.8	20.8
6	24.9	21.6
7	*8.9	0.9
8	15.1	-17.9
9	7.6	-6.2
10	11.0	14.8
11	7.8	-6.5
12	*10.2	-1.7
13	*10.2	0.1
4,1,L		
-15	*10.2	3.5
-14	*10.2	-5.5
-13	13.4	-12.5
-12	6.4	-4.0
-11	6.1	-8.0
-10	10.7	-14.3
-9	11.0	-13.3
-8	8.0	9.3
-7	28.7	31.2
-6	30.3	34.6
-5	16.6	14.6
-4	*5.5	4.6
-3	39.6	42.9
-2	*4.3	-2.0
-1	*3.6	-2.0
0	21.7	-24.2
1	48.1	-48.8
2	33.4	-29.2
3	11.4	-9.6
4	36.3	38.0
5	*8.0	-10.1
6	*8.7	-3.8
7	*9.4	-5.7
8	10.5	10.4
9	15.9	17.5
10	*10.2	-5.2
11	*10.2	1.6
12	*10.2	-9.0
13	*10.2	3.4
5,1,L		
-15	*10.2	3.8
-14	*10.2	2.6
-13	7.3	-11.3
-12	7.0	-8.4
-11	10.4	14.4
-10	8.8	13.4
-9	6.1	7.3
-8	10.6	-11.3
-7	8.5	-11.8
-6	7.7	-7.1
-5	8.7	13.0
-4	*5.8	-2.3
-3	19.5	-19.5
-2	*5.2	-7.1
-1	10.2	-12.4
0.	61.0	69.4
1	*5.8	-8.8
2	21.7	-22.5
3	*7.3	-1.3
4	28.2	-28.8
5	37.2	35.9
6	7.5	11.5
7	21.2	-17.8
8	*7.6	-9.3
9	*9.5	-5.6
10	9.9	3.5
11	12.5	14.5
12	*10.2	11.0
6,1,L		
-16	*10.2	-0.2
-15	*10.2	0.6
-14	*10.2	-5.7
-13	*10.2	-4.2
-12	5.8	-5.7
-11	16.5	-11.3
-10	13.8	13.0
-9	12.9	10.5
-8	17.2	14.5
-7	18.2	-14.9
-6	17.5	-12.1
-5	14.1	12.4
-4	26.3	22.1
-3	15.3	14.3
-2	25.9	-20.6
-1	25.8	26.2
0	*6.0	-8.8
1	*6.6	3.0
2	*7.2	2.6
3	7.5	-8.5
4	10.4	-7.0
5	25.1	-27.3
6	9.1	9.7
7	9.4	-7.3
8	9.7	3.0
9	10.0	-0.6
10	8.3	6.7
11	12.9	16.2
12	*10.2	-1.1
7,1,L		
-16	*10.2	-2.4
-15	*10.2	7.5
-14	*10.2	1.9
-13	*10.2	2.4
-12	*9.9	-0.7
-11	5.3	-7.9
-10	*9.1	-0.8
-9	*8.7	2.4
-8	*8.2	-4.0
-7	*7.7	0.4
-6	9.3	-11.1
-5	29.0	25.3
-4	19.3	21.8
-3	8.4	-8.2
-2	18.1	-18.3
-1	15.1	-12.5
0	*6.4	0.2
1	*7.0	3.0
2	30.3	22.9
3	31.6	-30.3
4	6.3	-8.2
5	10.2	9.6
6	6.6	-6.8
7	23.6	26.2
8	*10.0	1.4
9	12.3	-12.8
10	*10.2	1.7
11	*10.2	-0.2
8,1,L		
-15	*10.2	5.3
-14	*10.2	-1.8
-13	*10.2	-2.1
-12	12.1	-14.0
-11	*9.5	-5.3
-10	*9.1	2.9
-9	14.6	-13.9
-8	*8.2	2.2
-7	13.8	-13.3
-6	29.8	27.8
-5	20.0	20.0
-4	18.6	20.9
-3	23.0	22.2
-2	6.2	5.2
-1	*6.7	2.2
0	45.1	-38.5
1	*7.6	1.2
2	15.1	-18.2
3	15.0	-11.9
4	11.9	-11.2
5	*9.4	-5.8
6	11.4	11.1
7	*10.0	-2.4
8	16.9	19.2
9	10.8	4.9
10	*10.2	6.0
9,1,L		
-15	*10.2	-4.8
-14	*10.1	-1.8
-13	*10.0	-2.9
-12	11.0	-7.8
-11	*9.7	2.9
-10	10.3	6.2
-9	12.7	9.2
-8	10.9	12.0
-7	12.8	-8.7
-6	*7.9	-2.6
-5	*7.6	1.7
-4	25.7	-24.8
-3	24.4	14.3
-2	11.8	6.2
-1	13.3	-12.4
0	5.5	-2.3
1	9.0	-6.3
2	*8.5	-0.7
3	13.2	10.6
4	19.7	17.7
5	8.3	-9.4
6	*10.1	2.1
7	*10.2	-2.8
8	15.2	-15.3
9	9.2	10.4
10,1,L		
-15	8.5	4.0
-14	18.1	-9.0
-13	*10.0	-6.0
-12	17.2	-17.7
-11	*9.7	-0.2
-10	*9.4	0.9
-9	11.1	11.9
-8	17.3	20.6
-7	8.2	-3.4
-6	12.4	12.8
-5	13.4	-13.4
-4	25.0	25.3
-3	9.9	7.3
-2	17.4	-15.3
-1	26.0	-23.9
0	17.5	-18.9
1	14.5	16.6
2	*8.9	-6.7
3	8.4	9.0
4	*9.5	-7.1
5	*9.7	-2.7
6	*9.9	-0.7
7	*10.0	2.1
8	10.0	11.1
9	*10.2	-8.0
11,1,L		
-15	*10.2	1.2
-14	7.6	5.0
-13	*10.0	-4.2
-12	*9.9	3.5
-11	*9.7	4.3
-10	*9.5	2.6
-9	*9.4	-1.6
-8	13.2	-15.4
-7	*8.8	-0.4
-6	12.2	13.0
-5	7.6	-7.0
-4	10.1	8.5
-3	*8.2	-0.9
-2	23.9	-23.2
-1	20.7	17.8
0	11.3	7.9
1	*8.7	0.3
2	*9.0	2.7
3	13.8	-12.9
4	*9.9	-7.3
5	*10.0	4.4
6	*10.2	7.5
7	*10.2	-4.9
8	*10.2	4.7
12,1,L		
-15	*10.2	-7.4
-14	10.7	-11.5
-13	*10.2	5.2
-12	*10.1	0.2
-11	12.7	12.2
-10	11.4	-10.6
-9	*9.5	0.5
-8	7.6	-4.5
-7	*9.1	-0.5
-6	12.7	12.0
-5	*8.8	-3.5
-4	14.3	16.4
-3	15.7	-14.7
-2	7.9	9.4
-1	11.5	7.0
0	*8.2	0.1
1	*9.5	-8.4
2	14.3	-15.5
3	*9.9	7.0
4	9.9	-12.1
5	*10.2	-1.7
6	*10.2	-4.6
7	*10.2	5.9
13,1,L		
-14	*10.2	-3.1
-13	14.7	-15.3
-12	*10.2	-8.8
-11	9.8	6.3
-10	11.6	14.8
-9	*9.8	4.0
-8	*9.6	-2.8
-7	11.0	-10.2
-6	11.9	-11.1
-5	16.6	17.2
-4	19.2	18.8
-3	14.5	-13.9
-2	*9.4	-3.0
-1	10.5	-8.9
0	13.8	-12.0
1	17.0	16.2
2	*10.0	-2.8
3	*10.1	-2.1
4	*10.2	6.5
5	*10.2	-0.8
6	*10.2	2.1
14,1,L		
-13	*10.2	-6.3
-12	12.0	-12.6
-11	*10.1	1.8
-10	*10.0	-5.5
-9	15.1	13.2
-8	9.6	-7.3
-7	*9.7	7.7
-6	9.4	10.8
-5	11.7	11.4
-4	13.3	12.9
-3	11.3	-11.4
-2	*9.7	3.1
-1	18.4	-19.1
0	10.4	-5.6
1	*10.2	-2.1
2	*10.2	0.2
3	*10.2	-3.2
4	8.5	-10.3
15,1,L		
-13	*10.2	-3.3
-12	*10.2	-0.1
-11	*10.2	-4.0
-10	*10.2	3.3
-9	14.1	13.7
-8	*10.1	-1.3
-7	12.8	-12.5
-6	*10.0	-1.1
-5	*10.0	2.4
-4	*10.0	-1.1
-3	*10.0	3.7
-2	*10.0	-3.8
-1	15.4	-14.2
0	11.4	11.2
1	10.8	9.9
2	*10.2	0.6
3	*10.2	4.5
16,1,L		
-11	10.9	7.5
-10	*10.4	0.2
-9	*10.3	5.8
-8	8.5	-6.5
-7	14.6	15.3
-6	*10.2	-1.2
-5	*10.2	1.8
-4	*10.2	-4.4
-3	*10.2	-6.7
-2	*10.2	1.7
-1	*10.2	-9.0
0	8.5	10.8
1	*10.2	-1.7
17,1,L		
-10	14.7	10.9
-9	*10.3	-3.3
-8	9.4	-8.5
-7	*10.3	2.6
-6	*10.3	-5.2
-5	*10.3	0.8
-4	10.9	10.9
-3	*10.3	3.6
-2	*10.3	-4.4
-1	*10.2	-4.6
0,2,L		
0	8.2	-4.2
1	16.2	-15.5
2	51.5	-57.9
3	10.8	6.2
4	35.6	-31.4
5	22.2	23.1
6	59.3	65.3
7	*7.3	-9.4
8	12.9	12.1
9	7.6	-7.1
10	*8.2	1.5
11	6.0	-3.2
12	*10.5	-2.9
13	*10.5	2.0
14	*10.5	-9.9
1,2,L		
-14	*10.7	-0.9
-13	*10.7	4.6
-12	11.6	12.8
-11	13.8	-6.9
-10	6.8	6.5
-9	10.4	5.9
-8	*8.2	
-7	*8.2	5.1
-6	4.6	-3.4
-5	13.1	-14.2
-4	48.6	-52.2
-3	16.9	-17.7
-2	14.8	14.2
-1	33.5	35.9
0	21.9	23.4
1	9.4	7.8
2	5.3	-5.5
3	16.9	13.8
4	8.8	8.9
5	13.6	-8.5
6	6.1	3.7
7	17.6	-17.7
8	10.0	-9.5
9	6.4	-3.9
10	11.2	13.3
11	*9.9	2.5
12	*9.9	-4.5
13	8.1	6.8
14	*10.5	-4.9
2,2,L		
-15	*11.2	5.9
-14	14.6	-17.2
-13	*10.7	-3.8
-12	14.5	-17.6
-11	17.2	18.1
-10	28.2	29.8
-9	*8.9	5.1
-8	*9.5	-1.5
-7	8.8	-9.3
-6	20.8	18.6
-5	16.0	-14.8
-4	51.4	53.2
-3	40.4	-41.7
-2	68.5	-77.6
-1	16.3	13.7
0	29.8	-31.3
1	36.8	36.3
2	*3.0	-5.1
3	27.5	-24.3
4	*4.4	-5.6
5	36.5	35.3
6	7.7	-3.4
7	*9.3	-4.0
8	8.0	6.6
9	7.6	-11.6
10	16.5	16.8
11	*10.1	3.3
12	*10.1	4.7
13	*10.5	-7.6
3,2,L		
-15	*10.9	11.2
-14	*10.9	6.4
-13	*10.6	-0.3
-12	11.3	-12.4
-11	*9.8	-1.9
-10	15.9	16.0
-9	8.0	-7.3
-8	*7.7	-7.7
-7	11.6	-16.5
-6	6.1	-5.0
-5	7.3	8.9
-4	28.1	27.9
-3	28.4	28.2
-2	17.9	-16.4
-1	16.7	-13.3
0	10.2	-8.5
1	9.0	4.9
2	16.5	-15.1
3	7.0	-6.5
4	12.5	-12.2
5	22.2	21.0
6	10.9	14.8
7	14.1	-8.4
8	7.6	5.0
9	*9.5	0.2
10	*9.9	5.6
11	*10.2	-4.1
12	*10.5	0.5
13	*10.5	-9.3
4,2,L		
-15	*10.9	-6.8
-14	*10.6	1.0
-13	4.8	7.8
-12	8.6	-9.3
-11	19.5	18.1
-10	24.0	-24.3
-9	*10.3	4.5
-8	28.3	26.6
-7	10.1	-6.9
-6	16.8	17.6
-5	4.3	4.6
-4	13.1	10.5
-3	38.0	-31.1
-2	23.5	21.6
-1	28.0	-25.6
0	22.0	-19.4
1	11.3	11.0
2	53.7	-50.6
3	6.1	7.6
4	6.4	-6.8
5	*7.7	-0.6
6	18.9	17.8
7	28.9	27.5
8	*9.3	5.7
9	*9.6	-4.3
10	*9.9	-2.8
11	*10.3	-4.8
12	*10.6	7.1
5,2,L		
-15	*10.9	5.6
-14	*10.6	2.5
-13	*10.3	1.1
-12	8.3	-10.5
-11	*9.6	-3.3
-10	6.4	-4.0
-9	13.9	18.8
-8	33.9	33.5
-7	2.9	-4.9
-6	21.4	-22.0
-5	26.6	-25.5
-4	13.7	11.9
-3	*9.0	-0.5
-2	9.5	-9.2
-1	22.9	-23.5
0	19.3	-22.5
1	17.9	16.5
2	31.3	29.3
3	28.4	28.5
4	*7.0	-1.4
5	*7.8	-0.4
6	18.5	-17.2
7	7.3	9.2
8	*9.6	6.0
9	13.8	-14.1
10	*10.6	-6.0
11	*10.9	-5.3
12	*10.9	6.0
6,2,L		
-15	8.3	-8.1
-14	*10.9	-8.7
-13	12.4	-14.8
-12	*9.9	1.2
-11	12.7	14.8
-10	*8.9	4.6
-9	39.1	36.8
-8	35.1	-31.5
-7	*7.3	5.1
-6	43.9	44.1
-5	*5.9	-1.7
-4	16.5	13.2
-3	42.1	-43.1
-2	33.6	-32.3
-1	27.1	-25.1
0	*5.2	5.5
1	7.9	-7.5
2	*7.0	0.8
3	11.7	13.5
4	5.2	-5.2
5	23.8	27.2
6	11.4	9.8
7	*9.3	-4.3
8	6.0	-6.1
9	*10.3	1.7
10	*10.3	7.0
11	*10.6	-0.5
7,2,L		
-15	*10.9	0.3
-14	*10.6	2.4
-13	*10.3	7.3
-12	*9.9	0.5
-11	6.6	4.0
-10	5.8	-2.7
-9	19.3	14.6
-8	14.5	-10.0
-7	23.4	-19.8
-6	10.0	-14.1
-5	7.3	-12.2
-4	7.2	6.6
-3	*5.8	10.0
-2	28.6	29.7
-1	*5.8	-6.4
0	*6.0	-2.7
1	6.0	-2.4
2	6.3	6.1
3	14.8	13.4
4	4.7	-5.4
5	14.5	-13.0
6	10.4	-13.5
7	6.1	8.4
8	*10.3	-2.1
9	9.4	11.7
10	*10.9	6.6
11	*10.9	-8.4
8,2,L		
-15	9.4	-8.0
-14	7.3	5.9
-13	*9.9	-4.0
-12	*9.9	0.4
-11	8.7	10.0
-10	11.2	13.8
-9	*8.0	3.0
-8	10.8	-13.1
-7	35.4	29.5
-6	24.3	-20.0
-5	*6.8	2.7
-4	*6.5	6.8
-3	35.0	-36.5
-2	11.2	7.4
-1	10.4	-11.5
0	12.7	13.8
1	11.2	11.5
2	13.2	-11.1
3	15.8	-14.3
4	*8.5	-2.1
5	11.8	14.0
6	10.1	8.0
7	*9.9	7.0
8	*10.3	-1.6
9	*10.6	-1.9
10	*10.9	-3.1
9,2,L		
-15	*10.9	-2.5
-14	*10.6	4.8
-13	*10.3	-6.5
-12	8.3	-4.9
-11	*9.6	0.3
-10	*9.3	-1.5
-9	8.6	3.3
-8	14.4	-12.2
-7	15.7	16.7
-6	*7.7	3.0
-5	*7.6	-0.8
-4	*7.4	1.5
-3	*7.3	1.0
-2	*7.2	-1.5
-1	16.7	-14.9
0	*7.2	0.4
1	10.7	-11.4
2	9.0	9.0
3	*8.8	4.1
4	*9.3	2.5
5	8.6	6.5
6	*9.9	0.9
7	*10.3	0.3
8	*10.6	-1.7
9	*10.9	7.0
10,2,L		
-15	*10.9	2.7
-14	*10.6	2.5
-13	20.5	-21.7
-12	11.4	6.5
-11	9.5	8.7
-10	6.9	8.4
-9	8.6	10.7
-8	8.8	-5.3
-7	11.9	-10.5
-6	*7.9	-4.4
-5	40.7	39.0
-4	*7.5	4.0
-3	12.1	-11.6
-2	27.8	-25.8
-1	32.0	-33.0
0	16.5	16.9
1	*8.5	-0.2
2	*8.9	1.9
3	*9.3	1.6
4	*9.6	-5.0
5	9.7	10.8
6	11.4	9.4
7	*10.6	7.5
8	*10.9	2.2
11,2,L		
-14	*10.9	1.0
-13	16.5	-14.3
-12	9.3	6.0
-11	9.7	9.3
-10	14.4	15.1
-9	*9.1	4.0
-8	12.1	-10.2
-7	8.3	-7.6
-6	23.3	-21.4
-5	20.1	15.3
-4	10.5	4.4
-3	10.1	-8.8
-2	17.3	-14.1
-1	7.3	-3.3
0	16.2	13.7
1	18.8	14.1
2	13.0	13.7
3	9.2	-9.1
4	*9.5	-1.1
5	7.8	-6.0
6	*10.9	-3.4
7	*10.9	1.9
12,2,L		
-14	*10.9	-4.8
-13	6.7	4.3
-12	5.4	6.5
-11	18.0	-13.4
-10	15.5	16.6
-9	12.8	13.7
-8	4.7	4.9
-7	*8.8	5.8
-6	9.9	-9.1
-5	9.3	-7.5
-4	17.9	-15.2
-3	*7.7	-0.6
-2	*7.6	-3.8
-1	*7.6	-3.6
0	*8.5	-2.2
1	*8.9	-4.1
2	15.7	15.0
3	*9.9	-0.4
4	*10.2	0.2
5	*10.6	4.6
6	*10.9	0.7
13,2,L		
-14	*10.8	3.5
-13	*10.6	1.7
-12	10.2	5.7
-11	11.9	-13.1
-10	*10.6	1.7
-9	*9.7	-0.9
-8	*9.5	1.0
-7	9.6	-10.2
-6	*9.1	-7.4
-5	14.2	11.3
-4	*8.9	-2.5
-3	14.7	15.4
-2	*9.1	0.1
-1	9.6	-5.4
0	*9.3	-3.9
1	*9.6	0.8
2	8.2	5.0
3	*10.3	-2.1
4	10.3	-3.8
5	8.9	-10.4
14,2,L		
-13	*1.1	1.8
-12	*10.6	-3.5
-11	11.5	13.1
-10	13.0	12.6
-9	14.1	-12.4
-8	*9.8	-2.1
-7	*9.7	-5.1
-6	*9.5	1.6
-5	9.7	9.9
-4	*9.3	-3.5
-3	*9.3	-1.0
-2	14.9	-12.8
-1	7.9	-6.8
0	7.3	6.1
1	6.4	4.1
2	*10.3	0.9
3	*10.4	-5.8
4	*10.5	1.6
15,2,L		
-12	*10.9	0.4
-11	*10.6	-2.3
-10	13.3	8.4
-9	10.7	-8.4
-8	*9.9	2.9
-7	*9.9	1.9
-6	10.2	7.3
-5	*9.9	-3.8
-4	*9.9	-6.2
-3	*9.9	2.9
-2	15.5	-11.9
-1	*10.2	2.4
0	*10.2	-0.4
1	*10.2	4.8
2	7.9	6.0
16,2,L		
-11	*10.6	4.1
-10	*10.6	-0.5
-9	*10.6	9.8
-8	12.0	7.7
-7	*10.3	-3.1
-6	*10.3	-1.2
-5	9.9	-5.8
-4	*10.3	-1.2
-3	*10.3	-3.1
-2	7.6	-10.4
-1	*10.3	2.4
0	*10.3	-1.9
17,2,L		
-8	6.8	5.5
-7	*10.8	-3.9
-6	*10.8	0.9
-5	*10.8	-5.9

Table 2 (*cont.*)

L	OBS	CALC
17,2,L		
-4	*10.8	-0.3
-3	*10.8	-1.6
0,3,L		
1	29.4	29.9
2	36.8	36.2
3	*2.0	1.8
4	22.2	-25.3
5	25.2	18.4
6	7.7	12.8
7	7.4	8.6
8	*6.6	2.4
9	7.2	-10.6
10	8.0	-9.2
11	7.2	-4.8
12	6.4	9.9
13	9.4	-7.1
1,3,L		
-14	11.4	10.6
-13	15.8	-18.8
-12	*8.9	-2.3
-11	8.3	-6.1
-10	*7.9	3.2
-9	26.4	23.8
-8	15.7	-15.4
-7	11.9	11.0
-6	9.1	-4.8
-5	5.3	-1.6
-4	10.5	-11.5
-3	4.3	0.9
-2	8.0	-9.5
-1	25.9	-22.7
0	21.7	24.1
1	12.4	-11.7
2	38.4	40.1
3	20.5	-17.8
4	8.6	8.9
5	26.9	26.2
6	*4.7	-2.6
7	12.5	-9.6
8	17.4	-19.2
9	*7.7	-5.1
10	9.9	-11.1
11	11.8	12.2
12	*9.2	-1.8
13	*9.4	2.9
2,3,L		
-14	*9.9	8.8
-13	*9.4	1.8
-12	*8.9	-1.1
-11	8.7	6.8
-10	11.2	-11.3
-9	8.4	12.2
-8	16.1	17.8
-7	*5.0	2.1
-6	22.2	-24.0
-5	24.5	-25.7
-4	*4.0	-6.1
-3	19.7	-16.3
-2	24.5	22.4
-1	*2.0	-8.8
0	24.5	-25.4
1	*2.0	1.6
2	6.1	9.4
3	26.3	28.2
4	28.3	23.1
5	*4.4	3.2
6	12.1	-15.3
7	6.1	6.5
8	*6.9	2.5
9	6.5	-6.4
10	5.4	-6.3
11	*8.9	-6.5
12	*9.4	-0.8
13	*9.9	-3.5
3,3,L		
-14	*09.6	-3.1
-13	6.8	-6.7
-12	17.5	17.7
-11	*8.2	4.7
-10	15.1	16.0
-9	21.5	-21.2
-8	22.0	-24.5
-7	*5.0	1.7
-6	33.6	-30.0
-5	30.4	28.8
-4	*5.8	-2.3
-3	*5.5	-1.2
-2	6.0	-4.7
-1	12.9	14.5
0	38.4	38.8
3,3,L		
1	22.9	21.4
2	*5.7	1.2
3	31.4	-30.7
4	11.5	12.2
5	31.8	-31.5
6	9.5	-9.1
7	5.6	3.3
8	*7.6	-0.8
9	8.9	10.4
10	*8.4	0.3
11	*8.9	3.2
12	*9.4	-3.3
4,3,L		
-14	8.3	7.0
-13	*9.2	2.9
-12	7.1	11.4
-11	*8.2	-0.3
-10	6.1	-6.3
-9	24.7	19.5
-8	11.6	-11.7
-7	7.0	-3.9
-6	5.5	-4.3
-5	13.5	-16.8
-4	13.6	-14.8
-3	15.0	-12.7
-2	7.1	8.1
-1	18.6	-14.8
0	12.1	10.8
1	*5.5	3.7
2	8.0	6.8
3	26.4	26.2
4	*6.0	0.5
5	3.8	-2.3
6	*7.8	-1.3
7	*8.0	1.3
8	*8.7	-6.1
9	*9.1	1.2
10	*9.3	0.3
11	*9.5	-7.4
12	7.0	-6.0
5,3,L		
-14	*09.0	0.5
-13	9.2	-11.1
-12	*8.7	-4.4
-11	12.4	-12.9
-10	15.1	11.3
-9	5.6	-4.0
-8	*6.1	4.3
-7	26.7	-25.2
-6	*4.6	1.4
-5	42.2	39.3
-4	*6.0	-0.9
-3	26.2	25.7
-2	34.1	-30.4
-1	*1.6	-3.3
0	5.3	4.7
1	13.2	-9.7
2	*6.0	3.3
3	*8.6	-2.6
4	14.1	-9.5
5	*6.1	0.4
6	26.6	27.8
7	*7.6	-5.3
8	*8.2	-2.9
9	8.4	-10.0
10	*9.2	-6.1
11	6.2	8.6
6,3,L		
-14	7.0	4.1
-13	*9.2	-6.3
-12	*8.7	1.4
-11	*8.2	2.7
-10	17.0	18.4
-9	*6.9	2.3
-8	25.5	-22.6
-7	12.9	10.4
-6	20.9	-18.6
-5	6.8	-5.4
-4	*5.0	-0.3
-3	14.0	-14.7
-2	*3.8	1.2
-1	4.3	-6.2
0	15.3	13.8
1	*4.6	-2.7
2	7.8	7.7
3	7.4	6.6
4	4.7	2.4
5	16.7	17.0
6	*7.6	-2.9
7	11.9	-9.1
8	*8.7	-5.5
9	*9.2	2.0
6,3,L		
10	*09.2	-2.2
11	6.0	-4.1
7,3,L		
-14	13.3	-14.3
-13	*9.1	1.9
-12	*8.7	2.3
-11	7.9	6 6
-10	23.7	20.7
-9	20.7	-16.2
-8	8.7	-8.1
-7	15.0	-11.9
-6	20.0	16.3
-5	*4.4	0.6
-4	11.0	-6.7
-3	15.8	-11.7
-2	28.8	-23.9
-1	22.8	23.1
0	4.4	2.8
1	32.6	33.7
2	17.0	16.9
3	19.9	-19.0
4	8.9	-7.1
5	*7.3	-1.3
6	9.9	-6.3
7	*8.4	-2.7
8	*8.9	1.6
9	7.7	-3.7
10	*9.2	3.5
8,3,L		
-14	9.8	11.6
-13	5.7	-3.8
-12	*8.7	-0.1
-11	*8.2	-1.4
-10	7.9	9.4
-9	*7.1	6.3
-8	6.4	6.2
-7	10.4	-13.6
-6	34.5	-37.3
-5	20.5	15.8
-4	4.8	3.2
-3	*5.1	1.9
-2	11.6	-10.4
-1	26.7	-25.7
0	13.5	13.7
1	17.9	17.5
2	23.1	26.5
3	*6.6	-1.4
4	9.9	-9.5
5	*7.9	-2.3
6	*8.4	-3.3
7	8.4	12.5
8	*9.2	0.1
9	11.5	-10.7
9,3,L		
-14	5.4	-2.7
-13	*9.1	2.0
-12	11.4	-11.6
-11	13.4	12.0
-10	*7.7	-3.4
-9	9.3	-11.7
-8	5.6	1.1
-7	26.9	-25.6
-6	*6.1	3.3
-5	*5.9	6.7
-4	23.3	20.6
-3	17.2	13.7
-2	*6.1	-1.6
-1	8.5	-4.0
0	6.1	6.3
1	13.8	14.1
2	17.8	-20.6
3	8.4	-6.9
4	5.8	-5.0
5	8.0	-6.0
6	6.6	8.6
7	8.1	6.4
8	*9.4	-3.3
10,3,L		
-14	*9.2	-0.1
-13	*8.9	3.4
-12	9.2	7.7
-11	6.8	-7.8
-10	9.2	5.1
-9	6.1	5.9
-8	*6.9	2.0
-7	15.7	-12.7
-6	11.7	-13.4
-5	*6.2	-3.5
-4	13.4	-13.4
-3	18.3	21.1
10,3,L		
-2	*5.9	2.4
-1	*5.9	-4.1
0	*6.5	-0.9
1	5.1	-6.3
2	17.2	19.3
3	8.8	7.9
4	5.6	5.8
5	7.6	-9.8
6	6.2	-8.0
7	6.8	6.0
11,3,L		
-14	7.0	-7.6
-13	*9.1	-1.6
-12	*8.8	-0.1
-11	5.4	4.0
-10	13.9	-10.8
-9	4.9	5.6
-8	8.9	-9.7
-7	*7.1	2.4
-6	24.6	23.9
-5	*6.6	-
-4	*6.6	5.5
-3	22.9	-21.2
-2	15.4	-15.2
-1	13.2	11.3
0	10.0	8.9
1	*7.3	0.1
2	5.6	2.5
3	*8.2	-0.5
4	9.3	-8.3
5	5.6	6.0
6	6.8	5.6
12,3,L		
-13	8.6	10.0
-12	*8.9	0.2
-11	*8.5	-1.9
-10	9.7	5.3
-9	11.2	-10.1
-8	*7.6	-
-7	*7.4	
-6	*7.3	-1.7
-5	*7.1	-7.6
-4	*6.9	-6.9
-3	7.1	7.6
-2	9.4	-8.7
-1	5.7	6.5
0	*7.0	1.8
1	8.1	4.3
2	11.8	12.0
3	*8.4	-3.0
4	*8.7	2.6
5	*8.9	-5.4
13,3,L		
-12	5.7	-4.8
-11	6.7	5.0
-10	6.2	6.7
-9	*8.2	2.4
-8	13.7	-13.6
-7	4.9	-4.2
-6	11.3	-11.3
-5	5.7	4.0
-4	8.0	8.9
-3	5.5	-4.2
-2	13.7	14.7
-1	*8.1	-2.2
0	*7.9	0.4
1	14.1	13.9
2	*8.5	-0.6
3	12.8	-11.9
4	*9.2	-4.8
14,3,L		
-12	*09.2	3.9
-11	13.9	12.4
-10	*8.7	1.4
-9	9.8	-9.2
-8	*8.2	-2.4
-7	12.0	-10.5
-6	*7.9	-0.7
-5	*7.9	0.9
-4	*7.9	-0.2
-3	6.5	-3.3
-2	8.3	-5.5
-1	10.8	12.0
0	*8.4	0.8
1	7.1	6.5
2	*8.9	1.0
3	*9.2	-2.6
15,3,L		
-10	10.8	-10.3
-9	*8.7	-4.4
-8	*8.5	-0.9
-7	8.7	7.0
-6	8.0	6.6
-5	5.3	7.3
-4	5.3	-3.3
-3	*8.4	3.5
-2	*8.4	-2.6
-1	4.4	-4.3
0	11.8	10.4
1	5.7	-4.2
16,3,L		
-9	6.5	4.9
-8	*8.7	-1.4
-7	12.3	-10.9
-6	*8.7	-1.1
-5	*8.7	-0.6
-4	*8.7	3.2
-3	*8.7	-0.4
-2	*8.7	-1.7
0,4,L		
0	33.2	34.8
1	4.9	-4.0
2	3.5	-1.3
3	10.4	8.7
4	12.0	-9.6
5	7.6	8.7
6	*4.6	-0.5
7	7.4	-7.9
8	*4.8	-3.5
9	*4.6	-2.7
10	4.7	-4.0
11	*2.5	-1.1
1,4,L		
-11	*05.0	-4.4
-10	5.6	-2.8
-9	6.4	-8.3
-8	14.9	-14.2
-7	9.2	8.5
-6	13.6	16.5
-5	16.0	18.0
-4	15.5	15.6
-3	4.6	-4.0
-2	5.6	-4.9
-1	14.9	-14.0
0	22.1	21.3
1	*4.0	-3.7
2	35.1	-34.9
3	15.3	-16.3
4	*4.0	-0.9
5	14.6	13.5
6	7.3	5.8
7	10.8	9.4
8	*4.0	-1.5
9	*4.0	-2.5
10	*2.5	2.7
11	*4.0	-2.0
2,4,L		
-11	*04.3	-0.7
-10	10.9	10.2
-9	*4.6	-1.3
-8	14.2	-13.1
-7	9.7	-6.5
-6	14.1	-12.7
-5	4.9	-2.7
-4	11.7	8.1
-3	3.3	1.2
-2	*4.4	1.4
-1	15.5	15.7
0	*2.2	4.5
1	5.4	3.1
2	31.3	26.8
3	*4.3	-3.2
4	5.2	2.5
5	*4.4	-1.5
6	24.4	-23.8
7	*4.2	-0.5
8	8.6	-7.5
9	*3.8	-1.4
10	4.8	7.0
3,4,L		
-12	*03.0	-5.4
-11	4.8	-2.0
-10	8.7	12.1
-9	*4.4	6.6
-8	*4.4	4.7
-7	*4.4	-5.8
3,4,L		
-6	17.0	-17.1
-5	17.2	15.0
-4	6.9	8.6
-3	*4.0	0.6
-2	16.7	-16.4
-1	11.4	-12.4
0	17.1	14.6
1	10.9	-5.6
2	28.3	27.3
3	8.4	5.9
4	20.6	-18.5
5	11.0	-12.1
6	*4.6	-0.3
7	4.1	4.2
8	*3.2	-6.9
9	*2.5	-1.5
10	*2.5	-3.7
4,4,L		
-12	8.7	-6.9
-11	5.7	7.7
-10	*4.3	3.3
-9	7.2	-11.3
-8	8.7	-11.6
-7	18.5	-17.8
-6	*4.6	-1.1
-5	25.1	24.2
-4	3.3	-2.6
-3	*3.0	-0.7
-2	*3.0	2.0
-1	*2.5	0.7
0	27.3	28.0
1	18.9	15.7
2	6.8	-7.1
3	17.5	-18.6
4	6.2	-5.6
5	7.5	-4.7
6	5.5	7.9
7	*4.4	2.7
8	11.4	-14.4
9	6.7	4.4
10	4.8	-4.6
5,4,L		
-12	8.0	-10.2
-11	*4.8	-7.6
-10	5.5	2.5
-9	6.8	-3.0
-8	4.7	1.3
-7	14.1	-13.5
-6	*4.7	-3.4
-5	17.7	14.9
-4	*4.3	4.8
-3	23.2	24.9
-2	*4.3	1.0
-1	6.8	3.4
0	9.0	-9.9
1	*4.3	-0.2
2	14.1	12.4
3	40.3	-37.4
4	9.4	-11.3
5	*4.7	-1.7
6	5.4	6.5
7	11.5	10.4
8	*3.8	4.8
6,4,L		
-13	9.9	-11.3
-12	5.5	4.0
-11	*3.2	2.1
-10	*3.8	3.2
-9	*4.3	2.3
-8	15.2	-16.9
-7	9.2	-12.5
-6	*4.6	1.1
-5	*4.6	5.9
-4	15.8	14.6
-3	13.3	11.3
-2	23.4	-22.3
-1	4.6	-1.5
0	9.0	9.7
1	9.4	7.2
2	21.2	20.5
3	5.6	-6.8
4	9.9	-8.3
5	7.2	-7.8
6	*4.1	-2.3
7	*4.1	-0.3
8	*4.1	
10	8.3	-9.1
7,4,L		
-11	*04.7	-4.4
-10	5.2	-7.4
7,4,L		
-9	6.6	-3.6
-8	16.1	18.9
-7	4.7	6.1
-6	10.5	10.9
-5	8.3	-8.1
-4	*4.7	-0.4
-3	25.8	22.3
-2	13.9	-10.7
-1	11.4	-11.0
0	23.7	-24.8
1	5.7	4.3
2	*4.8	-0.2
3	5.6	7.2
4	19.9	20.0
5	16.9	-19.5
6	*3.8	-1.3
7	*5.0	2.4
9	6.9	3.4
8,4,L		
-11	*03.2	-2.9
-10	6.7	7.7
-9	11.1	-10.7
-8	9.5	-9.3
-7	*4.7	-5.3
-6	6.3	-8.6
-5	15.4	13.6
-4	*4.8	3.4
-3	7.5	-5.5
-2	*4.8	1.7
-1	11.5	12.3
0	8.8	8.4
1	18.2	15.6
2	*4.7	-3.4
3	15.7	-18.2
4	5.8	-2.8
5	9.4	-9.6
6	*5.0	6.9
9,4,L		
-11	*2.6	2.7
-10	*3.2	0.7
-9	*3.8	2.7
-8	*4.3	0.9
-7	10.6	-8.8
-6	9.4	9.1
-5	10.4	-9.6
-4	8.7	7.4
-3	10.2	-8.3
-2	10.4	-6.8
-1	28.8	27.0
	5.8	4.1
1	*4.6	-0.0
2	14.9	-17.1
3	*4.1	1.9
4	7.4	-6.6
5	*5.0	-4.4
10,4,L		
-10	*03.2	-3.2
-9	10.1	-13.0
-8	5.2	-5.7
-7	7.6	-9.4
-6	12.8	11.9
-5	13.0	12.6
-4	*4.7	-1.9
-3	6.7	8.6
-2	9.4	-6.9
-1	*4.1	0.1
	7.6	6.7
1	*4.3	4.6
2	*3.8	-1.3
3	*3.2	-1.9
4	11.5	-8.4
11,4,L		
-10	*4.0	-3.2
-9	*3.2	1.3
-8	5.3	-3.1
-7	*4.0	-0.6
-6	8.8	7.9
-5	*4.4	5.2
-4	17.4	19.6
-3	8.8	-7.0
-2	8.0	8.4
-1	9.1	-9.8
	20.3	-21.5
1	9.7	8.6
2	*3.2	-2.6
3	2.6	1.4
12,4,L		
-9	*6.0	-7.1
12,4,L		
-8	*02.6	-4.3
-7	4.3	-3.1
-6	*3.6	6.0
-5	*3.8	0.7
-4	5.5	4.5
-3	3.5	-4.7
-2	5.7	-6.9
-1	10.8	15.7
	6.8	6.7
1	5.4	7.3
13,4,L		
-7	*02.6	2.0
-6	4.0	-5.4
-5	3.1	-6.3
-4	3.7	3.0
-3	*2.8	-0.8
-2	*2.6	5.9
-1	14.6	-19.4
0,5,L		
1	3.5	-2.5
2	27.5	24.5
3	27.3	-27.4
4	16.5	-17.9
5	20.5	-17.6
6	*4.6	3.4
7	8.8	10.8
8	9.9	9.3
9	9.6	9.5
1,5,L		
-10	*02.6	1.6
-9	6.2	3.3
-8	*4.1	-1.3
-7	11.6	-11.1
-6	12.8	13.7
-5	10.5	-6.2
-4	16.7	15.0
-3	3.5	-3.7
-2	17.7	-17.3
-1	22.5	22.3
0	6.1	-2.6
1	7.8	-5.7
2	19.5	-20.8
3	6.7	2.0
4	5.5	-6.7
5	6.7	5.6
6	9.2	10.4
7	7.0	-5.5
8	13.6	11.1
9	*	6.2
2,5,L		
-12	7.8	-10.8
-10	*4.6	0.2
-9	*4.0	-1.3
-8	16.9	18.1
-7	8.7	-7.3
-6	*4.6	-0.8
-5	10.0	8.4
-4	9.1	7.9
-3	10.4	12.2
-2	13.2	-9.5
-1	5.3	2.0
0	27.5	-25.9
1	16.5	13.6
2	*3.8	4.5
3	14.4	-12.7
4	12.9	9.3
5	16.3	-15.3
6	8.8	8.2
7	4.1	5.4
8	*3.6	2.1
9	*5.5	-6.3
10	7.8	-9.3
3,5,L		
-12	13.8	-15.6
-10	4.7	6.2
-9	5.8	8.6
-8	*4.3	3.2
-7	5.4	-3.9
-6	5.5	4.8
-5	6.9	7.5
-4	21.4	20.0
-3	18.9	-19.6
-2	*3.6	-2.7
-1	21.4	-21.1
0	28.6	-25.3
1	25.9	23.3
2	4.7	3.2
3	3.5	3.2
3,5,L		
4	13.2	-13.0
5	*4.6	4.1
6	11.7	12.1
7	10.5	10.8
8	*3.0	2.2
9	7.9	-9.0
4,5,L		
-12	6.5	7.3
-10	5.8	-4.9
-9	*3.8	-2.7
-8	5.4	-5.5
-7	11.3	-10.3
-6	23.5	21.0
-5	*4.4	-2.5
-4	*4.3	3.5
-3	*4.3	4.1
-2	6.7	-4.5
-1	11.0	9.6
0	*4.0	0.4
1	19.3	15.8
2	15.6	-13.3
3	*4.6	0.5
4	13.8	-11.2
5	17.3	-19.2
6	9.2	7.8
7	*9.1	-3.3
8	6.4	9.2
5,5,L		
-11	17.7	17.8
-10	*3.0	-3.5
-9	7.0	6.4
-8	5.6	-2.6
-7	*4.6	2.2
-6	10.5	7.4
-5	11.1	-11.9
-4	6.2	-7.4
-3	*4.3	-2.9
-2	8.8	8.7
-1	15.8	-12.4
0	13.6	11.2
1	*4.4	-2.6
2	13.3	-12.9
3	12.2	11.6
4	*4.0	-2.0
5	7.9	8.8
6	5.4	-7.6
7	4.9	-5.6
8	7.0	6.0
6,5,L		
-12	7.3	-10.6
-11	4.6	-5.8
-10	5.3	2.5
-9	*3.8	-1.4
-8	6.4	-5.7
-7	9.8	5.8
-6	10.0	6.9
-5	*4.4	1.1
-4	24.7	24.3
-3	9.3	-4.9
-2	*4.0	0.8
-1	*4.0	0.1
0	7.2	-7.7
1	*4.2	-0.5
2	17.6	-15.5
3	*4.6	-2.1
4	14.5	-11.9
5	10.2	10.1
6	10.1	11.0
7,5,L		
-10	*02.6	-0.8
-9	6.4	4.0
-8	10.9	-12.3
-7	4.8	7.7
-6	5.5	0.6
-5	11.5	10.7
-4	24.6	16.8
-3	15.3	-15.7
-2	16.5	-14.7
-1	4.9	-4.6
0	9.5	8.8
1	4.9	-4.9
2	*4.4	-0.2
3	6.4	-7.6
4	5.4	-5.6
5	6.0	8.8
7	14.3	15.7
8,5,L		
-9	3.2	1.0
8,5,L		
-8	4.7	8.3
-7	*4.1	3.4
-6	*4.3	-0.9
-5	11.8	8.9
-4	*4.4	1.8
-3	17.4	-11.6
-2	*4.6	3.2
-1	11.6	-10.8
0	11.2	6.0
1	7.6	7.6
2	*4.1	-0.6
3	*3.6	-1.8
4	12.3	-12.5
9,5,L		
-9	4.1	5.0
-8	*3.6	-3.9
-7	*3.6	-1.4
-6	*4.0	0.4
-5	16.9	15.8
-4	10.1	-11.6
-3	12.8	-15.2
-2	*4.6	-1.7
-1	8.2	-7.5
0	*4.0	4.3
1	*4.0	1.8
2	4.9	3.5
3	*2.8	-1.2
10,5,L		
-8	*3.6	3.0
-7	*3.6	-2.4
-6	*3.6	-2.9
-5	*3.8	-1.9
-4	*4.0	3.2
-3	15.5	17.2
-2	7.2	8.7
-1	8.3	-7.5
0	*3.0	-3.2
1	11.6	-12.6
2	*3.6	-0.6
11,5,L		
-6	*03.0	-2.5
-5	5.2	-1.4
-4	2.9	2.4
-3	9.8	7.5
-2	9.7	-11.5
-1	8.4	-8.3
0	*4.0	6.3
0,6,L		
0	12.2	9.6
1	*9.6	-3.0
2	*9.6	1.3
3	*9.6	-2.3
4	*9.6	-6.0
5	*9.5	-1.6
6	*9.2	-2.5
7	*9.2	2.1
8	*8.7	5.4
9	*7.2	3.2
1,6,L		
-10	*09.2	2.7
-9	9.1	8.9
-8	6.5	-7.4
-7	*10.2	1.8
-6	*10.2	-2.7
-5	*10.2	-2.0
-4	*10.2	-1.4
-3	13.5	-12.8
-2	6.4	3.1
-1	11.8	9.8
0	*10.2	3.5
1	12.7	11.8
2	*10.2	7.3
3	9.6	9.3
4	17.8	-15.1
5	*10.2	-2.5
6	8.6	5.9
7	12.2	11.1
8	*9.9	2.8
9	10.3	-12.6
2,6,L		
-10	*8.7	-1.7
-9	*10.6	-1.2
-8	*10.6	-6.8
-7	*10.6	7.5
-6	5.5	8.6
-5	11.0	9.4
2,6,L		
-4	*10.6	-0.8
-3	*10.6	-2.8
-2	*10.4	-0.9
-1	*10.4	3.5
0	9.2	-8.4
1	14.0	-14.7
2	*10.2	-6.8
3	9.3	-8.8
4	10.2	12.3
5	*10.6	5.2
6	*10.6	4.1
7	8.8	6.1
8	*10.6	-0.7
3,6,L		
-10	15.1	14.8
-9	*8.8	-1.7
-8	13.6	-11.5
-7	*8.6	-5.6
-6	*8.5	-6.6
-5	11.2	8.5
-4	5.6	5.4
-3	*8.1	-1.7
-2	8.7	-7.8
-1	18.7	-18.6
0	15.3	12.3
1	8.3	7.2
2	*8.1	5.6
3	*8.2	1.5
4	12.2	-10.7
5	12.9	12.2
6	*8.5	1.8
7	*8.5	-0.2
8	*8.5	-1.1
4,6,L		
-10	9.5	11.4
-9	*9.1	-1.9
-8	*9.1	3.2
-7	*8.8	4.4
-6	*8.8	8.0
-5	6.6	6.0
-4	13.2	-13.3
-3	5.2	-10.5
-2	11.1	-13.4
-1	*8.1	-0.1
0	7.3	7.5
1	8.0	7.5
2	*8.4	-6.9
3	*8.6	-2.7
4	*8.8	4.4
5	*9.0	4.3
6	12.6	15.7
7	6.8	-8.6
5,6,L		
-10	9.9	-6.7
-9	*9.0	1.1
-8	9.5	8.9
-7	*8.8	2.7
-6	7.1	-8.7
-5	*8.6	-1.0
-4	*8.4	-3.9
-3	6.2	-3.5
-2	7.6	-3.7
-1	*8.0	-1.8
0	9.6	7.8
1	*8.4	0.2
2	4.3	12.8
3	*8.8	-2.2
4	*8.9	-2.8
5	*9.0	1.3
6	6.9	-3.6
7	13.2	15.2
6,6,L		
-10	*8.2	4.6
-9	*8.2	0.8
-8	*8.2	-0.3
-7	8.4	-9.9
-6	*7.9	-0.7
-5	*7.9	-0.6
-4	13.0	11.8
-3	4.7	3.5
-2	11.5	-11.3
-1	*7.3	2.8
0	6.5	-3.0
1	4.8	3.7
2	*7.7	-1.3
3	6.2	-6.0
4	6.1	-8.0
5	*8.8	4.4
6	*8.8	3.6
7,6,L		
-10	*08.0	3.0
-9	10.1	4.5
-8	8.8	-7.9
-7	12.4	-10.6
-6	*7.4	-1.4
-5	9.2	6.0
-4	*7.0	-3.5
-3	5.7	-3.6
-2	*7.0	-1.5
-1	*7.0	-1.8
0	6.3	4.2
1	5.8	4.4
2	*7.1	6.9
3	*7.1	-2.3
4	*7.1	2.9
5	*7.1	-4.0
8,6,L		
-9	6.3	6.1
-8	10.7	10.9
-7	*7.1	2.5
-6	*7.8	3.0
-5	*7.8	-0.8
-4	*7.8	1.9
-3	7.3	-9.0
-2	*7.8	2.1
-1	8.9	-8.3
0	13.3	-12.3
1	*7.8	3.8
2	*7.8	-4.7
3	9.1	7.2
4	6.4	5.0
9,6,L		
-9	*7.1	1.6
-8	*7.1	0.3
-7	12.2	10.0
-6	*7.1	-2.4
-5	14.7	-13.7
-4	7.5	-7.7
-3	7.0	5.8
-2	*7.1	2.6
-1	*7.1	1.7
0	*7.1	1.5
1	*7.1	-2.5
2	*7.1	4.2
3	*7.1	-0.4
10,6,L		
-8	6.7	-4.6
-7	*7.5	-0.7
-6	*7.5	4.2
-5	8.6	-8.4
-4	*7.5	-4.8
-3	*7.5	-3.1
-2	*7.5	1.7
-1	5.4	-4.4
0	6.7	7.7
1	*7.5	-1.9
0,7,L		
1	*09.5	3.2
2	14.7	14.5
3	10.7	10.2
4	8.4	-5.7
5	*8.4	2.1
6	4.0	-4.7
1,7,L		
-7	*1.1	-2.1
-6	*10.5	-0.6
-5	*10.5	0.2
-4	*10.5	2.5
-3	*10.5	3.7
-2	10.3	-8.3
-1	*10.5	2.0
0	16.1	17.3
1	*10.4	2.1
2	8.1	6.9
3	10.5	-11.7
4	15.7	-14.1
5	9.6	10.7
6	*10.2	5.7
2,7,L		
-7	5.5	-7.3
-6	6.8	-6.8
-5	*10.2	-3.7
-4	*10.7	3.7
-3	*10.7	-2.9
-2	11.2	9.1
-1	*10.7	-2.4
2,7,L		
0	*10.7	-0.7
1	6.8	8.2
2	*10.7	2.6
3	8.3	-8.3
4	*10.8	-3.4
5	*10.8	1.6
6	*10.8	1.8
3,7,L		
-7	5.7	4.7
-6	8.6	-7.8
-5	6.4	5.0
-4	*8.8	-1.0
-3	7.8	-4.2
-2	*8.7	3.5
-1	7.7	5.6
0	*8.8	2.9
1	*8.8	2.1
2	*8.8	3.0
3	9.4	-7.7
4	8.2	6.8
5	*8.9	-0.4
4,7,L		
-7	*9.0	4.0
-6	*9.4	2.0
-5	*9.4	3.7
-4	*9.4	4.1
-3	*9.4	-1.6
-2	8.0	-9.9
-1	13.5	-15.2
0	8.7	5.8
1	*9.0	4.9
2	*9.0	4.2
3	*9.0	1.7
4	*9.0	0.3
5,7,L		
-7	9.9	-7.4
-6	*9.3	-1.3
-5	*9.3	-0.3
-4	9.5	-9.6
-3	10.0	9.3
-2	*9.1	5.7
-1	*9.1	3.5
0	6.3	6.1
1	*9.1	-3.2
2	*9.2	0.6
3	*9.3	0.4
6,7,L		
-6	16.1	-18.3
-5	*8.6	-3.1
-4	*8.6	-0.3
-3	*8.6	-1.4
-2	*8.6	0.1
-1	*8.6	2.3
0	*8.6	2.1
1	*8.6	0.8
2	7.7	9.0
7,7,L		
-6	6.4	6.9
-5	*8.0	-3.1
-4	*8.0	-3.8
-3	*8.0	1.3
-2	*8.0	1.0
-1	12.6	14.5
0	*8.0	2.0
1	7.2	-5.6

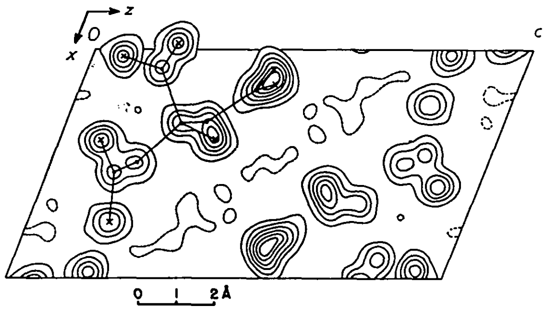


Fig. 1. Anhydrous citric acid: $h0l$ projection. Contour interval: 3 e.Å^{-2}. Zero contour dotted.

After two or three trials an $hk0$ projection was then found which could rapidly be refined by essentially the same procedure. The $h0l$ and $hk0$ projections were then refined by F_o-F_c syntheses to R values of 0·16 and 0·22 respectively. The $h0l$ projection is shown in Fig. 1.

Three-dimensional refinement of structure

Although no attempt was made to refine the coordinates of hydrogen atoms, the aliphatic hydrogen atoms were included in the structure factor calculations at the beginning of the least-squares procedure, and the three carboxyl hydrogens were added after the third complete least-squares cycle.

Two cycles of three-dimensional least-squares refinement were carried out, using a random selection of three tenths of the data. Viewed in terms of the final values, this procedure seemed profitable since twenty-nine coordinates were improved or left unchanged while ten were made worse.

In all, six cycles of least-squares refinement were carried out with all observed reflections all equally weighted. In parallel with the sixth least-squares refinement, a Booth differential synthesis and a back shift calculation were run. The final coordinates for oxygen and carbon are given in Table 1. These were obtained from an average of the final least-squares shifts and those obtained from the differential synthesis and the backshifts. The shifts predicted by the two methods for the last step agreed very well. The largest shift in the final least-squares calculation was 1·5(×10^{-3} Å) for oxygen and 1·6 for carbon. The r.m.s. values for these shifts were respectively 0·7 and 0·8. As was to be expected, the differential synthesis and backshift approach gave larger shifts at this stage since all earlier refinements were least squares. The largest shifts for oxygen and carbon were 9·8 and 9·5 while the corresponding r.m.s. values were 3·8 and 3·1(×10^{-3} Å).

Table 2 lists the observed F values together with the estimated upper limits for the photographed but unobserved reflections. The calculated values in this table assume the coordinates of Table 1 for oxygen and carbon and the hydrogen positions of Table 5. The R value is 0·14 for all observed reflections and 0·16 with the unobserved included at one-half their estimated upper limit.

Accuracy of the analysis

We have examined the accuracy of our analysis from several points of view and have summarized the results of this examination in Table 3. According to the Fourier Series approach suggested by Booth (1948) and developed by Cruickshank (1949) we have found an overall value of $\sigma(x)=2{\cdot}5(\times10^{-3}$ Å) for oxygen and 2·8 for carbon, consistent values being obtained from the three coordinates. From the least-squares approach (Hughes, 1941) with unit weighting we have obtained the much larger values of 5·9 and 8·6 for oxygen and carbon respectively. This discrepancy is quite surprising in view of some results which have seemed to indicate the equivalence of the two approaches. Some of this discrepancy may have been due to the weighting which we used but there is indication in the literature that these two approaches do not always agree (e.g. Abrahams, 1954). We attempted an estimate of error by the method of Luzzati (1952) and found that the plot of R versus $\sin\theta$ did not follow his curves, indicating that the residual error was not due to a random displacement of atomic positions but lay rather in the experimental error in the data.

Table 3. *Error estimates for citric acid*

	Carbon		Oxygen
Coordinates (σx)(×10^{-3} Å)			
Fourier series	2·8		2·5
Least squares	8·6		5·9
Electron density ($\sigma\varrho$) (e.Å^{-3})			
Fourier series		0·23	
XRAC background		0·16	
Peak height fluctuation	0·59		0·41

Table 4. *Peak densities and curvatures*

Measured on XRAC Fourier

	ϱ (e.Å^{-3})	ϱ'' (e.Å^{-5})
O_1	19·2	231
O_2	19·2	231
O_3	15·5	169
O_4	18·5	207
O_5	18·5	221
O_6	16·8	208
O_7	19·3	255
XRAC average	18·1 ± 1·4	217 ± 25
Series average		196
C_1	14·8	197
C_2	14·3	193
C_3	15·5	206
C_4	13·8	182
C_5	14·0	183
C_6	14·3	209
XRAC average	14·4 ± 0·6	195 ± 10
Series average		173

Temperature factor: $B=1{\cdot}30$ Å^2

Table 5. *Location of hydrogen atoms*

Hydrogen atom	Bonded to	Assumed for structure factor refinement			Found on difference Fourier		
		X	*Y*	*Z*	*X*	*Y*	*Z*
H_1	C_2	0·125	0·554	0·244	0·15	0·55	0·25
H_2	C_2	0·242	0·478	0·371	0·25	0·50	0·38
H_3	C_4	0·214	0·329	0·117	0·21	0·30	0·12
H_4	C_4	0·322	0·290	0·257	0·32	0·27	0·24
H_5	O_1	0·032	0·690	0·487	0·06	0·68	0·51
H_6	O_3	0·420	−0·228	0·187	0·38	−0·23	0·16
H_7	O_5	−0·062	0·236	0·007	−0·06	0·25	0·02
H_8	O_7	0·137	−0·100	0·372	See text		

In attempting to reconcile the two differing estimates of error we examined the curvature term which we obtained by calculation of the Fourier series for this quantity at the atomic sites. In Table 4, the values obtained in this manner are compared with those obtained by extrapolation from the radii of the contours near the peaks on the XRAC density map, and show reasonable agreement. The values for ϱ and ϱ'' are higher than many which have been published but we believe this to be satisfactorily accounted for by the low value of the temperature factor for this substance (cf. Atoji, 1957).

In connection with the possible improvement in accuracy of this analysis, there are several comments which can be made. There seems to be strong evidence that there is extinction in our data since in ten of the eleven strongest reflections the calculated value exceeds the observed. In the difference Fourier computed on XRAC all six carbon positions lie on peaks indicating that the temperature factor we have used is too large for these atoms. All the oxygen atoms on the other hand lie in holes or troughs indicating that for these atoms the isotropic temperature factor is too small. Clearly the assumption of individual isotropic temperature factors or of individual anisotropic temperature factors would improve the fit of our calculated structure factors to the observed. We have not undertaken any of the refinements suggested in this paragraph since we feel that the use of any one of them would be inadvisable without a re-examination of the accuracy of the experimental data, and the consequent revision of the weighting of the least-squares analysis.

In discussing the precision of the structure which we present we have taken the most conservative point of view with regard to the error in coordinates (Table 3) and have assumed the following contributions from this source to the standard errors for the distances between atoms:

$$\text{O–O},\ 0{\cdot}83\times10^{-2}\ \text{Å};\ \text{O–C},\ 1{\cdot}04\times10^{-2}\ \text{Å};$$
$$\text{C–C},\ 1{\cdot}21\times10^{-2}\ \text{Å}.$$

These contributions must be combined with an error of 0·2% of the distance due to the error in cell dimensions. The probable error in bond angles we have taken to be about 0·7°.

Hydrogen positions

As mentioned above, the hydrogens were located for the most part by theoretical considerations in the positions given in Table 5. Atoms H_1–H_4 are aliphatic hydrogens and were located early in the refinement. Atoms H_5–H_7 are the carboxyl hydrogens and could be located as soon as the carboxyl oxygens were differential as noted below. The hydroxyl hydrogen H_8 has been the most difficult to locate. The coordinate assumed in Table 5 is that dictated by the hydrogen bonding which we have postulated for the hydroxyl group. As indicated in the table, atoms H_1–H_7 have all appeared quite clearly on the difference Fourier computed on XRAC. There is density near the coordinates which we have postulated for H_8, but unfortunately there is other density elsewhere in the neighborhood of O_7 of the same magnitude.

Molecular structure

The bond lengths and bond angles are shown in Fig. 2 and all the interatomic distances within the molecule are given in Table 6. The C–C bonds around C_3 are not significantly different from one another and do not depart from the single bond value. The terminal bonds C_1C_2 and C_4C_5 are perhaps significantly shorter than the others. The C_3O_7 bond length is in close agreement with the usually accepted values for the C–OH bond. The dimensions of the three carboxyl groups are not significantly different from one another. The hydroxyl oxygens O_1, O_3, O_5 are clearly indicated by their bond lengths which are here slightly larger than those usually found for the 'long' bonds of carboxyl groups but perhaps not significantly.

The aliphatic chain C_1–C_5 is approximately planar, but is slightly bent away from the carboxyl $O_5C_6O_6$. The largest departure from planarity in the chain is 0·12 Å. All carboxyls are coplanar with their α-carbons within limits far below the experimental error discussed above. The carboxyl $O_1C_1O_2$ makes an angle of 3·3° with the plane of the aliphatic chain, while the carboxyl $O_3C_5O_4$ is approximately at right angles to that plane.

The approximate parallelism between the plane of the carboxyl $O_5C_6O_6$ to the plane $C_6C_3O_7$ containing

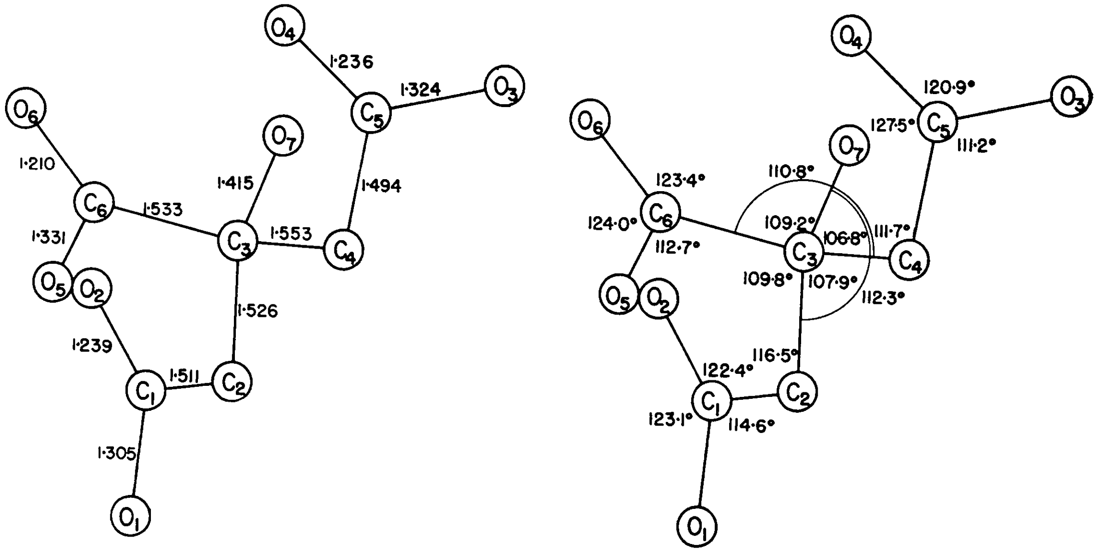


Fig. 2. Bond lengths and bond angles in the molecule of citric acid.

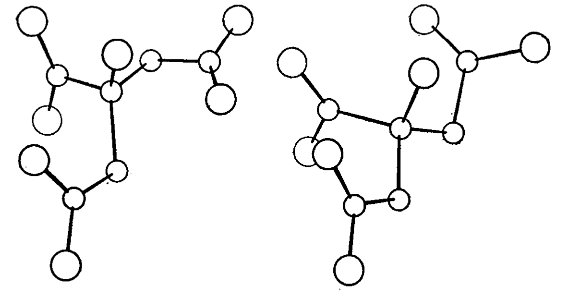
Table 6. *Intramolecular distances*

Atoms are numbered as in Table 1 and Fig. 2. Bond lengths are boxed. Distances between next nearest neighbors, fixed by two adjacent bonds, are underlined in dots. Values singly and doubly underlined correspond to distances between third and fourth nearest neighbors having one and two degrees of freedom respectively. Distances corresponding to three and four degrees of freedom are unmarked. The estimated standard deviations, due to the error in coordinate determination, are for the O–O and C–C distances 0·008 Å and 0·012 Å respectively; and for the C–O distances 0·010 Å. These are representative of the overall error for the shorter distances, but for the longer distances they must be combined with an error of 0·2% of the bond length due to the error in cell dimensions

HO_1	O_2	HO_3	O_4	HO_5	O_6	HO_7	C_1	H_2C_2	C_3	H_2C_4	C_5	C_6	
-	$\underset{\cdots}{2.237}$	6.968	6.749	4.603	4.759	$\underline{\underline{4.259}}$	$\boxed{1.306}$	$\underset{\cdots}{2.372}$	$\underline{3.736}$	$\underline{\underline{4.859}}$	6.178	$\underline{\underline{4.236}}$	HO_1
	-	6.199	5.588	4.014	3.133	$\underline{\underline{2.864}}$	$\boxed{1.239}$	$\underset{\cdots}{2.413}$	$\underline{2.875}$	$\underline{\underline{4.383}}$	5.304	$\underline{\underline{3.102}}$	O_2
		-	$\underset{\cdots}{2.227}$	4.773	4.981	$\underline{\underline{3.492}}$	5.930	$\underline{\underline{4.645}}$	$\underline{3.516}$	$\underset{\cdots}{2.328}$	$\boxed{1.324}$	$\underline{\underline{4.341}}$	HO_3
			-	3.408	3.366	$\underline{\underline{3.201}}$	5.573	$\underline{\underline{4.492}}$	$\underline{3.030}$	$\underset{\cdots}{2.455}$	$\boxed{1.236}$	$\underline{\underline{2.999}}$	O_4
				-	$\underset{\cdots}{2.243}$	$\underline{3.580}$	$\underline{\underline{3.737}}$	$\underline{2.970}$	$\underset{\cdots}{2.386}$	$\underline{2.850}$	$\underline{\underline{3.495}}$	$\boxed{1.331}$	HO_5
					-	$\underline{2.695}$	$\underline{\underline{3.581}}$	$\underline{3.394}$	$\underset{\cdots}{2.419}$	$\underline{3.543}$	$\underline{\underline{3.754}}$	$\boxed{1.210}$	O_6
						-	$\underline{3.011}$	$\underset{\cdots}{2.443}$	$\boxed{1.415}$	$\underset{\cdots}{2.384}$	$\underline{2.780}$	$\underset{\cdots}{2.404}$	HO_7
							-	$\boxed{1.511}$	$\underset{\cdots}{2.582}$	$\underline{3.892}$	$\underline{\underline{5.081}}$	$\underline{3.109}$	C_1
								-	$\boxed{1.526}$	$\underset{\cdots}{2.489}$	$\underline{3.828}$	$\underset{\cdots}{2.502}$	H_2C_2
									-	$\boxed{1.553}$	$\underset{\cdots}{2.522}$	$\boxed{1.533}$	C_3
										-	$\boxed{1.494}$	$\underset{\cdots}{2.541}$	H_2C_4
											-	$\underline{3.081}$	C_5
												-	C_6

the α-hydroxyl (Jeffrey & Parry, 1952, 1955) is repeated here as in rubidium dihydrogen citrate (Nordman, Weldon & Patterson, 1960). In the present case the angle between these planes is 11·3°.

In comparing the molecule of citric acid with its primary ion in rubidium dihydrogen citrate we notice (Fig. 3) that the two molecules are closely alike with the exception of one carboxyl group $O_3C_5O_4$. In citric acid the carbon C_5 lies in the main aliphatic chain, while in the citrate this group is completely out of the



Dihydrogen citrate ion viewed down[102]

Citric acid molecule viewed down[001]

Fig. 3. Comparison of the dihydrogen citrate ion in rubidium dihydrogen citrate with the molecule of citric acid.

chain. It would be interesting to speculate on the reason for this rotation, but we shall postpone such discussion until we have examined some other citrates.

Intermolecular distances and hydrogen bonding

The shorter intermolecular distances are listed in Table 7 with references to Fig. 4. The latter indicates the hydrogen bonding of the crystal. The carboxyl group $O_1C_1O_2$ is connected by two equivalent hydrogen bonds to an equivalent group across a center of symmetry. The bond length of $2{\cdot}63_3$ Å is characteristic of this 'dimer' type bond (Donohue, 1952; Ubbelohde & Gallagher, 1955). The other two carboxyls form a part of a continuous spiral chain of hydrogen bonds which extend along a screw axis. The two non-equivalent hydrogen bonds have the not unexpected lengths of $2{\cdot}65_9$ and $2{\cdot}73_0$ Å. There seems also to be a fourth hydrogen bond of length $2{\cdot}84_2$ Å between the hydroxyl oxygen O_7 of one molecule and the carboxylate hydroxyl oxygen O_1 of an equivalent molecule removed from the first by a *b* translation.

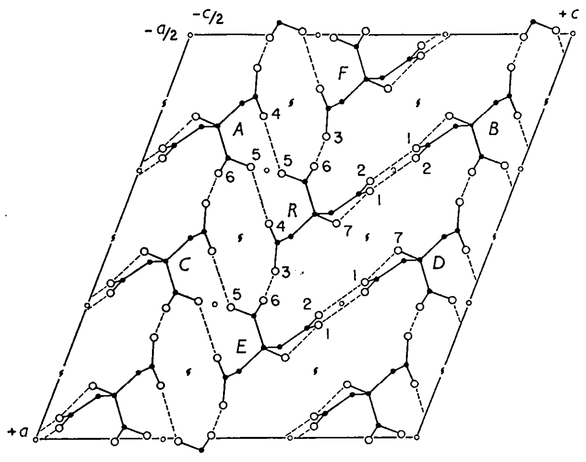


Fig. 4. Hydrogen bonding and molecular packing in anhydrous citric acid. Intermolecular close approaches are listed in Table 7. Oxygen atoms, open circles; carbon atoms, filled circles.

The hydrogen bond system of the crystal can be described in terms of two non-equivalent systems of spirals about the screw axes. One type of spiral about the screw axis $\frac{1}{4}$, *v*, 0 (and also its centrosymmetrical

Table 7. *Intermolecular close approaches* (*shorter than* 3·30 Å)

The reference molecule (*R*) is that of Table 1 (see also Fig. 4). The neighboring molecules are related to the reference molecule by the following symmetry operations:

A: $\bar{1}$ at 0, 0, 0; *B*: $\bar{1}$ at 0, $\frac{1}{2}$, $\frac{1}{2}$;
C: 2_1 in $\frac{1}{4}$, *v*, 0 with translation +0, $\frac{1}{2}$, 0; *D*: 2_1 in $\frac{1}{4}$, *v*, $\frac{1}{2}$ with translation +0, $\frac{1}{2}$, 0;
E: *a* in *u*, $\frac{1}{4}$, *w* with translation +$\frac{1}{2}$, 0, 0; *F*: *a* in *u*, $\frac{1}{4}$, *w* with translation −$\frac{1}{2}$, 0, 0.

Molecules related to the above by *b* translation are indicated as *Ab*, $R\bar{b}$, etc. The closest approach of molecules *C* or $C\bar{b}$ to *R* is 3·57 Å. The shortest intermolecular approaches of C_2–C_6 are greater than 3·36 Å. Hydrogen bond lengths are underlined. Standard deviations as for Table 6

Atoms on reference molecule	Atoms in neighboring molecules								Next shortest
	HO_1	O_2	HO_3	O_4	HO_5	O_6	HO_7	C_1	
HO_1		$\underline{2{\cdot}633}B$	$3{\cdot}118F$			$3{\cdot}190Rb$	$\underline{2{\cdot}842}Rb$ $3{\cdot}130D$		3·437
O_2	$\underline{2{\cdot}633}B$		$3{\cdot}259F$						3·410
HO_3	$3{\cdot}118E$	$3{\cdot}259E$			$3{\cdot}116E$	$\underline{2{\cdot}659}E\bar{b}$		$2{\cdot}994E$	3·361
O_4					$\underline{2{\cdot}730}A$				3·406
HO_5			$3{\cdot}116F$	$\underline{2{\cdot}730}A$	$2{\cdot}959Ab$	$3{\cdot}224A$			3·400
O_6	$3{\cdot}190R\bar{b}$		$\underline{2{\cdot}659}F\bar{b}$		$3{\cdot}224A$				3·406
HO_7	$\underline{2{\cdot}842}R\bar{b}$ $3{\cdot}130D\bar{b}$							$3{\cdot}156D\bar{b}$	3·581
C_1			$2{\cdot}994F$				$3{\cdot}156D$		3·416

equivalent about $\frac{3}{4}, v, 0$) involves both the hydrogen bonds of the terminal carboxyl $O_3C_5O_4$ and both the hydrogen bonds of the central carboxyl group $O_5C_6O_6$. The two equivalent pairs of spirals are tied together in the *a* direction by a pair of centrosymmetrically related molecules. The second family of spirals about the screw axes at $\frac{1}{4}, v, \frac{1}{2}$ and $\frac{3}{4}, v, \frac{1}{2}$ involve the bonds O_3–O_6 which formed part of the first spirals and the dimer type pair of O_1–O_2 bonds. The successive turns of the second type of spiral are linked by the O_1–O_7 hydrogen bonds.

The rigidity imparted to the crystal by this highly interlocked hydrogen bond system is evidenced by the low value of the temperature factor *B*. The intermolecular distances not involved in the hydrogen bonding are well in accord with the accepted van der Waals radii for structures of this type.

References

Abrahams, S. C. (1954). *Acta Cryst.* **7**, 423.

Atoji, M. (1957). *Acta Cryst.* **10**, 291.

Booth, A. D. (1948). *Fourier Technique in X-ray Organic Structure Analysis*, Chap. VII. Cambridge: University Press.

Burns, D. M. & Iball, J. (1954). *Acta Cryst.* **7**, 137.

Crowfoot, D., Bunn, C. W., Rogers-Low, B. W. & Turner-Jones, A. (1949), in *The Chemistry of Penicillin*, p. 310. Princeton: University Press.

Cruickshank, D. W. J. (1949). *Acta Cryst.* **2**, 65.

Donohue, J. (1952). *J. Phys. Chem.* **56**, 502.

Gillis, J. (1948). *Acta Cryst.* **1**, 174.

Grenville-Wells, H. J. & Abrahams, S. C. (1952). *Rev. Sci. Instrum.* **23**, 328.

Groth, P. (1910). *Chemische Kristallographie*, Vol. 3, p. 475. Leipzig: Engelmann.

Harker, D. & Kasper, J. S. (1948). *Acta Cryst.* **1**, 70.

Hughes, E. W. (1941). *J. Amer. Chem. Soc.* **63**, 1737.

Jeffrey, G. A. & Parry, G. S. (1952). *Nature, Lond.* **169**, 1105.

Luzzati, V. (1952). *Acta Cryst.* **5**, 802.

Nordman, C. E., Patterson, A. L., Weldon, A. S. & Supper, C. (1955). *Rev. Sci. Instrum.* **26**, 690.

Nordman, C. E., Weldon, A. S. & Patterson, A. L. (1960). *Acta Cryst.* **13**, 414.

Ubbelohde, A. R. & Gallagher, K. J. (1955). *Acta Cryst.* **8**, 71.

Waser, J. (1951). *Rev. Sci. Instrum.* **22**, 563, 567.

Wiebenga, E. H. & Smits, D. W. (1950). *Acta Cryst.* **3**, 265.

Zachariasen, W. H. (1952). *Acta Cryst.* **5**, 68.

Acta Cryst. (1960). **13**, 426

X-ray Crystal Analysis of the Substrates of Aconitase.*
III. Crystallization, Cell Constants, and Space Groups of Some Alkali Citrates

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Cell dimensions and space groups have been determined for twelve alkali citrates and are listed with five others determined previously. The *p*H of the dissolved crystal is used in each case as a check on the ionic content of each crystal type.

The cell constants and space groups of the alkali citrates listed in Table 1 have been determined as part of a study of the structure of the substrates of the enzyme aconitase (Nordman, Weldon & Patterson, 1960*a*, *b*). In this paper the alkali citrates are represented by symbolic formulae of the type M_nH_{3-n}cit in which '*M*' stands for the metal and 'cit' stands for the tri-ionized citrate ion

$$^{-}OOC.CH_2.C(OH)(COO^{-}).CH_2.COO^{-}.$$

Crystallization

The method of crystallization used in each case is given in Table 1. Aqueous salt solutions were made by allowing 1·0*M* citric acid to react with weighed amounts of the appropriate alkali carbonate. In some cases slow evaporation to dryness led to usable crystals. In other cases crystals were grown from an aqueous citrate solution layered under either ethanol or acetone, i.e., under hydrophilic solvents in which citrate salts are nearly insoluble (cf. King, Magdoff, Adelman & Harker, 1956). Portions of the solution 2 c.c. in volume, were layered under 10–20 c.c. of the organic solvent in 16 × 150 mm. test tubes. Crystals grew in a week or two in stoppered tubes.

Using lithium, sodium, potassium, rubidium, and

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