[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Raman Spectra of *l*-Ascorbic Acid, Tetronic Acid and Related Compounds[1]

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Studies of the effects of ionization on the Raman spectra of carboxylic acids[2] and amines[3a,b] have revealed highly characteristic changes in spectra resulting from the addition or removal of an acidic proton. In this communication we report similar studies on a series of compounds containing acidic hydroxyl groups. These include *l*-ascorbic acid, *d*-glucoascorbic acid, isoascorbic acid, tetronic acid, α-nitrotetronic acid and phenol. All were studied in aqueous solution, both as undissociated acids and in the form of their sodium salts. In the case of the salts, the observed spectra arise from the anions (conjugate bases) of the acids. The sodium ion makes no contribution to the spectra, since it is well known that only structures bound by covalent linkages give rise to Raman lines.[4] All these compounds give very intense Raman spectra, and the three ascorbic acids studied show a characteristic shift of one intense spectral line on ionization. Apart from the effects of ionization, the data furnish a well defined set of physical constants, which may be used in the characterization of these important substances.

Experimental Methods

The Raman spectra were excited by the blue and violet lines of the mercury arc. The apparatus was closely similar to that employed by Wood,[5] and has been described in previous publications from this Laboratory.[2,3] Wherever possible, it is desirable to obtain for each substance at least one spectrum excited by the mercury *e* line (4358 Å.; 22938 cm.$^{-1}$), and one excited by the *k* line (4047 Å.; 24705 cm.$^{-1}$). Comparison of the two spectra renders the results more accurate than those obtained from either one alone.[6]

To isolate the *k* line, a filter was employed consisting of a dilute solution of sodium nitrite, to remove the ultraviolet, and Corning red purple ultra glass (2 mm. thick) to remove radiation of wave length longer than 4100 Å. To isolate the *e* line, an alcoholic solution containing about two per cent. of *p*-nitrotoluene and a very small amount of Rhodamine 5 GDN Extra was employed.[7] The concentration of rhodamine was generally near 0.02 g./l. of solvent, but in order to eliminate continuous background a concentration as high as 0.05 g./l. sometimes was employed. The filter solution was contained in a Pyrex tube 30 mm. in diameter; this acted as a condensing lens to focus the light of the mercury arc on the Raman tube.

The spectra were photographed on Eastman Kodak Co. Spectroscopic plates, type I-O and type III-O, using a Hilger E-439 glass spectrograph.

Materials

***l*-Ascorbic acid** was obtained from Hoffman–LaRoche. Several different samples obtained from this source gave correct equivalent weights by iodine titration[8]; m. p. 190–192° (dec.) (values reported in the literature 190–192° (dec.)).

The free acid was studied in aqueous solution, at concentrations ranging from 1 to 1.4 *m*. Sodium *l*-ascorbate solution was prepared by adding sodium hydroxide in solution (0.95 to 0.98 equivalent) to a solution of ascorbic acid. The *p*H of such a solution lies near 6. Complete neutralization of the acid with sodium hydroxide was not desired, since if the *p*H is alkaline to 7, oxidation of the ascorbic acid by molecular oxygen takes place at a fairly rapid rate.[9] The very small amount of undissociated ascorbic acid present at *p*H 6 made no observable contribution to the observed spectrum, since even the most intense line in its spectrum, near 1700 cm.$^{-1}$, could not be observed in these solutions.

To obtain optically clear solutions, suitable for spectroscopic study, it was necessary to filter repeatedly through a fine grain filter paper, after previous adsorption of impurities with norit, directly into the Raman tube, which was then tightly stoppered and exposed to the filtered light of the mercury arc. The same procedure was adopted with all the substances studied in this investigation. Fox and Levy,[10] however, have reported that the oxidation of ascorbic acid to dehydroascorbic acid is catalyzed by norit. If appreciable oxidation had occurred in our studies, it would naturally have completely altered the interpretation of the results. Iodine titrations of the solution removed from the Raman tube after the spectrum had been photographed, however, showed no detectable

(1) A preliminary account of some of the studies on *l*-ascorbic acid was given by one of us in 1936 (*Proc. Am. Soc. Biol. Chem.*; see *J. Biol. Chem.*, **114**, Proc. XXVIII (1936)). All subsequent work has confirmed the findings, there very briefly reported; but publication of the data has been withheld until a group of related compounds could be studied.

(2) J. T. Edsall, *J. Chem. Phys.*, **4**, 1 (1936); **5**, 508 (1937).

(3) (a) J. T. Edsall, *ibid.*, **5**, 225 (1937); (b) J. T. Edsall and H. Scheinberg, *ibid.*, **8**, 520 (1940).

(4) K. W. F. Kohlrausch, "Der Smekal-Raman Effekt," Berlin, Julius Springer, 1931; Ergänzungsband, Berlin, 1937, p. 111.

(5) R. W. Wood, "Physical Optics," third edition, The Macmillan Co., New York, N. Y., 1934, Chapter XIV.

(6) In the case of α-nitrotetronic acid and its sodium salt, satisfactory results could not be obtained using the *k* line as the exciting line, since the initially colorless solution soon became yellow when exposed to this radiation. Sodium phenolate also could not be studied with the mercury *k* line, because of traces of fluorescent impurities in the solution. No such difficulties were encountered with the other substances studied.

(7) J. T. Edsall and E. B. Wilson, Jr., *J. Chem. Phys.*, **6**, 124 (1938). The rhodamine dye was supplied by E. I. du Pont de Nemours and Co.

(8) We are indebted to Dr. F. W. Klemperer for these titrations.

(9) E. S. G. Barron, R. H. de Meio and F. Klemperer, *J. Biol. Chem.*, **112**, 625 (1935–36).

(10) F. W. Fox and L. F. Levy, *Biochem. J.*, **30**, 208 (1936).

TABLE I

RAMAN SPECTRA OF ASCORBIC ACIDS AND THEIR SODIUM SALTS IN AQUEOUS SOLUTION

Frequency shifts of Raman lines are expressed in wave numbers. Numbers in parentheses give rough estimates of relative intensities; b denotes a broad line; vb a very broad line.

l-Ascorbic acid	Sodium *l*-ascorbate	*d*-Glucoascorbic acid[b]	Sodium *d*-Glucoascorbate[a]	Isoascorbic acid[c]	Sodium isoascorbate
237 (1/2)					
318 (1/2)	318 (1)		312 (1/2)	309 (1b)	330 (2b)
588 (2)	603 (2)	597 (1b)	609 (3)	574 (4b)	507 (1/2)
636 (1)	643 (1)			638 (1/2)	601 (5)
				653 (1/2)	666 (2)
668 (1)	672 (1)	668 (1b)	665 (1/2)	688 (1/2)	716 (1)
706 (1)	710 (1)		709 (1/2)	756 (0?)	
828 (4)	835 (4)	834 (4)	841 (4b)	822 (3b)	825 (4)
884 (1/2)	878 (1/2)			915 (1/2)	
946 (1/2)	935 (1)	907 (1b)	907 (1)	942 (1/2)	943 (1)
1061 (2)	1054 (2b)	1053 (1)	1038 (1b)	1055 (1b)	1055 (2)
1153 (2)	1147 (2b)	1146 (3b)	1133 (1)	1155 (2)	1161 (1)
1225 (1)	1260 (1)	1230 (2)	1290 (1vb)	1228 (1)	
1300 (2)	1301 (2)	1302 (5)		1302 (2)	1273 ± 30 (1vb)
1361 (1)		1373 (1/2)	1422 (1vb)	1357 (1)	1422 ± 30 (1vb)
1477 (1/2)	1454 (1vb)	1474 (0)		1472 (0)	
1699 (15vb)	1596 (15vb)	1703 (15b)	1592 (12b)	1699 (20b)	1595 (20b)
1765 (1/2)	1726 (2)		1723 (2)	1767 (1/2)	1727 (2)
2956 (3b)	2964 (2)	2970 (3)	2950 (2)	2958 (3b)	

[a] Very weak and questionable frequencies at 427 and 517 were observed for sodium *d*-glucoascorbate. [b] Spectrum incomplete. [c] A faint line at 1110 was also observed in isoascorbic acid.

decrease in the amount of ascorbic acid present, resulting from treatment with norit, filtration, and exposure to light. Our solutions were very concentrated, while those of Fox and Levy were very dilute. Therefore, while a little oxidation may have occurred in our experiments, we conclude that it must have involved only a very small fraction of the total ascorbic acid present.

In other experiments, this point was tested further by adding potassium cyanide (0.005 *m*) to the solution before treatment with norit. Cyanide ion (even at 0.001 *m*) has been shown[9] to inhibit completely the "autoxidation" of ascorbic acid at *p*H values acid to 7, by combining with Cu^{++} ion, which is the effective catalyst for this oxidation. The spectra obtained in the presence of cyanide were identical with those obtained without it[11]; from this and the other findings reported above, we conclude that no appreciable amount of oxidation had occurred in any of our experiments. This might not have been true at *p*H values alkaline to 7, at which ascorbic acid (or rather ascorbate ion) is truly autoxidizable[9]; but since the pK value[12] of the acid is 4.12, conversion of the acid to the ion is more than 99% complete at *p*H 6.2, and it was possible to study both the acid and its conjugate base in solutions sufficiently acid to insure stability.

d-Glucoascorbic acid and isoascorbic acid (*d*-araboascorbic acid) were obtained from Eastman Kodak Co They gave correct equivalent weights on titration and were used without further purification.

(11) Cyanide ion gives only one Raman line, of a frequency widely different from any of those characteristic of ascorbic acid. The cyanide in our experiments was so dilute that even this line could not be observed.

(12) W. D. Kumler and T. C. Daniels, THIS JOURNAL, **57**, 1929 (1935).

TABLE II

RAMAN SPECTRA OF TETRONIC ACID, α-NITROTETRONIC ACID AND THEIR SODIUM SALTS

All these spectra, except the last, are of aqueous solutions.

Tetronic Acid	Sodium tetronate[a]	α-Nitro-tetronic acid in H_2O[b]	Sodium α-nitro-tetronate[b]	α-Nitro-tetronic acid in CH_3OH[b]
		236 (2)	236 (2)	236 (2)
		364 (3)	368 (2b)	366 (3)
		440 (1/2)	438 (4)	433 (1)
		490 (2)	493 (3)	485 (2)
550 (0)	562 (1)	564 (0)	566 (0)	
610 (1/2)	629 (2)	619 (2)	623 (2)	619 (1)
			660 (1/2)	647 (1)
708 (4)	708 (5)	710 (1)	716 (1)	715 (1)
846 (2)	845 (2)	822 (10)	823 (10)	820 (10)
930 (1/2)	909 (5)			[1037 (9)]
1040 (1)	1037 (1)	1078 (10)	1082 (10)	1070 (9)
1168 (7b)	1172 (7)			
1229 (1/2)	1264 (0?)	1257 (2)	1263 (2)	1259 (2)
1351 (5)	1360 (7)	1312 (8b)	1316 (9)	1310 (10b)
		1346 (3)	1346 (4)	
1452 (3b)	1454 (1b)	1401 (8b)	1402 (10)	1404 (10b)
		1440 (3)	1442 (4)	[1468 (8b)]
1584 (10vb)	1581 (10vb)	1567 (1)	1573 (1)	1572 (1)
1679 (1)	1684 (4)	1655 (6b)	1660 (4b)	1664 (7)
1727 (2)		1734 (4)	1739 (2)	1742 (4)
2950 (5)	2942 (4)			[2836] [2945]

[a] Very weak lines were also observed at 753 and 790 in sodium tetronate solution. [b] The line near 1260 in nitrotetronic acid and its sodium salt may correspond to the intense line near 1320, excited by Hg 22995 cm.$^{-1}$. Likewise there is a line of rather low intensity in these spectra, which we have assumed to represent the frequency shift $\Delta\nu$ 822 cm.$^{-1}$, excited by Hg 22995, but may represent $\Delta\nu$ 764 cm.$^{-1}$, excited by Hg 22938. Lines enclosed in square brackets in the last column arise from CH_3OH.

TABLE III

RAMAN SPECTRA OF PHENOL AND SODIUM PHENOLATE IN WATER

Phenol (6.5%)	Sodium phenolate (25%)
.....	461 (0)
532 (1)	549 (1)
620 (1/2)	618 (1)
770 (1/2)	773 (1)
816 (5)	826 (6)
861 (1/2)	896 (1/2)
946 (1/2)	939 (1/2)
1002 (7)	998 (8)
1026 (3)	1026 (5)
1069 (1/2)	1072 (1/2)
1163 (3b)	1162 (5)
1253 (3b)	1280 (5)
1600 (6b)	1592 (8)
3074 (10b)	3039 (4b)[a]

[a] This value is based on only one measurement, under adverse conditions, and is probably about 30 cm.$^{-1}$ too low.

TABLE IV

RAMAN SPECTRA OF *l*-ASCORBIC ACID IN CH_3OH AND IN D_2O

(a) 5% Ascorbic Acid in Absolute CH_3OH[a]

$\Delta\nu$	$\Delta\nu$	$\Delta\nu$
589 (1/2)	942 (1/2)	1380 (1)
632 (1/2)	[1037 (10b)]	[1472 (10)]
703 (1/2)	[1120 (2b)]	1703 (5)
827 (1)	[1154 (1)]	[2830 (10b)]
877 (1/2)	1287 (1)	[2930 (10b)]

(b) 20% Ascorbic Acid in 99.65% D_2O[b]

$\Delta\nu$	$\Delta\nu$	$\Delta\nu$
312 (0b)	1060 (0)	1406 (2)
583 (2)	1116 (3)	1473 (1)
633 (3)	1197 (1)	1695 (20b)
668 (1vb)	1235 (1/2)	1768 (2)
824 (6)	1300 (5)	2952 (7)
862 (1)	1355 (3)	

[a] Frequencies in brackets arise chiefly or entirely from the solvent. The ascorbic acid lines found show no significant difference from their values in water. [b] The D_2O bands near 2500 were also of course clearly visible. The above spectrum is nearly identical with that found in water (see Table I), except for the frequencies at 1116 and 1406. The line at 1197 may arise from D_2O. The line at 1153, present in H_2O solution, is not found in D_2O. The line at 706 in H_2O may also be present in D_2O, but is very faint.

A sample of **tetronic acid** was supplied by Professor W. D. Kumler. It was a portion of the same preparation employed by him[13] in determining the dissociation constant; m. p. 141° (literature 141°); equivalent weight found, 100; calculated, 100.

α-Nitrotetronic acid was also supplied to us by Professor Kumler. The material was from the preparation reported in his recent paper on the dissociation constant and the dipole moment of this substance.[14]

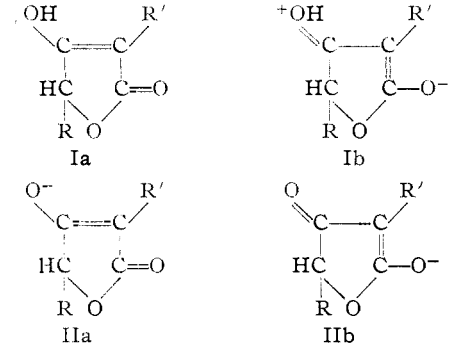
Phenol was Baker c. p. material, m. p. 40.5°, used without further purification.

Experimental Results

The observed spectra are listed in Tables I to IV inclusive. The values reported here for each substance are averages of measurements on several different spectra.

Discussion

All the substances studied, except phenol, are described, in the acid form, by formula Ia, with some contribution from the resonating structure Ib.



The conjugate bases (anions) resonate between the structures IIa and IIb, which probably do not differ very much in energy, so that the resonance energy for the anions should be large. In the undissociated acids, however, the form Ib is a dipolar ion, and its internal energy must be considerably larger than that of Ia. Hence the actual state of the undissociated acids should approximate much more closely to formula Ia than to Ib. Thus resonance energy is much greater for the anion than for its conjugate acid, and will tend to stabilize the former relatively to the latter. This largely explains the high dissociation constants of these substances, which are so much more strongly acidic than most enols.[13]

The simplest substance of this group is tetronic acid, for which R and R′ in formulas Ia, Ib, IIa and IIb are both H atoms. For α-nitrotetronic acid, R′ is a nitro group. For the three ascorbic acids, R′ = OH and R = $(CHOH)_nCH_2OH$, n being 1 for ascorbic and isoascorbic acids, and 2 for glucoascorbic acid. For nitrotetronic acid, it is possible to write additional resonating structures, involving resonance between the nitro group and the adjoining ring.[15] The recent work of Kumler,[14] on the dissociation constant and

(13) W. D. Kumler, THIS JOURNAL, **60**, 859 (1938).

(14) W. D. Kumler, *ibid.*, **64**, 1948 (1942).

(15) Compare the discussion of nitrophenols and related compounds by L. Pauling, "The Nature of the Chemical Bond," Second edition, Cornell University Press, Ithaca, 1940, p. 205 ff.

dipole moment of this substance, suggests that a strong hydrogen bond may exist between the acidic hydrogen and the adjoining oxygen of the nitro group. The same study also indicates that the normal form of nitrotetronic acid is the enol structure (Ia), and not the isomeric isonitro form.

In what follows we shall not attempt to assign the observed frequencies to modes of molecular vibration, but shall merely point out some empirical correlations between Raman spectrum and structure. First we may note the high intensity of the spectra. An excellent spectrum of any of these substances, in molar aqueous solution, may be obtained in about three hours with our apparatus; while aliphatic compounds, such as amino acids, of similar molecular weight and at the same molar concentration in water, require at least three or four times as long an exposure to give a comparable spectrum.[16] This is not surprising, for ascorbic acid shows an intense ultraviolet absorption band with a maximum near 2600 Å., and appreciable absorption even at 3500 Å. and beyond.[17] The intensity of scattered radiation is inversely proportional to $(\nu_e^2 - \nu^2)^2$, where ν is the frequency of the incident radiation and ν_e is the frequency of the nearest neighboring electronic absorption band.[18] It is obvious that for the ascorbic acids this term is much smaller than the corresponding term for simple aliphatic molecules, with absorption bands near 2200 Å. or below; and this factor in the expression for the intensity is, therefore, correspondingly larger. So far as we are aware, no ultraviolet absorption measurements are available for tetronic acid or α-nitrotetronic acid. The intensity of their spectra, however,—especially the extremely high intensity of the spectrum of nitrotetronic acid—indicates that they also show strong absorption in the near ultraviolet.

The strong ultraviolet absorption of the ascorbic acids clearly arises from the ring structure, not from the aliphatic side chains. Correspondingly the Raman lines arising from this structure are probably more intense than those arising from vibrations of the side chains. It is notable that the three ascorbic acids studied possess almost identical Raman spectra, suggesting that nearly all the observed lines arise from the ring framework and the attached oxygen atoms; and also, of course, from the nitro group in the case of α-nitrotetronic acid. The study of α-hydroxytetronic acid, which possesses the ring structure of the ascorbic acids, without a side chain, should throw valuable light on this problem. Unfortunately, we have not yet been able to obtain a sufficient amount of this substance for study.

All the ascorbic acids show a broad Raman line near 1700 cm.$^{-1}$, which is by far the most intense line in the entire spectrum. The intensity and position of this line are unchanged by dissolving *l*-ascorbic acid in methanol or in deuterium oxide (Table IV). When the molecule loses a proton, this line disappears from the spectrum, although a considerably weaker line appears near 1725; and a new and very intense line appears at about 1595. In its breadth and intensity, this 1595 line in the ascorbate anions corresponds exactly to the line at 1700 in the undissociated ascorbic acids. This effect of ionization on this very intense line is apparently specific for the ring structure of the ascorbic acids, which contains the $-C(OH)=C(OH)-C\overset{O}{\diagup\!\!\diagdown}$ grouping. In tetronic acid, with the $-C(OH)=CH-C\overset{O}{\diagup\!\!\diagdown}$ grouping, the effect of ionization is entirely different and much less pronounced. Tetronic acid shows a line of moderate intensity at 1727, which is not observed in the spectrum of the tetronate ion. The line at 1680, which is very weak in tetronic acid, becomes considerably more intense in the ion. The very intense line near 1580 is not at all affected by ionization. Other differences between the spectra of tetronic acid and the tetronate ion are so small that they can scarcely be taken as significant.

Williams and Rogers[19] have studied the infrared spectrum of *l*-ascorbic acid in water. They find absorption bands near 1280, 1330, 1450, 1540, 1610, 1700 and 2500 cm.$^{-1}$; and a series of bands, due to C–H and O–H vibrations, between 2850 and 3300 cm.$^{-}$.[1] The bands near 1610 and 1700 are very strong; the latter coincides with the very intense Raman line found by us, but we have never seen any line near 1610 in *undissociated* ascorbic acid. The infrared bands near 1540 and 2500 do not correspond to any Raman lines; they may represent harmonics or combination frequencies. The other infrared

(16) With a more powerful mercury arc, recently employed by us, these exposures are reduced to a third or a quarter of their previous values.

(17) H. Mohler and H. Lohr, *Helv. Chim. Acta*, **21**, 485, 1036 (1938); G. E. Carpeni, *ibid.*, **21**, 1031 (1938).

(18) See Kohlrausch, ref. 4, especially Ergänzungsband, p. 110.

(19) D. Williams and L. H. Rogers, THIS JOURNAL, **59**, 1422 (1937).

bands reported correspond fairly closely to Raman lines.

The spectrum of nitrotetronic acid and that of the nitrotetronate ion are practically identical within the limits of experimental error. This finding was a surprise to us, since all previous studies[2,3] of the ionization of acids and bases have shown marked changes in Raman spectrum on ionization. Nitrotetronic acid is by far the strongest of the acids studied by us. It was conceivable that it was mostly dissociated, even in acid solution, and that we were really observing the spectrum of the anion, even when no alkali was added to the solution. The pK value of nitrotetronic acid,[14] however, is 1.68, and at the concentration employed by us (20% by weight, in water) calculation shows that about 12% of the acid should be dissociated. This estimate is subject to some uncertainty, since the activity coefficients are unknown at this high concentration; but the percentage dissociation is almost certainly not higher than twenty. Hence the observed spectrum must be mostly that of the undissociated acid. This conclusion was further confirmed by studying nitrotetronic acid in methyl alcohol, in which its degree of dissociation must be far less than in water. No change could be found from the spectrum observed in water; hence we must conclude that no detectable difference was found between the spectrum of α-nitrotetronic acid and that of the α-nitrotetronate ion.

A study of phenol was made for comparison to test this point further. The lines found by us for phenol in aqueous solution are all identical with those already reported for pure liquid phenol.[20] Furthermore, sodium phenolate shows no significant difference from phenol in its Raman spectrum. Therefore, there appear to be some substances containing acidic hydroxyl groups in which the change of Raman spectrum on ionization is inappreciable, so far as present studies reveal.

It should be pointed out that the OH frequencies near 3400 cm.$^{-1}$, which are presumably present in nitrotetronic acid and phenol, could not be observed in our studies, since they are obscured by the OH frequencies of the solvent. Studies of these acids and their salts in a solvent containing no hydroxyl groups should reveal an OH frequency in the acid which would vanish on ionization.

(20) See for instance Landolt–Börnstein, "Tabellen," dritter Ergänzungsband, zweiter Teil, p. 1058.

Acknowledgment.—We are indebted to Professor W. D. Kumler for supplying us with pure samples of tetronic and α-nitrotetronic acids, and also for valuable discussions. Also we are indebted to Mr. Herbert Scheinberg for valuable aid in the studies on glucoascorbic and isoascorbic acids.

Summary

1. The Raman spectra of three ascorbic acids, of tetronic and α-nitrotetronic acids, and of phenol have been studied in aqueous solution. The spectra of the sodium salts of all these compounds were also determined.

2. All these substances yield very intense Raman spectra, which is to be expected from the character of their ultraviolet absorption.

3. All the undissociated ascorbic acids show a very intense Raman line near 1700 cm.$^{-1}$, which is shifted to about 1595 cm.$^{-1}$ on ionization. This change appears to be characteristic of the ring structure found in the ascorbic acids, since the effect of ionization on tetronic acid is entirely different and much smaller.

4. α-Nitrotetronic acid and phenol show no appreciable change in the observed Raman spectrum on ionization.

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