339. *The Rotatory Dispersive Power of Organic Compounds. Part XXIV. Ascorbic Acid.*

By T. M. LOWRY and S. A. PEARMAN.

THROUGH the kindness of Professor Szent-Györgyi, we have been enabled to study the rotatory power and related properties of ascorbic acid, on similar lines to the investigations described in preceding papers of this series.

1. *Mutarotation.*—Preliminary observations suggested that measurements of rotatory dispersion would be complicated by the occurrence of mutarotation, in the same way as in aqueous solutions of the sugars or sugar-lactones (compare Lowry and Krieble, *Z. physikal. Chem.*, Bodenstein Festband, 1931, 881), since the specific rotatory power of a dilute aqueous solution fell from $[\alpha]_{5461} = 30{\cdot}2°$ to 24·7° in 7 days; but observations on a more concentrated solution, in which the mutarotation of a sugar would have been immediately obvious, showed that, when air was excluded as far as possible, mutarotation was arrested completely over a period of 3 days, and only set in when access of oxygen was permitted. This result is in harmony with the statement of Cox, Hirst, and Reynolds (*Nature*, 1932, **130**, 888) that " its solutions do not display mutarotation," although a rapid diminution in the intensity of the absorption band at 2600 Å.U. had previously been recorded by Herbert and Hirst (*ibid.*, p. 205).

2. *Rotatory Dispersion.*—The rotatory dispersion of an aqueous solution of ascorbic acid containing 10 g./100 c.c. is shown in Table I, and is plotted in Fig. 1. The dispersion is anomalous, since the specific rotatory power rises to a maximum $[\alpha] = +\,27{\cdot}65°$ at or near the violet mercury line Hg 4358. The observed rotations obviously include a *positive* term of high characteristic frequency and a *negative* term of lower frequency. The low-frequency term can be identified with the optical activity of the well-known band at 2650 Å.U. (Bowden and Snow, *Nature*, 1932, **129**, 720; Herbert and Hirst, *loc. cit.*). It would therefore have been our normal procedure to follow the rotatory dispersion into the region covered by this absorption band, as has recently been done in the case of three aldehydic sugars (Hudson, Wolfrom, and Lowry, this vol., p. 1179). Unfortunately, as in the case of nicotine, the absorption is too intense for this to be practicable, since adequate transmission of light would only be possible at dilutions at which the rotatory power would be too small to be detected. The form of the curve can, however, be expressed by means

of a Drude equation, containing two terms of opposite sign. Since our data were not extensive enough to enable us to calculate four independent constants, a three-constant equation was used, in which the high-frequency dispersion constant was ignored, as in Drude's equation for quartz. An empirical equation of this type,

$$[\alpha] = -8{\cdot}464/(\lambda^2 - 0{\cdot}05412) + 17{\cdot}142/\lambda^2,$$

expresses the data in a satisfactory way, as can be seen from the smallness of the errors tabulated in col. 4 of Table I.

The rotatory dispersion of a more dilute solution, containing only 0·615 g./100 c.c. of ascorbic acid, is shown as a broken line in Fig. 1. The rotations are uniformly higher, and show a maximum at a rather longer wave-length. The readings were carried a little further into the ultra-violet, but the observed rotations were too small to provide accurate data for a mathematical analysis of the curve.

FIG. 1.

Rotatory dispersion of ascorbic acid : (1) 10 *G. per* 100 *c.c.* (*full curve*) *;* (2) 0·615 *g. per* 100 *c.c.* (*broken curve*).

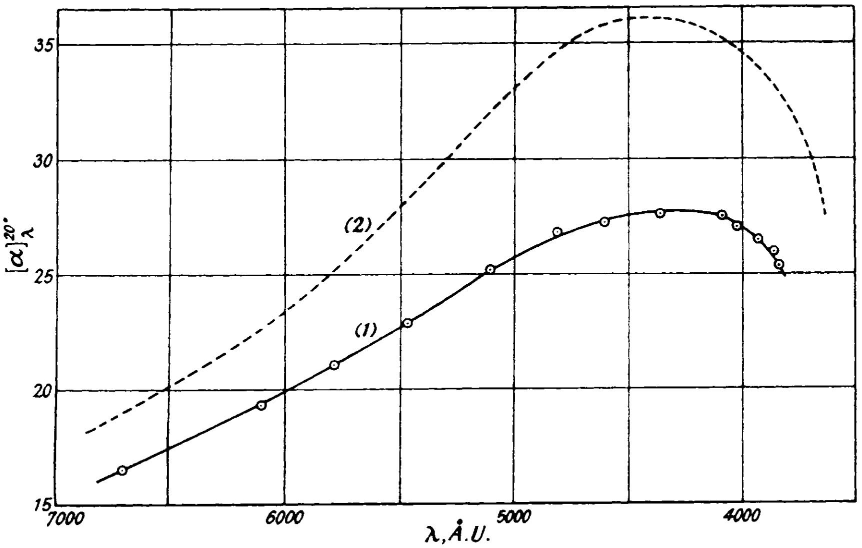


TABLE I.

Rotatory Dispersion of Ascorbic Acid in Water.

(c = 10 g./100 c.c.; l = 2 dm.; t = 20°.)

$$[a]_{\text{calc.}} = 5a = -\frac{8{\cdot}464}{\lambda^2 - 0{\cdot}05412} + \frac{17{\cdot}142}{\lambda^2}.$$

		$a_{\text{obs.}}$	$a_{\text{calc.}}$	Diff. (O − C).	$[a]_{\text{obs.}}$
Li	6708	+3·31°	+3·34°	−0·03°	+16·55°
Li	6104	+3·87	+3·87	±	+19·35
Hg	5780	+4·22	+4·22	±	+21·10
Hg	5461	+4·58	+4·56	+0·02	+22·90
Cu	5105	+5·05	+4·94	+0·11	+25·25
Zn	4811	+5·37	+5·27	+0·10	+26·85
Li	4602	+5·46	+5·45	+0·01	+27·30
Hg	4358	+5·53°	+5·59°	−0·06°	+27·65°
Fe	*4085* *	+5·52	+5·50	+0·02	+27·60
Fe	*4022* *	+5·42	+5·47	−0·05	+27·10
Fe	*3928* *	+5·31	+5·32	−0·01	+26·55
Fe	*3860* *	+5·20	+5·17	+0·03	+26·00
Fe	*3847* *	+5·09	+5·13	−0·04	+25·45

* Photographic values, using iron arc.

3. *Influence of Dilution.*—The broken curve of Fig. 1 shows that an increase of rotatory power on dilution takes place throughout the whole of the spectral range covered by the observations. Fuller data for the influence of dilution on the rotatory power of ascorbic

acid for five different wave-lengths over a range of concentrations from 10 to 0·6 g./100 c.c. are set out in Table II, and are plotted in Fig. 2. In the range from 10 to 5% the changes of specific rotatory power are not important, but a rapid increase is shown in the range from 2 to 0·6%. This increase may perhaps be attributed to a reversible hydration (cf. many compounds of the sugar series), but a proof of this cannot yet be offered.

TABLE II.

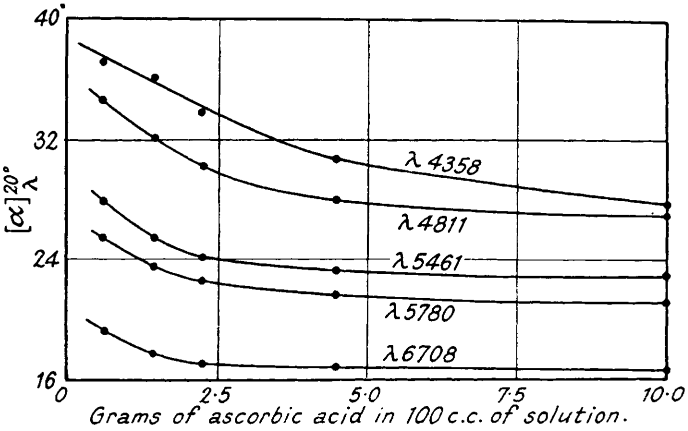
Influence of Concentration on the Specific Rotation of Ascorbic Acid.

Solution.	l (dm.).	c (g./100 c.c.).	$[\alpha]_{6708}$.	$[\alpha]_{5780}$.	$[\alpha]_{5461}$.	$[\alpha]_{4811}$.	$[\alpha]_{4358}$.
A	2	10·00	+16·55°	+21·10°	+22·90°	+26·85°	+27·65°
B	2	4·47	+16·80	+21·73	+23·40	+27·90	+30·80
C	2	2·23	+17·05	+22·66	+24·22	+30·27	+33·85
D	4	1·43	+17·72	+23·48	+25·43	+32·08	+36·13
E	6	0·61	+19·23	+25·47	+27·92	+34·67	+37·13

4. *Influence of Alkali.*—(a) *Mutarotation.* Addition of alkali produces a large increase in the optical rotatory power of ascorbic acid (Cox, Hirst, and Reynolds, *Nature*, 1932, **130**, 888) and facilitates its oxidation so much that the compound is relatively unstable in alkaline solutions (Szent-Györgyi, *Biochem. J.*, 1928, **22**, 1387). The abrupt and very large increase of rotatory power which results from the addition of alkali to ascorbic acid is shown in a striking way in Fig. 3. Since oxygen was not rigidly excluded from the system, this increase was followed by a progressive decrease of rotatory power, due to oxidation, which led to a reversal of sign in 28 days and to a negative rotation $[\alpha]_{5461}^{20°} = -21·5°$ at the end of 51 days. Hirst (*Chem. and Ind.*, March 10, 1933, 221) records a similar negative rotation $[\alpha]_D = -6°$ in a solution which had been oxidised with sodium hypochlorite and then allowed to undergo mutarotation.

FIG. 2.

Influence of dilution on the specific rotatory power of ascorbic acid at five wave-lengths.



Although our preliminary measurements thus showed a rapid mutarotation in presence of alkali, no immediate mutarotation was observed when an aqueous solution, containing 1·44 equiv. of sodium hydroxide, was kept in an atmosphere of nitrogen, since a complete curve of rotatory dispersion was plotted without any correction for mutarotation being necessary. Under the conditions of our experiment it would, however, have been impossible to detect either the ordinary mutarotation of a reducing sugar, which would be excessively rapid in presence of so large a proportion of free alkali, or a transformation of the Lobry de Bruyn type, which would have given rise to an excessively slow mutarotation.

(b) *Rotatory dispersion.* In order to measure the rotatory dispersion of ascorbic acid in presence of an excess of alkali two series of observations were made with unoxidised solutions, the optical rotatory power of which was checked repeatedly for three mercury lines as a control on the stability of the solutions. The concordant observations obtained in this way are set out in Table III, and are plotted in Fig. 4. The broken curve in Fig. 4 shows the decrease of rotatory power, *throughout the whole spectral range of the observations,* which resulted from partial oxidation of one of the solutions.

FIG. 3.

Change in rotatory power in an alkaline solution of ascorbic acid.

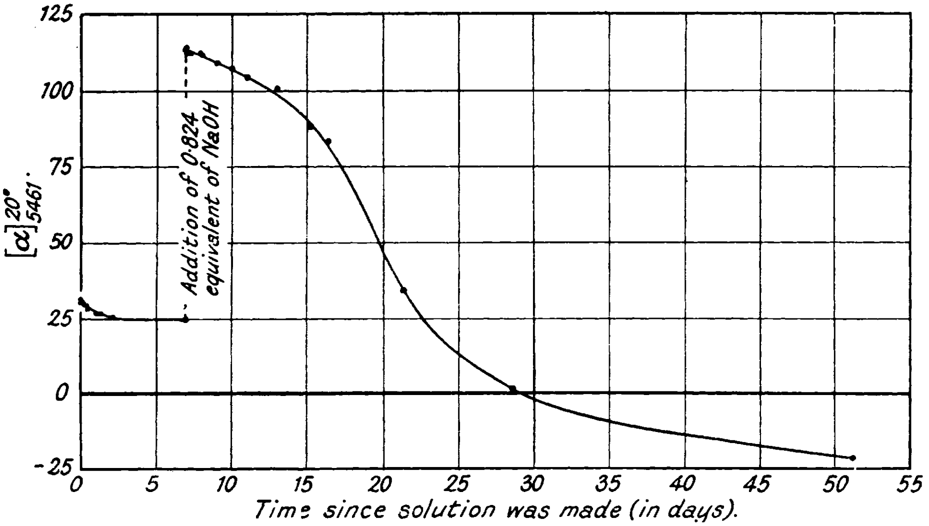
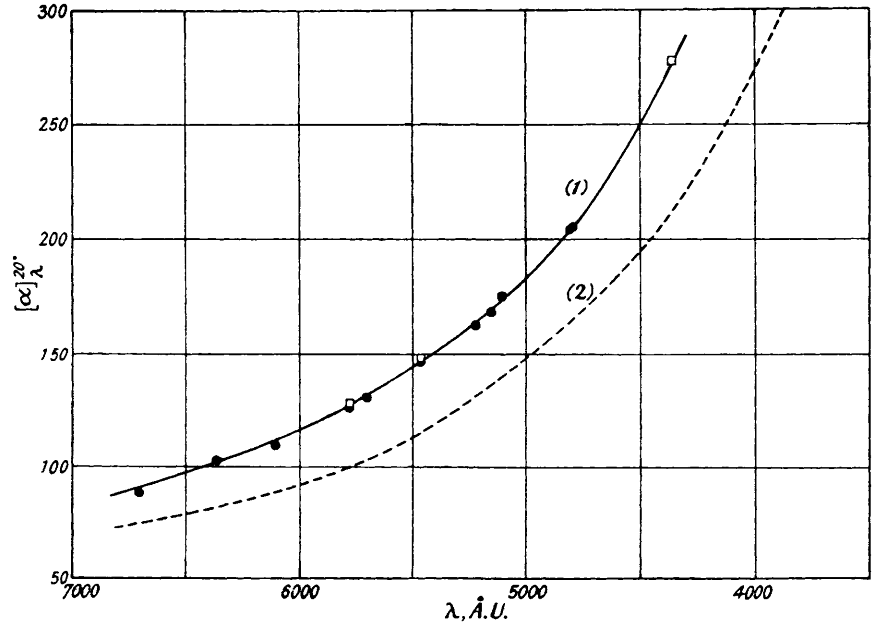


FIG. 4.

Rotatory dispersion of ascorbic acid in an alkaline solution : (1) *No oxidation* (*full curve*) *;* (2) *partial oxidation* (*broken curve*).



The anomalous rotatory dispersion of ascorbic acid disappears completely in the alkaline solutions, the dispersion of which is not only normal but apparently simple.* This is seen clearly in Table III, where the differences tabulated in col. 5 show the deviation of the observed values from those calculated by means of the equation $[\alpha] = 33{\cdot}81/(\lambda^2 - 0{\cdot}06589)$.

TABLE III.

Rotatory Dispersion of Ascorbic Acid in Alkaline Solution.

(1·44 equivalents of NaOH.)
Solution (*a*) $c = 0{\cdot}6154$ g./100 c.c.; $l = 6$ dm.; $t = 20°$.
(*b*) $c = 0{\cdot}5964$ g./100 c.c.; $l = 4$ dm.; $t = 20°$.
$[\alpha]_{\text{calc.}} = 33{\cdot}81/(\lambda^2 - 0{\cdot}06589)$.

λ.	Soln.	$\alpha_{\text{obs.}}$	$\alpha_{\text{calc.}}$	Diff. (O − C).	$[\alpha]_{\text{obs.}}$
Li 6708	a	3·16°	3·14°	+0·02°	88·6°
Zn 6362	a	3·67	3·56	+0·12	102·9
Li 6104	a	3·92	3·93	−0·01	109·9
Cu 5782	a	4·50	4·49	+0·01	126·2
Hg 5780	a	4·54	4·50	+0·04	127·3
Hg 5780	b	3·04	3·01	+0·03	127·5
Cu 5700	a	4·65	4·66	−0·01	130·4
Hg 5461	a	5·22	5·19	+0·03	146·4
Hg 5461	b	3·52°	3·47°	+0·05°	147·5°
Cu 5219	a	5·79	5·84	−0·05	162·4
Cu 5153	a	6·01	6·04	−0·03	168·5
Cu 5105	a	6·23	6·19	+0·04	174·7
Zn 4811	a	7·28	7·29	−0·01	204·1
Cd 4800	a	7·31	7·35	−0·04	204·9
Hg 4358	b	6·62	6·50	+0·12	277·5

(c) *Progressive neutralisation.* Birch and Harris (*Biochem. J.*, 1933, **27**, 595) by a micro-electrometric titration have established the existence of "neutralisation" points at $p_{K_a} = 4{\cdot}17$ and 11·57, the former in weakly acid and the latter in strongly alkaline solutions. The influence of progressive neutralisation on the rotatory power of ascorbic acid is shown in Fig. 5. Up to one equivalent the increase of rotatory power is roughly proportional to the amount of alkali added; but the increase continues up to 1·5 equivalents, and even then has scarcely reached a maximum. This further increase might be due to a reversible neutralisation, but other factors would require careful study before such a conclusion could be definitely established.

EXPERIMENTAL.

Materials.—The two samples of ascorbic acid (designated I and II) supplied by Professor Szent-Györgyi, on micro-analysis (by Dr. A. Schoeller of Berlin), gave the following figures:

I. C, 40·86, 40·88; H, 4·58, 4·68; residue on ignition, 0·19%.
II. C, 40·75, 40·76; H, 4·80, 4·71; residue on ignition, 0·10%.

Calc. for $C_6H_8O_6$: C, 40·91; H, 4·57%.

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* [*Note added, November 6th.*]

In a paper which has appeared since the present communication was made, Hirst and others have shown (this vol., p. 1280) that the well-known absorption band of ascorbic acid at 2650 Å.U. is also characteristic of the sodium salt. The contrast between the anomalous rotatory dispersion of the acid and the apparently simple rotatory dispersion of its sodium salt is therefore not due to any marked change in the ultra-violet absorption of the acid on neutralisation, although profound alterations in the Schumann region could not be detected by the methods which are now in general use. The data now recorded suggest that the optically active absorption band, which is associated with the low-frequency negative partial rotation of ascorbic acid in aqueous solutions, becomes relatively inactive or may even undergo a reversal of sign in the sodium salt.

A similar observation has already been made in the case of nicotine, where the absorption band at 2650 Å.U., which dominates the strong lævorotation of the base, becomes inactive in the kation of the zinc chloride compound, the simple dextrorotation of which has a characteristic frequency in the Schumann region at 1480 Å.U., although the ultra-violet band is actually twenty times stronger than that of the base. In the present instance the strong simple dextrorotation of the salt, which has a characteristic frequency at 2567 Å.U., can probably be associated with the absorption band at 2650 Å.U.; but the origin of the lævorotatory partial rotation of the acid is still uncertain, as its absorption is too intense to permit of observations within the band, which are essential for a complete analysis of its complex rotatory dispersion.

On the basis of these observations it now appears likely that the conversion of the complex dispersion of tartaric acid and its esters into a simple dispersion in certain cyclic derivatives (Brynmor Jones, this vol., pp. 788, 951) may be due to a change in the optical activity of the ultra-violet absorption bands rather than to any profound change in the absorption bands themselves. T. M. L.

I consisted of heavy white crystals and appeared to be almost completely pure, II was pale cream and the crystals were lighter than I; it appeared less pure than I, since its specific rotation in water under comparable conditions was definitely lower :

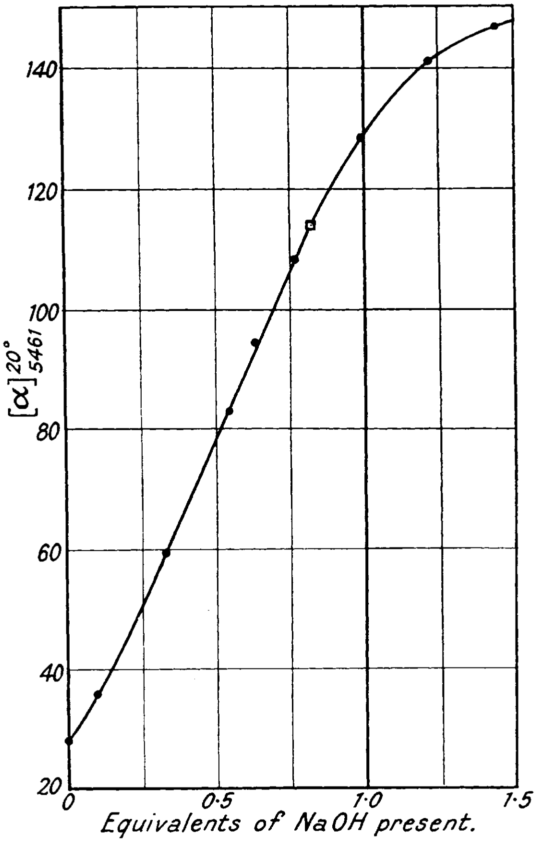
I. 0·3077 g. in 50 c.c. ($l = 6$) gave $\alpha_{5461} = 1{\cdot}03°$, $[\alpha]^{20}_{5461} = 27{\cdot}9°$.
II. 0·1568 ,, ,, 26·1 ,, ($l = 4$) gave $\alpha_{5461} = 0{\cdot}62°$, $[\alpha]^{20}_{5461} = 25{\cdot}8°$.

In consequence of this, all the work described in this paper was carried out on sample I.

Methods of Measurement.—The measurements of optical rotatory power were made by methods which have already been described. Rigorous exclusion of oxygen was essential, however, especially from the alkaline solutions. The aqueous solutions were therefore prepared with water which had been boiled and cooled in a current of nitrogen; and a stream of damp nitrogen was also passed continuously over the surface of the solution in the polarimeter tube, through a small aperture in a rubber stopper. The broken curve of Fig. 4 shows the effect of the oxidation which took place when, owing to leakage at an end-plate, an alkaline solution was transferred to a stoppered flask over-night, without maintaining the current of nitrogen.

FIG. 5.

Changes of specific rotatory power of ascorbic acid on progressive addition of sodium hydroxide.



SUMMARY.

1. Aqueous solutions of ascorbic acid do not undergo mutarotation in the absence of oxygen, but they exhibit anomalous rotatory dispersion, with a maximum in the violet.

2. Alkaline solutions also do not undergo mutarotation in the absence of oxygen, but the rotatory power is much higher and the rotatory dispersion is apparently simple.

3. The rotatory power of ascorbic acid is increased by dilution and by the addition of alkali, but that of alkaline solutions diminishes and then becomes negative on atmospheric oxidation.

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