370. *Optical Rotatory Dispersion in the Carbohydrate Group. Part II. Ascorbic Acid.**

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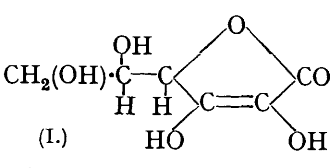
It became evident when the structure (I) was definitely allocated to ascorbic acid (Herbert, Hirst, Percival, Reynolds, and Smith, this vol., p. 1270) that the optical rotatory dispersion displayed by this substance would be of special interest in connection with recent views on the origin of optical activity. It was obvious also that an examination of the dispersion was desirable in view of the unusual relationship between the rotatory power of the acid ($[\alpha]_D + 23°$) and that of the sodium salt ($[\alpha]_D + 102°$ in neutral solution; much greater in alkaline solutions). We have accordingly examined solutions of ascorbic acid and of its sodium salt in water, and in addition we have investigated the dispersion in non-ionising solvents in the hope that some correlation might be found between the character of the dispersion and the marked difference in chemical reactivity exhibited by ascorbic acid in water and in non-aqueous solvents.

Dilute solutions of ascorbic acid in water and in methyl alcohol are characterised by intense absorption bands at λ 260—265 mμ. The absorption does not follow Beer's law, and it was doubtful, therefore, whether the much stronger solutions used for polarimetric work would show absorption bands at this wave-length, and until this point was settled any correlation between the observed absorption bands and those calculated from the rotational data might well be fallacious. We accordingly undertook measurements of the absorption shown by very thin layers of concentrated solutions and found that under these conditions the head of the absorption band moved to λ 240—245 mμ. It was already known that the band in acidified aqueous or methyl-alcoholic solutions of ascorbic acid was at λ 245 mμ and we have now found that it occurs at the same wave-length for acidified concentrated solutions. In dioxan also (c, 3·5) it is found at λ 245 mμ. Dilute or concentrated solutions of the sodium salt of ascorbic acid have the band at λ 265 mμ. It is of interest that, to a first approximation, the molecular extinction coefficients of the dilute and concentrated solutions are of the same order of magnitude, and it follows that the marked deviations from Beer's law which are encountered in aqueous and methyl-alcoholic solutions of ascorbic acid are at any rate partly attributable to movement of the position of the band as the concentration of the solution is altered.


(I.)

If the observed absorption bands contribute actively to the optical rotatory power of solutions of ascorbic acid, it is to be expected that the equation expressing the rotatory dispersion in regions of transparency would contain a term of the type $k_2/(\lambda^2 - \lambda_2^2)$, in which λ_2^2 would have the value 0·06 approx. In addition, a term covering the contributions derived from bands in the Schumann region (λ_1^2, approx. 0·02) is likely to be

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* We had intended to discuss the dispersion of ascorbic acid and of its derivatives in the light of results we have obtained with derivatives of the γ- and δ-lactones of monocarboxylic acids of the sugar group. In view, however, of Lowry and Pearman's paper (published, this vol., p. 1444, while this was being printed) on the dispersion of ascorbic acid, we have decided to present the results obtained with the free acid now, and to discuss the rotation of derivatives in a later paper.

[*Added, December 4th.*] Comparison of our results with those of Lowry and Pearman shows that our observations have been carried further into the ultra-violet region. The equation for the rotatory dispersion of ascorbic acid developed by these authors does not fit our extended data. A point of special interest is that the dispersion of sodium ascorbate is apparently simple both in neutral solution (our observations) and in alkaline solution (Lowry and Pearman) but with the value of λ_0^2 greater for the alkaline solution. We find that, as the alkalinity of the solution increases, λ_0^2 becomes larger and the observed absorption band moves towards the red. Simultaneously the numerical value of the specific rotation increases (see this vol., p. 1279) and since all these changes depend on the p_H of the solution and not primarily on the number of equivalents of alkali present, they are presumably related to the reversible formation of the disodium salt of ascorbic acid.

present, and the total rotation would be expressible by a two-term Drude equation of the type (1).

$$\alpha_\lambda = k_1/(\lambda^2 - \lambda_1^2) + k_2/(\lambda^2 - \lambda_2^2) \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In certain circumstances it may happen that the partial rotations derived from the high-frequency bands may cancel one another, leaving only the contribution of the low-frequency term. One such case has recently been examined (Hudson, Wolfrom, and Lowry, this vol., p. 1179), and it will be seen below that the simple rotatory dispersion shown by aqueous solutions of the sodium salt of ascorbic acid conforms to this type.

On the other hand, the rotatory dispersion of ascorbic acid in aqueous solution is highly anomalous, and analysis of the observations shows that the rotation may be accurately represented by equation (2), which contains a negative low-frequency term, corresponding to a band at λ 2480, and a positive high-frequency term, the corresponding

$$\alpha_\lambda = 2{\cdot}518/(\lambda^2 - 0{\cdot}0195) - 1{\cdot}390/(\lambda^2 - 0{\cdot}063) \quad . \quad . \quad . \quad . \quad (2)$$

band being at λ 1400. The wave-length of the low-frequency band is in good agreement with the observed value (λ 2450) and it seems reasonable to suppose that the low-frequency term in (2) is definitely associated with the observed absorption band.

In view of this result it was surprising to find that the dispersions shown by ascorbic acid in methyl alcohol and in dioxan were apparently simple in character, and could be represented by positive single-term equations with λ_0^2 0·012 and 0·025 respectively. If the dispersion is genuinely simple in these solvents, it follows that the rotational activity of the band at λ 2450 is completely suppressed in the non-ionising media. It is necessary to remember, however, that the observed simple character of the dispersions in these solvents may be apparent rather than real, in that a two-term Drude equation with suitable constants may simulate simplicity over a considerable range of the spectrum. In the present instances we have been able to analyse the rotational data for the methyl alcohol and the dioxan solutions, and have derived two-term equations containing high- and low-frequency terms of opposite sign, the latter being negative and containing a constant ($\lambda_2^2 = 0{\cdot}06$) which is almost identical with the corresponding constant in equation (2). These equations which are analogous to (2) fit the data equally as well as the simple equation (see Experimental), and from this point of view it is not possible to choose between the single and the two-term equations. The value of λ_1^2 in the high-frequency term corresponds with a band at λ 2000, and it might be objected that the observed absorption of ascorbic acid gives little indication of a band in this region. This objection, however, is not necessarily valid, inasmuch as we have found several instances amongst lactones of the hexonic acids in which the rotatory dispersion is anomalous and the rotation contains a low-frequency term corresponding to a band at λ 2600 which, if present at all, is so weak as to be incapable of detection by the usual experimental procedure. The real character of the dispersion in methyl alcohol and in dioxan is therefore not yet decided. Further experiments are in progress in this connection and the results will be discussed when the details of the work on the acid lactones are communicated.

Further important problems are raised by the character of the dispersion shown by the sodium salt of ascorbic acid in aqueous solution. Here the dispersion is simple and may be represented by equation (3), in which λ_0^2 corresponds with a band at λ 2450.

$$\alpha_\lambda = 1{\cdot}916/(\lambda^2 - 0{\cdot}060) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

The observed band is situated at λ 2650. The difference of 200 Å. between the observed and the calculated position of the band is not unusual and is probably accounted for by the incomplete cancelling out of the partial rotations of the bands of higher frequency. The point of particular interest is, however, the fact that the low-frequency term which controls the rotation of the sodium salt is positive in sign, whereas the term of precisely similar frequency exhibited by ascorbic acid in water is negative. In the latter case the observed rotation is almost entirely that of un-ionised ascorbic acid, but with the salt the rotation is that of the ion of ascorbic acid. Whilst it is possible that we have to deal here with an altogether exceptional case in which the charge on the ion reverses the sign of the circular dichroism without altering the intensity of the observed absorption band, we feel that the alteration in the nature of the dispersion is of such a profound character as to warrant the consideration of intramolecular change during ionisation as a possible explanation. It is well known that the ascorbic acid molecule can be formulated in a variety of tautomeric modifications, and the situation here may be analogous to that encountered amongst certain indicators in which the salts and the un-ionised molecules are structurally different.

If isomerisation of this kind occurs with ascorbic acid, it must be instantaneously reversible, since after the examination of the sodium salt we acidified the solution and immediately re-examined the dispersion; the measurements then obtained were expressible exactly by equation (2) (multiplied by a factor to allow for the different concentration), and it follows that the dispersion of ascorbic acid in the presence of an excess of hydrogen ion is identical with that shown by concentrated solutions of the acid itself.

EXPERIMENTAL.

The *l*-ascorbic acid used in these experiments was a sample prepared from paprica by Prof. Szent-Györgyi of the University of Szeged. It was specially purified for the polarimetric work by successive crystallisations from a mixture of acetone, methyl alcohol, and light petroleum, m. p. 190° (decomp.). Its absorption spectra in dilute (*c*, 0·002) aqueous (acidic and alkaline) and in methyl-alcoholic solution were identical with those previously recorded (*loc. cit.*). For more concentrated solutions the following new observations were recorded. In aqueous solution (*c*, 1·0; *l*, 0·02 mm.) the head of the band was at λ 240—245 mμ; in acidified aqueous solution (*c*, 1·0) it was at λ 240—245 mμ. Neutral solutions of the sodium salt (*c*, 1·0) had a band at λ 265 mμ and methyl-alcoholic solutions of ascorbic acid (*c*, 1·0) a band at λ 245 mμ. The absorptions were measured with a Hilger Spekker photometer using specially constructed quartz cells of length approx. 0·02 mm. The polarimetric observations were made by the methods detailed in Part I (J., 1932, 2112).

l-*Ascorbic Acid in Water* [equation (2)].

$c = 2{\cdot}0967$ g. in 15 c.c.; $l = 1$ dm.; $t = 20°$; $[a^{20°}]_{\lambda} = 7{\cdot}16a_{\lambda}$.

λ.	$a_{obs.}$	$a_{calc.}$	Diff.	λ.	$a_{obs.}$	$a_{calc.}$	Diff.	λ.	$a_{obs.}$	$a_{calc.}$	Diff.
6708	+2·27°	+2·26°	+0·01°	4400	+3·82°	+3·82°	±0°	3815	+3·15°	+3·15°	±0°
6294	2·55	2·52	+0·03	4300	3·80	3·82	−0·02	3780	2·95	3·01	−0·06
6104	2·65	2·64	+0·01	4135	3·75	3·75	±0	3720	2·75	2·74	+0·01
5893	2·79	2·79	±0	4065	3·70	3·68	+0·02	3600	1·95	2·00	−0·05
5805	2·85	2·86	−0·01	4012	3·58	3·61	−0·03	3445	+0·45	0·43	+0·02
5515	3·10	3·08	+0·02	3966	3·55	3·53	+0·02	3415	0·00	0·00	±0
5225	3·35	3·31	+0·04	3945	3·50	3·49	+0·01	3353	−1·05	−1·02	−0·03
4887	3·58	3·58	±0	3935	3·45	3·47	−0·02	3310	−1·80	−1·89	+0·09
4755	3·70	3·66	+0·04	3892	3·35	3·37	−0·02	3280	−2·55	−2·59	+0·04
4500	3·80	3·80	±0	3840	3·25	3·22	+0·03				

In aqueous solution the rotatory dispersion was anomalous. The calculated maximum value of the rotation was 3·82° at λ 4345 (obs., 3·82° at λ 4300—4400). The point of inflexion occurred at λ 5400 (calc., λ 5400) and the crossing of the axis was at λ 3415 (calc., λ 3415). The value of $[\alpha]^{20°}_{D}$ for $c = 13{\cdot}97$ (+ 20·0°) was very little removed from that (+ 23°) previously reported for much lower concentrations ($c = 1{\cdot}0$—$3{\cdot}0$).

Sodium Salt of l-*Ascorbic Acid in Water.*—0·8717 G. of ascorbic acid was dissolved in a little oxygen-free distilled water. After addition of the calculated volume of *N*-sodium hydroxide, the volume was made up to 15 c.c. with distilled water. The concentration of sodium salt was therefore 6·54.

$l = 1$; $[a]^{20°}_{\lambda} = {}_{-}15{\cdot}28\, a_{\lambda}$; $a_{\lambda} = 1{\cdot}916/(\lambda^2 - 0{\cdot}060)$; $[a]^{20°}_{D}$ +102°.

λ.	$a_{obs.}$	$a_{calc.}$	Diff.	λ.	$a_{obs.}$	$a_{calc.}$	Diff.	λ.	$a_{obs.}$	$a_{calc.}$	Diff.
6708	+4·92°	4·91°	+0·01°	5225	+ 9·02°	9·00°	+0·02°	4390	+14·45°	14·44°	+0·01°
6294	5·72	5·70	+0·02	4887	10·71	10·73	−0·02	4286	15·45	15·49	−0·04
6104	6·14	6·13	+0·01	4770	11·45	11·44	+0·01	4160	16·95	16·95	±0
5893	6·67	6·67	±0	4625	12·45	12·45	±0	4045	18·45	18·45	±0
5805	6·93	6·92	+0·01	4500	13·45	13·45	±0	3990	19·20	19·21	−0·01
5515	7·87	7·85	+0·02								

Within this range of wave-lengths the dispersion is simple, the corresponding value of λ_0 being λ 2450.

The above solution was acidified by addition of an excess of sulphuric acid and the dispersion measured again. The solution now had greater transparency and resembled the aqueous solution of ascorbic acid described above. Polarimetric measurements from λ 6708 to λ **3440** were made, and it was found that the results could be accurately expressed by the formula $\alpha_\lambda = 0{\cdot}31\alpha_\lambda'$, where α_λ' is the value for the rotation of ascorbic acid in water given in the preceding section.

l-*Ascorbic Acid in Methyl Alcohol.*

$c = 5{\cdot}9304$; $t = 20°$; $l = 1$ dm.; $[\alpha]_D^{20°} = +50{\cdot}8°$; $[\alpha]_\lambda^{20°} = 16{\cdot}85\alpha_\lambda$.

$\alpha_\lambda = 1{\cdot}012/(\lambda^2 - 0{\cdot}012)$ (A); $\alpha_\lambda = 1{\cdot}796/(\lambda^2 - 0{\cdot}04) - 0{\cdot}816/(\lambda^2 - 0{\cdot}06)$ (B).

λ.	$\alpha_{\text{obs.}}$	$\alpha_{\text{calc.}}$ (A).	Diff.	$\alpha_{\text{calc.}}$ (B).	Diff.	λ.	$\alpha_{\text{obs.}}$	$\alpha_{\text{calc.}}$ (A).	Diff.	$\alpha_{\text{calc.}}$ (B).	Diff.
6708	+2·32°	2·31°	+0·01°	2·29°	+0·03°	4529	+5·25°	5·24°	+0·01°	5·25°	±0°
6294	2·63	2·64	−0·01	2·62	+0·01	4447	5·45	5·45	±0	5·46	−0·01
6104	2·79	3·81	−0·02	2·80	−0·01	4367	5·65	5·66	−0·01	5·68	−0·03
5893	3·02	3·02	±0	3·00	+0·02	4307	5·85	5·83	+0·02	5·85	±0
5805	3·13	3·12	+0·01	3·10	+0·03	4227	6·05	6·07	−0·02	6·08	−0·03
5515	3·48	3·46	+0·02	3·45	+0·03	4172	6·25	6·24	+0·01	6·24	+0·01
5225	3·89	3·88	+0·01	3·88	+0·01	4107	6·45	6·46	−0·01	6·45	±0
4895	4·45	4·45	±0	4·45	±0	4049	6·65	6·66	−0·01	6·64	+0·01
4785	4·65	4·66	−0·01	4·68	−0·03	3969	6·95	6·95	±0	6·92	+0·03
4706	4·85	4·83	+0·02	4·85	±0	3900	7·20	7·22	−0·02	7·19	+0·01
4602	5·05	5·07	−0·02	5·08	−0·03	3830	7·45	7·51	−0·06	7·46	−0·01

l-*Ascorbic Acid in Dioxan.*

$c = 3{\cdot}536$; $t = 20°$; $l = 1$ dm.; $[\alpha]_D^{20°} = +54{\cdot}3°$; $[\alpha]_\lambda^{20°} = +28{\cdot}3\alpha_\lambda$.

$\alpha_\lambda = 0{\cdot}62/(\lambda^2 - 0{\cdot}025)$ (A). $\alpha_\lambda = 0{\cdot}871/(\lambda^2 - 0{\cdot}04) - 0{\cdot}265/(\lambda^2 - 0{\cdot}06)$ (B).

λ.	$\alpha_{\text{obs.}}$	$\alpha_{\text{calc.}}$ (A).	Diff.	$\alpha_{\text{calc.}}$ (B).	Diff.	λ.	$\alpha_{\text{obs.}}$	$\alpha_{\text{calc.}}$ (A).	Diff.	$\alpha_{\text{calc.}}$ (B).	Diff.
6708	+1·45°	1·46°	−0·01°	1·44°	+0·01°	4652	+3·25°	+3·24°	+0·01°	+3·24°	+0·01°
6294	1·68	1·67	+0·01	1·67	+0·01	4415	3·65	3·65	±0	3·66	−0·01
6104	1·78	1·78	±0	1·78	±0	4230	4·05	4·03	+0·02	4·05	±0
5893	1·92	1·92	±0	1·91	+0·01	4047	4·45	4·47	−0·02	4·48	−0·03
5805	2·00	1·99	+0·01	1·99	+0·01	3877	4·95	4·95	±0	4·96	−0·01
5515	2·23	2·22	+0·01	2·21	+0·02	3729	5·45	5·44	+0·01	5·43	+0·02
5225	2·51	2·50	+0·01	2·49	+0·02	3597	5·95	5·94	+0·01	5·95	±0
4887	2·90	2·90	±0	2·91	−0·01	3477	6·45	6·47	−0·02	6·42	+0·03
4935	2·83	2·84	−0·01	2·84	−0·01	3374	6·95	6·98	−0·03	6·87	+0·08

Solutions of ascorbic acid in dioxan (*c*, 3·536) show an intense single absorption band with head at λ 2450 approx., the shape of the band being exactly similar to that of the corresponding band in methyl-alcoholic solution.

The authors are indebted to Professor Szent-Györgyi for the supply of ascorbic acid.

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[*Received, November 9th,* 1933.]