299. *The Constitution of Ascorbic Acid.**

By R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds, and F. Smith.

Investigations into oxidation systems of biological interest led Szent-Györgyi (*Biochem. J.*, 1928, **22**, 1387) to the discovery of a crystalline substance, $C_6H_8O_6$, which, widely distributed in plants and animals, possesses chemical and physiological properties of outstanding importance. On account of its acidic character, strong reducing power, and colour reactions, which recalled those given by carbohydrates, the substance was named hexuronic acid, but the present work has demonstrated that it is not in reality a member of the uronic acid class. In view of the fact that all specimens which have been examined possess strong antiscorbutic properties (Svirbely and Szent-Györgyi, *Nature*, 1932, **129**, 576, 690 ; *Biochem. J.*, 1932, **26**, 865; 1933, **27**, 279; Birch, Harris, and Ray, *Nature*, 1933, **131**, 273; Tillmanns, Hirsch, and Vaubel, *Z. Unters. Lebensm.*, 1933, **65**, 145. See also Hirst and Zilva, *Biochem. J.*, 1933, in the press) the name has been changed to ascorbic acid (Haworth and Szent-Györgyi, *Nature*, 1933, **131**, 24). Much evidence has been accumulated concerning the relationship between this substance and the antiscorbutic factor (vitamin C) (for a summary of the evidence, see Szent-Györgyi, *Nature*, 1933, **131**, 225) and the view is held by many workers that ascorbic acid is vitamin C in a pure crystalline condition. The biological problem is, however, one of great complexity and it is

* Summaries of the work now presented have already been published as follows: The General Properties of Ascorbic Acid and Oxidation to Threonic Acid (with R. J. W. Reynolds; *Nature*, 1932, **129**, 576; **130**, 888). Absorption Spectrum of Ascorbic Acid (with R. W. Herbert; *ibid.*, 1932, **129**, 205). Configuration of Ascorbic Acid and Proposal of a Lactone Formula (*J. Soc. Chem. Ind.*, 1933, **52**, 221). Investigation of Methylated Derivatives of Ascorbic Acid (with E. G. V. Percival and F. Smith; *Nature*, 1933, **131**, 617).—E. L. Hirst.

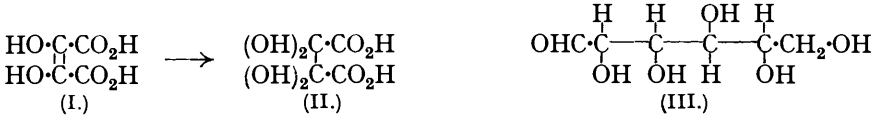
necessary to proceed cautiously before the claim that ascorbic acid is to be regarded as the only antiscorbutic agent can be considered as definitely established.

The present paper deals with the chemical constitution of ascorbic acid and describes work carried out with material derived both from animal (adrenal glands) and from plant (paprica) sources. We confirmed in the first place, using rigorously purified material, m. p. 192°, the early observations of Szent-Györgyi, who assigned the elementary formula $C_6H_8O_6$ (M.W. 176). We found that the substance behaved on titration as a weak organic acid, giving salts of the type $C_6H_7O_6M$. Ascorbic acid is not, therefore, a lactone of an acid $C_6H_{10}O_7$, as was formerly supposed, and on the other hand it cannot be lactonised. It does not exhibit mutarotation in aqueous solutions and the magnitude of the rotation, $[\alpha]_{5780}$ +24°, is not appreciably affected by the acidity of the solution. By contrast the salts display a high positive rotation (*ca.* + 100°) and the value varies greatly with the alkalinity, increasing to over 160° in 2*N*-alkali. These high rotations are not indicative of decomposition, since on acidification the value falls immediately to that of ascorbic acid.

Ascorbic acid is a powerful reducing agent. In cold neutral or acid solution it is attacked immediately by iodine, ozone, silver nitrate, copper acetate, and potassium permanganate. It reduces Fehling's solution vigorously in the cold, and in alkaline solution it is rapidly attacked by gaseous oxygen. Alkaline solutions of ascorbic acid are, however, relatively stable in an inert atmosphere and acidified solutions are only slightly affected by oxygen. The reducing properties are much less evident in non-aqueous media, iodine, for example, being entirely devoid of action upon alcoholic solutions of the acid. It does, however, react with permanganate in acetone. These properties and the ease of reaction with phenylhydrazine, which readily gives a red crystalline derivative, m. p. 187°, point to the presence of at least one carbonyl group capable of enolisation, and this conclusion is supported by the nature of the ultra-violet absorption spectrum, which resembles that given by many labile ketonic substances. The colour reactions with ferric chloride and sodium nitroprusside also indicate the presence of an enolic group, and the work of Karrer, Salomon, Schöpp, and Morf (*Vierteljahrsch., Naturforsch. Ges. Zurich*, 1933, **78**, 9; *Helv. Chim. Acta*, 1933, **16**, 181; *Biochem. Z.*, 1933, **258**, 4) lends further support to this view. Since no colour is given with Schiff's reagent, it is unlikely that a free aldehyde group is present. That five at least of the six carbon atoms are present as an unbranched chain follows from the observation that ascorbic acid yields furfuraldehyde quantitatively on treatment with boiling hydrochloric acid.

Insight into the structure of ascorbic acid was gained from a quantitative study of its behaviour towards oxidising agents. Two well-defined stages mark the course of the oxidation. When the substance is oxidised by iodine in acid solution, two atomic proportions of iodine are required, and two molecules of hydriodic acid are liberated during the reaction, which in effect consists in the addition of two hydroxyl groups to a double bond. The intervention of water is essential, alcoholic iodine being without action. The newly formed product does not display selective absorption and does not yield furfuraldehyde with hydrochloric acid. It is neutral in character and behaves towards water and towards alkalis as the lactone of a monobasic hydroxy-acid. No disintegration of the molecule takes place during this stage of the oxidation inasmuch as the product can be converted quantitatively into ascorbic acid by reducing agents such as hydrogen sulphide or hydriodic acid.

These observations show clearly that there is no free carboxyl group in ascorbic acid and that the acidic properties are due to the presence of an activated –CH·OH group situated next to a carbonyl group. The reactive group would be of the type –C(OH):C(OH)–,

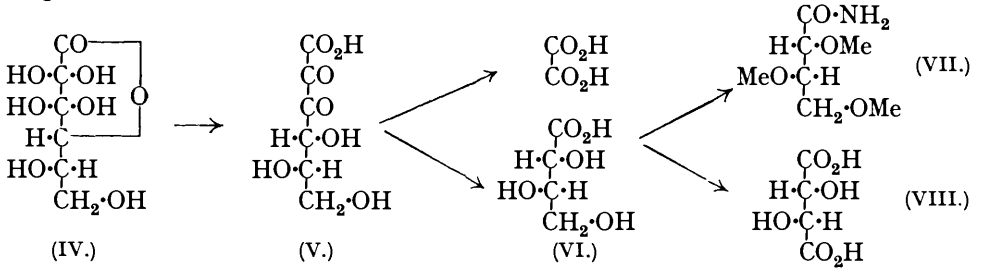


(I.) (II.) (III.)

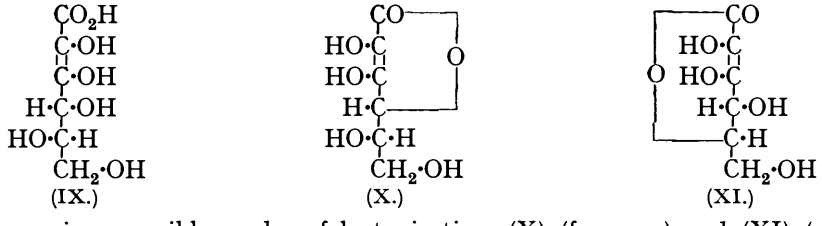
giving on oxidation in the presence of water –$C(OH)_2$·$C(OH)_2$–. Such a system is present in dihydroxymaleic acid and it is of special interest to find that dihydroxymaleic acid (I)

reacts in acid solution with iodine (2 atoms), giving dihydroxytartaric acid (II). The product can be quantitatively reduced with hydriodic acid. During the oxidation the intense absorption band of dihydroxymaleic acid, which closely resembles that of ascorbic acid, disappears and the product does not show selective absorption. This analogy may be extended in that the two enolic groups in ascorbic acid and in dihydroxymaleic acid react with diazomethane, giving methylated derivatives whose absorption spectra stand in a similar close relationship with those of the parent substances. Since we advocated this type of linking for ascorbic acid in our preliminary note (Hirst, *J. Soc. Chem. Ind., loc. cit.*) the same kind of group has also been suggested by von Euler and Martius (*Arkiv Kemi Min. Geol.*, 1933, **11**, *B*, 1) from analogies with gluco-reductone [CHO·C(OH):CH·OH], which reacts with acid iodine, is acidic in character without possessing a carboxyl group, and shows a strong absorption band in the ultra-violet region.

The first oxidation product of ascorbic acid still possesses reducing power, especially in alkaline solution, and on treatment with alkaline sodium hypoiodite it takes up one atomic proportion of oxygen and is transformed quantitatively into oxalic acid and a trihydroxybutyric acid (VI). The latter substance was recognised in the form of the crystalline amide of its trimethyl derivative, which was proved to be trimethyl *l*-threonamide (VII). The identity of the trihydroxybutyric acid as *l*-threonic acid was further established by its conversion, on oxidation with nitric acid, into *d*-tartaric acid (VIII). The same product (*l*-threonic acid) was obtained when ascorbic acid was oxidised directly with acid permanganate.



These observations demonstrate that ascorbic acid is a derivative of *l*-gulose (III) and that its first oxidation product (which, as we shall show later, has structure IV) must be capable of reacting in the form of structure (V) (2 : 3-diketo-*l*-gulonic acid). The evidence given above indicates that the primary oxidation product is a lactone of (V), the carbonyl groups being in all probability hydrated. Ascorbic acid is the reduced form of this lactone and in view of its enolic character is to be represented as a lactone of the acid (IX) (3-keto-*l*-gulonolactone).

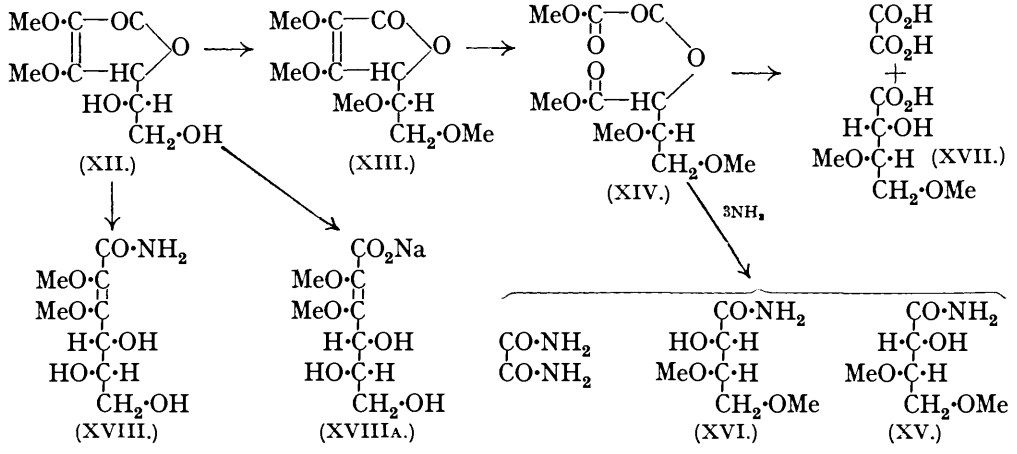


Of the various possible modes of lactonisation, (X) (furanose) and (XI) (pyranose) represent the two most probable types, but the evidence already outlined above does not permit a definite allocation of ring structure to be made, and in view of the unknown effect on ring formation of the active groups at C_2 or C_3 it is impossible to rule out at this stage the formation of a 1 : 6-lactone ring.

The nature of the ring system in ascorbic acid was determined by a study of the methylated derivatives of the acid. By this means complete confirmation was obtained of the accuracy of the views advanced above concerning the stereochemical configuration of the molecule and the nature of the reactive enolic groups. The results enabled a clear decision

to be made in favour of the furanose structure (X) for ascorbic acid, which may therefore be designated 3-keto-*l*-gulofuranolactone.

By the action of diazomethane on ascorbic acid a dimethyl derivative (XII) is readily obtained (Karrer, Salomon, Schöpp, and Morf, *loc. cit.*; Micheel and Kraft, *Z. physiol. Chem.*, **1933**, **215**, 222). We have found that both the methoxyl groups so introduced are enolic in origin. In addition there are two other hydroxyl groups which can be methylated by Purdie's reagents, giving tetramethyl ascorbic acid (XIII). This substance reacts easily with ozone, two atoms of oxygen being added with formation of a neutral product (XIV) which we identified as methyl 3 : 4-dimethyl *l*-threonate substituted in position 2 by a methyl oxalate residue. This reaction proceeds similarly to the ozonisation of di-



p-nitrobenzoyl dimethyl ascorbic acid studied by Micheel and Kraft (*loc. cit.*). On treatment with methyl-alcoholic ammonia the neutral ester (XIV) gives immediately oxamide and 3 : 4-dimethyl *l*-threonamide (XV) together with a small quantity of the epimeric 3 : 4-dimethyl erythronamide (XVI). Both amides give rise to sodium *iso*cyanate with sodium hypochlorite (Weerman reaction) and must therefore possess a hydroxyl group in the α-position to the $-CO\cdot NH_2$ group. Reference to our earlier papers on the use of this test will suffice to show that it is of general application in the aliphatic group and quite diagnostic. The criticism of Micheel and Kraft (*J. physiol. Chem.*, 1933, **218**, 280) does not apply, since the only example they have quoted to the contrary is that of mandelamide, which is not a comparable case. Hydrolysis of (XIV) with barium hydroxide gave barium oxalate and the barium salt of 3 : 4-dimethyl *l*-threonic acid, again admixed with a small quantity of 3 : 4-dimethyl *l*-erythronic acid. From the 3 : 4-dimethyl *l*-threonic acid (XVII) there was obtained by methylation methyl 2 : 3 : 4-trimethyl *l*-threonate, and this was converted into the same crystalline amide (VII) which we had previously derived from ascorbic acid as the result of oxidation, followed by methylation of the *l*-threonic acid so produced.

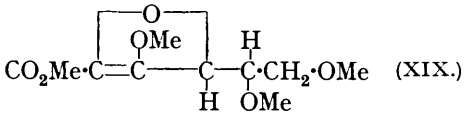
Proof of the identity of this amide (VII) was provided in the following ways. The analytical data, molecular weight, and the fact that the substance was optically active were sufficient to show that it was a 2 : 3 : 4-trimethoxy-*n*-butyramide. Four possibilities then arose, namely, the *d*- and *l*-forms of trimethyl erythronamide and the *d*- and *l*-forms of trimethyl threonamide. Now trimethyl *d*-erythronamide was already known (Avery, Haworth, and Hirst, J., 1927, 2308) and was definitely not identical with (VII). *X*-Ray examination confirmed that (VII) was different in structure from *d* (or *l*)-trimethyl erythronamide. The substance was therefore either *d*- or *l*-trimethyl threonamide, and conclusive proof that it was in fact the *l*-enantiomorph was provided by the observation that the unmethylated acid corresponding to (VII) gave *d*-tartaric acid on oxidation with nitric acid and must therefore be *l*-threonic acid. It is of interest to observe that the sign of the

rotation of (VII) is positive and that in consequence this substance follows the amide rotation rule.

The identity of the 3 : 4-dimethyl *l*-threonic acid was determined by the following considerations. On methylation it gave methyl trimethyl *l*-threonate, showing that the partly methylated derivative was 2 : 3-, 2 : 4-, or 3 : 4-dimethyl *l*-threonic acid. The first two of these were ruled out by the observation that the dimethyl ester yielded an amide which gave a positive Weerman reaction. The substance was therefore 3 : 4-dimethyl *l*-threonic acid, and this view is in agreement with the fact that methylation was accompanied by a large increase in dextrorotation.

The isomeric amide which was also isolated differed from 3 : 4-dimethyl *l*-threonamide and was not the enantiomorph of (XV). Since the analytical data, the optical activity, and positive Weerman reaction sufficed to establish its structure as 2-hydroxy-3 : 4-dimethoxy-*n*-butyramide, it followed that it must be either *d*- or *l*-3 : 4-dimethyl erythronamide. Since inversion of the groups attached to the penultimate carbon atom is most improbable, we designate it 3 : 4-dimethyl *l*-erythronamide. In agreement with this is the fact that the amide is lævorotatory in accordance with the amide rotation rule which appears to hold generally in this series.

The reaction between tetramethyl ascorbic acid (XIII) and ozone involved the addition of two oxygen atoms with formation of a neutral ester (XIV), and the breaking of the bond between the two carbon atoms which were united by a double linkage did not result in the formation of a substance containing a smaller number of carbon atoms. It follows, therefore, that a ring system was present in tetramethyl ascorbic acid and the nature of the reaction leaves open only two possibilities for the structure of (XIII), for which the alternative is the following (XIX) containing a propylene oxide ring. The latter is in-



(XIX.)

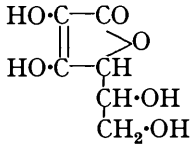
herently improbable owing to the strained nature of the ring and furthermore the properties of dimethyl ascorbic acid and the fact that the primary oxidation product of ascorbic acid is a lactone and not an acid provide decisive evidence in favour of (XIII).

Dimethyl ascorbic acid is a neutral substance which reacts with one equivalent of sodium hydroxide, giving a sodium salt. This reaction was claimed by Karrer (*loc. cit.*) and by Micheel (*loc. cit.*) as proof that one of the methoxyl groups was esteric in character. A closer examination of the reaction has revealed that the sodium salt is formed without elimination of methyl alcohol and proceeds in the cold in a manner similar to the opening of a lactone ring. The formation of a sodium salt from (XIX) would necessarily involve ester hydrolysis and this structure may therefore be discarded.

The origin of the small quantity of 3 : 4-dimethyl *l*-erythronic acid which accompanies the 3 : 4-dimethyl *l*-threonic acid is at present obscure. Its formation involves inversion of the groups attached to C_4 of the original ascorbic acid. This may have occurred during the reaction with diazomethane or during the subsequent methylation with silver oxide, or more probably during the treatment of the ozonised product with alkali, epimerisation of α-hydroxy-acids by alkali being of well-known and frequent occurrence. We have considered the possibility that the ascorbic acid used might have contained two isomerides, but have rejected it on the ground that no *i*-tartaric acid could be discovered in a rigorous search for traces of this material amongst the *d*-tartaric acid produced by direct oxidation of *l*-ascorbic acid. It follows that 3 : 4-dimethyl *l*-erythronic acid had been formed by an indirect route involving isomerisation and that *l*-ascorbic acid is configurationally related to *l*-gulose and *l*-sorbose.

Tetramethyl ascorbic acid has therefore the structure represented by (XIII) and the form of ascorbic acid which reacted with diazomethane must be (XX). It is obvious that various tautomeric modifications of this structure are possible, for example (XXI), (XXII) and (XXIII), and the versatile character of ascorbic acid suggests that it can indeed react

in more than one of these forms. Two of those illustrated (XXII) and (XXIII) (3-keto-*l*-sorbosone) should give rise to two stereochemical isomerides and, apart from the fact that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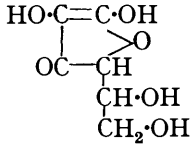
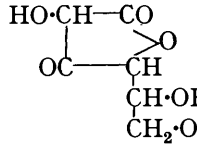
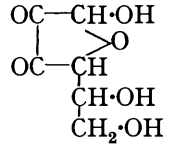
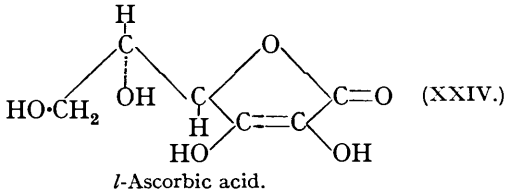

(XXI.)


(XXII.)


(XXIII.)

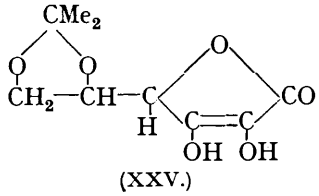
no such modifications of ascorbic acid have been observed, a structure of this type cannot be reconciled with the *X*-ray data of the crystalline material (Cox, *Nature*, 1932, **130**, 205), which demand an extraordinarily flat molecule. The very close similarity between the absorption spectra of ascorbic acid and its dimethyl derivative, taken in conjunction with the similar relationship existing in the dihydroxymaleic acid series, provides a strong indication that in solution, whether in acid, neutral or alkaline media, ascorbic acid is essentially in the condition represented by (XX) (enolic form of 3-keto-*l*-gulofurano-lactone, which, despite the views of Micheel and Kraft, *J. physiol. Chem.*, 1933, **218**, 280, is in no sense equivalent to the 2-keto-isomeride). It is surprising to find that a structure of this type should remain intact in the presence of alkali. The stability of the lactone ring in the free acid would appear to be connected in some way with the ionised condition of the hydroxyl group responsible for salt formation. If a non-ionised ether group replaces the ionised hydroxyl, the lactone ring appears to be very much less stable and opens readily in the cold under the influence of dilute alkali. A search for analogies has revealed that this behaviour is simulated by certain other substances and a detailed investigation, now in progress in these laboratories, of the very remarkable mannosaccharodilactone has indicated that a lactone containing strongly reducing groups may yield a sodium salt without opening of the lactone ring.

A critical discussion of the *X*-ray data, details of which will be published later by Mr. E. G. Cox (for summary, see Cox, *Nature*, 1932, **130**, 205), reveals that (XX) accounts satisfactorily for the crystallographic properties. Reference to the model, a diagrammatic representation of which is given in (XXIV), shows that of the total of 12 carbon and oxygen atoms all but one can be accommodated in one plane without appreciable valency strain, whilst the remaining carbon (C_5) lies less than 1 Å. above the plane.


(XXIV.)

l-Ascorbic acid.

Structures may now be assigned to various derivatives of ascorbic acid. Vargha's monoacetone derivative will be represented by structure (XXV) (*Nature*, 1932, **130**, 847), which is transformed by diazomethane into dimethyl monoacetone ascorbic acid (Karrer, Salomon, Schöpp, and Morf, *loc. cit.*). This is in agreement with Karrer's observation that the acetone body retains unimpaired its enolic character. Micheel and Kraft's dimethyl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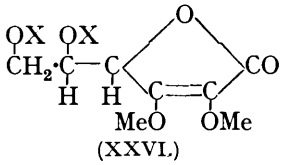
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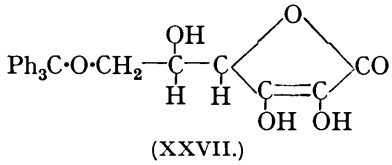
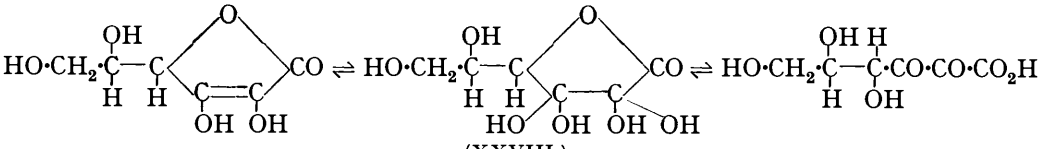

(XXVII.)

di-*p*-nitrobenzoyl derivative is (XXVI). The observation of the latter authors that (XXVI) gives oxalic acid and *l*-threonic acid on ozonisation, followed by hydrolysis, is

in exact agreement with the structural views now advocated, although a different explanation, rendered untenable by the present results, was adopted by them. The structure (XXIV) contains a primary alcoholic group on C_6 and so accounts for the formation of a triphenylmethyl derivative (XXVII) (Vargha, *Nature*, 1933, **131**, 363).

It is necessary to discuss in greater detail the properties of the first oxidation product of ascorbic acid. It has already been mentioned that when newly formed this substance is neutral in reaction and possesses no trace of the intense selective absorption characteristic of ascorbic acid. If, however, the aqueous solution of the oxidation product is kept at room temperature, the rotation gradually changes from + 56° to − 6° in the course of 70 hours, a longer time being required for the attainment of equilibrium if the mineral acid (2 mols.) formed during the oxidation is neutralised. The fall in rotation is accompanied by a gradual development of a weak absorption band at λ 290 mμ, which attains its maximum intensity when the solution has reached equilibrium, in which condition at least 80% of the oxidation product is present as free acid. No disintegration of the molecule has occurred during these changes, since on reduction with hydriodic acid the " equilibrium " product gives rise to ascorbic acid in good yield. As will be seen later, however, with hydrogen sulphide the reduction proceeds only to the extent of about 10%. The freshly prepared oxidation product gives an orange derivative, m. p. 216°, with phenylhydrazine, the analytical data for which indicate that it is obtained by the condensation of a molecule $C_6H_6O_6$ with two molecules of the base. A different phenylhydrazine deriv-



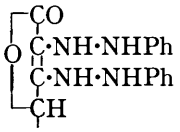
(XXVIII.)

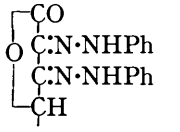
ative (yellow, m. p. 210°), which nevertheless has the same empirical formula, is obtained from the " equilibrium " solution and also from the oxidised product after opening of the lactone ring by salt formation. The rotation of the neutral sodium salt of the oxidation product is $[\alpha]_{5780}$ − 26°. The value depends on the p_H of the solution and approaches − 100° in *N*-alkali. These alkaline solutions are yellow and extraordinarily unstable in the presence of oxidising agents, including gaseous oxygen. Some decomposition takes place, even in an inert atmosphere, with formation of oxalic acid and the series of changes which takes place appears to be highly complex. For instance, slightly alkaline solutions of the oxidation product display relatively intense absorption bands at 265 mμ and 340 mμ, which, on acidification of the solution, move to 245 mμ and 300 mμ respectively. Only slight decomposition takes place under these conditions, since the re-acidified solution gives the yellow phenylhydrazine derivative in good yield. Iodine, however, is now taken up to an extent consonant with the idea that the band at 245 mμ is due to ascorbic acid. If this is indeed the case, it appears that amongst the changes which take place in alkaline solution there occurs to a small extent self-oxidation and reduction of the oxidation product with partial regeneration of ascorbic acid. A tentative explanation of these phenomena may be offered on the following lines. The primary oxidation product (XXVIII) would not show selective absorption, but if on opening of the lactone ring one (or both) of the hydrated keto-groups resumes its normal form the carbonyl band at 290 mμ would be expected to appear. It is readily understandable also that the lactone form would be more readily reduced to ascorbic acid than the open-chain form, since the latter, which must lactonise during the regeneration of ascorbic acid, can conceivably react both as a furanose and as a pyranose sugar. The facility with which hydriodic acid effects the lactonisation and reduction is probably due to the fact that the experiment is carried out by evaporating to dryness a solution of the oxidation product containing the requisite amount of reducing agent. The complex behaviour of the oxidation product towards alkalis is normal for reactive keto-hydroxy-compounds of this type.

The possibilities for structural isomerism and stereoisomerism amongst phenylhydrazine

derivatives of ascorbic acid and its reversible oxidation product are so numerous that the allocation of precise structures is a matter of extreme difficulty. The highly coloured nature of the ascorbic acid derivatives renders it doubtful whether a true osazone structure is present. The analytical data point to a condensation product derived from $C_6H_8O_6$ rather than from $C_6H_6O_6$, but with phenylhydrazine compounds it is difficult to discriminate by analysis between formulæ so closely related. On the basis of the formula $C_6H_8O_4(N{\cdot}NHPh)_2$ it is perhaps possible that the structure of the red derivative, m. p. 187°, from ascorbic acid is (XXIX). The orange derivative, m. p. 216°, obtained from the lactone form of the oxidised substance may be a true osazone (XXX). The yellow deriv-

(XXIX.)





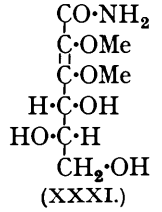
(XXX.)

ative, m. p. 210°, may also be an osazone and in this case condensation probably takes place with the free acid, followed by ring closure. In addition to alternative methods of ring closure, various tautomeric and stereoisomeric modifications are theoretically possible. The various derivatives with phenylhydrazine, *p*-nitrophenylhydrazine, *p*-bromophenylhydrazine, and 2 : 4-dinitrophenylhydrazine form well-defined series of characteristic compounds obtainable in most instances in excellent yield.

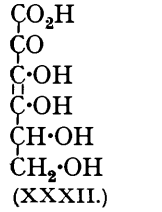
Ascorbic acid reacts also with *o*-tolylenediamine, giving slowly and in small yield a yellow condensation product which, however, does not appear to be homogeneous. The analytical data suggest that it consists mainly of a condensation product derived by the elimination of one molecule of water from one molecule of ascorbic acid and one of *o*-tolylenediamine. Its structure is obscure and its formation lends no support to the view at one time held that ascorbic acid contains two carbonyl groups.

Another derivative of special interest from the point of view of structure is the crystalline substance obtained by Micheel and Kraft by the action of methyl-alcoholic ammonia on dimethyl ascorbic acid. No loss of methyl groups occurs during the reaction, which involves the addition of ammonia. These authors denied the amidic character of the product, but we have found that it gives the usual amide reactions and on the basis of the above structure for dimethyl ascorbic acid its formation can be simply represented as the ordinary addition of ammonia to a lactone, giving a product of structure (XXXI). The reaction is analogous to the opening of the lactone ring by alkali and it is significant that precisely the same type of rotation change occurs during the transformation of dimethyl ascorbic acid into the sodium salt and into the amide. Furthermore, both products are devoid of selective absorption in the ultra-violet region, although dimethyl ascorbic acid has a strong band at λ 230 mμ. The disappearance of the band may be ascribed to various causes, but little stress can be laid on this point, since a slight movement, towards the ultra-violet, of the band at 230 mμ would place it in a region of wave-length (< 200 mμ) where observations are impossible.

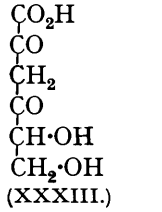
The evidence which has been cited above is inconsistent with the various alternative formulæ which have been suggested for ascorbic acid. The earliest of these (XXXII) was proposed as an attempt to derive a reduced form of the first oxidation product (V) which would satisfy both the chemical and the crystallographic data. It was discarded in favour of a ring structure (Hirst, *J. Soc. Chem. Ind.*, 1932, **52**, 221) as soon as it became evident that the newly formed first oxidation product was a lactone and not an acid.



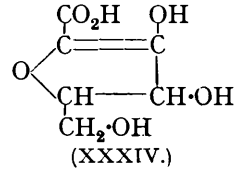
(XXXI.)



(XXXII.)



(XXXIII.)



(XXXIV.)

Formulæ of the type (XXXIII), including various tautomeric ring forms, were proposed by Karrer almost simultaneously with our suggestion of (XXXII). These also suffer from the same disability as regards the presence of a carboxyl group. Moreover, we find that acetylpyruvic acid is not oxidised by iodine in acid solution and on the other hand is very readily hydrolysed by alkali. Recently a synthesis of a substance closely allied to (XXXIII) has been achieved (Fischer and Baer, *Helv. Chim. Acta*, 1933, **16**, 534), but its properties do not favour in any way this type of structure for ascorbic acid. The structure (XXXIV), in which ascorbic acid is represented as a furancarboxylic acid, was later advocated by Micheel and Kraft (*Nature*, 1933, **131**, 274 and *loc. cit.*) and postulated as an alternative to (XXXIII) by Karrer. We had long been aware that this particular formulation served to explain many of the chemical properties of ascorbic acid, but we did not advance it because of its incompatibility with the crystallographic and *X*-ray requirements, which sufficed to exclude it (Cox, *loc. cit.*; Cox and Hirst, *Nature*, 1933, **131**, 402). The first oxidation product derived from a substance of this structure would have a free carboxyl group, the properties of its dimethyl derivative would be quite different from those observed, and ozonisation of the tetramethyl derivative would yield a product giving on hydrolysis oxalic acid and 2 : 4-dimethyl *l*-threonic acid instead of the 3 : 4-dimethyl *l*-threonic acid we actually found. On the other hand the facts reported in Micheel and Kraft's papers find a ready and natural interpretation (which has since been accepted by these authors, *Z. physiol. Chem.*, 1933, **218**, 280) in terms of the structure (XXIV) here advocated for ascorbic acid. It should be mentioned also that the synthetic experiments of Reichstein, Grüssner, and Oppenauer (*Helv. Chim. Acta*, 1933, **16**, 561) offer no support to the furancarboxylic acid structure for ascorbic acid (see Haworth, *J. Soc. Chem. Ind.*, 1933, **52**, 482), but on the contrary a synthesis of ascorbic acid by a similar procedure furnishes evidence of the accuracy of the constitution herein assigned (Haworth and Hirst, *ibid.*, p. 645).

Experimental.

Properties of Ascorbic Acid.—Three samples of ascorbic acid were examined : (a) Crude material from adrenal glands. Yellow powder, m. p. 170—175° after previous softening. $[\alpha]_{5780}$ + 23° in water. After recrystallisation from methyl alcohol–ether–light petroleum it had m. p. 190—191° (decomp.), $[\alpha]_{5780}$ + 24° in water. Spectrophotometric examination indicated that the crude material was very nearly pure (Found : C, 41·0; H, 4·7. Calc. for $C_6H_8O_6$: C, 40·9; H, 4·6%). (b) Material from adrenal glands, prepared at Mayo Clinic, Rochester, U.S.A. Cream-coloured powder, m. p. 182—184°. $[\alpha]_D$ + 24° in water (Found : C, 41·2; H, 4·8%). After recrystallisation this had m. p. 190—191°. (c) Material from paprica. White crystalline powder, m. p. 192°. $[\alpha]_{5780}$ + 24° in water (Found : C, 41·1; H, 4·6%). The properties of this material did not alter on recrystallisation from acetone, dioxan, alcohol, or methyl alcohol–ether–petroleum. The properties of recrystallised ascorbic acid from adrenal glands and from paprica were identical in all respects except that when heated the material of animal origin turned pink (on subsequent cooling the colour disappeared) whereas the paprica material remained colourless up to the m. p. The slight trace of impurity responsible for this could not be removed by crystallisation. The following experiments were mainly carried out with ascorbic acid from paprica, but were duplicated in many instances by similar experiments with material of animal origin. In no case was any difference in behaviour noticed. Pure ascorbic acid has m. p. 192° without previous darkening and rapidly decomposes with effervescence just above the m. p. When heated slowly, it begins to liberate carbon dioxide at 170° without darkening and without melting.

The mean of several analyses gave the figures C, 41·0; H, 4·7%. *M* 170 by *X*-ray analysis, 172 by titration with sodium hydroxide (calculated as monobasic acid), 176 by iodine titration. The substance contained no nitrogen, sulphur, or methoxyl. It reduced Fehling's solution in the cold. Neutral silver nitrate and neutral permanganate were reduced instantaneously. It decolorised aqueous bromine and iodine at once. Alcoholic iodine was not attacked, but it reduced potassium permanganate in acetone solution. It did not restore the colour to Schiff's reagent, nor did it give the naphtharesorcinol test for glycuronic acid. When heated with boiling 12% hydrochloric acid, it readily gave furfuraldehyde, which was estimated in the usual way as the phloroglucide (yield, 87% of the theoretical). It reacted vigorously with carbonates, giving salts. The salts gave with ferric chloride an intense violet colour (fleeting colour only

with the free acid). Alkaline solutions of ascorbic acid gave with sodium nitroprusside a deep blue colour, changing to green and then to red.

The *calcium* salt was prepared by adding a slight excess of calcium carbonate to an aqueous solution of ascorbic acid, filtering the solution, and evaporating it to dryness in a vacuum desiccator. On trituration with alcohol the neutral salt was obtained as a pale yellow powder. $[\alpha]_D^{19°}$ + 91° in water (*c*, 0·3) [Found : Ca, 9·9. $(C_6H_7O_6)_2Ca$ requires Ca, 10·2%].

The *brucine* salt of ascorbic acid was prepared by warming at 70° for 5 minutes an aqueous solution of ascorbic acid with an alcoholic solution containing the calculated amount of brucine. On evaporation a syrup was obtained which soon crystallised. After recrystallisation from hot alcohol it was a cream-coloured crystalline powder, soluble in water and in hot alcohol. M. p. 216—217° (decomp.) (Found : C, 60·6; H, 6·6; OMe, 12·2; N, 5·0. $C_{29}H_{34}O_{10}N_2,C_2H_5{\cdot}OH$ requires C, 60·4; H, 6·5; OMe, 15·1; N, 4·6%).

Rotation of Ascorbic Acid and its Sodium Salt.—$[\alpha]_{5780}^{19°}$ + 24° in water (*c*, 3·0), 25° (*c*, 0·5), 24° (*c*, 1·1) (contrast Karrer, von Euler, and Hellström, *Arkiv Kemi Min. Geol.*, 1933, **11**, *B*, No. 6). No mutarotation. Unless air-free water and hard-glass vessels were used, a gradual rise, followed by a fall in rotation, was observed. This was traced to partial salt formation caused by alkali dissolved from the glass, followed by oxidation. $[\alpha]_D^{17°}$ + 22° in *N*/20-hydrochloric acid or *N*-sulphuric acid. No mutarotation. The sodium salt of ascorbic acid had $[\alpha]_{5780}^{18°}$ + 116° in neutral aqueous solution (rotation calculated on concentration of ascorbic acid), + 130° in *N*/20-sodium hydroxide, + 149° in *N*/7-sodium hydroxide, 155° in *N*/2-sodium hydroxide, 161° in 2*N*-sodium hydroxide (constant for 1 hour; value after acidification + 21°) (all solutions made up in an atmosphere of nitrogen). The alkaline solutions were yellow, the acidified solution colourless. In acid solutions the rotation value remained almost unchanged for some hours when oxygen was passed through the solution; but rapid decomposition took place when oxygen was bubbled through alkaline solutions, oxalic acid being formed.

In 50% aqueous acetic acid ascorbic acid reacts rapidly (10—15 minutes) with ozone, giving the same product (" first oxidation product," $[\alpha]_{5780}$ + 56°) as that produced by oxidation with iodine or chlorine in acid solutions. The continued action of ozone results in decomposition with formation of oxalic acid.

Titration of Ascorbic Acid with Iodine and Chlorine.—0·1000 G. of ascorbic acid, dissolved in water, required 11·4 c.c. of neutral *N*/10-iodine to complete the first stage of the oxidation (calc., 11·4 c.c. for 2 atomic proportions of iodine). After this no more iodine was taken up. The solution, which showed $[\alpha]_{5780}$ + 56° (calc. on concentration of ascorbic acid), was then strongly acid and required, when titrated immediately, 12 c.c. of *N*/10-sodium hydroxide to restore neutrality. Since 2 mols. of hydrogen iodide are liberated during the oxidation (see below), 11·4 c.c. of *N*/10-alkali were required for the hydriodic acid and 0·6 c.c. for the organic acid. The end-point was indefinite and the titration proceeded as for a lactone until in all 17 c.c. of alkali were added (calc. for 2 mols. HI + 1 mol. of a monobasic organic acid, 17·0 c.c.). The end-point was now sharp and any further addition of alkali produced a yellow colour. No oxalic acid was formed during this stage of the oxidation. At the neutral point the rotation was $[\alpha]_{5780}$ − 26°. The oxidation was continued with iodine in alkaline solution. An excess of iodine and sodium hydroxide was added and the solution was kept for 15 minutes at room temperature. An excess of acid was then added, and the remaining iodine titrated with thiosulphate. The iodine used in the second stage of the oxidation was 11·6 c.c. (calc., 11·4 c.c. for 2 atomic proportions). Titration of the excess of acid showed that for the second stage of the oxidation 22·8 c.c. of *N*/10-alkali were required. Now the 11·6 c.c. of iodine utilised as an oxidising agent account for 11·6 c.c. of alkali, leaving 11·2 c.c. of alkali utilised in neutralising the carboxyl groups produced during the oxidation (calc. for two CO_2H groups, 11·4 c.c.). These two groups are additional to the one present as a lactone in the newly formed first oxidation product. At the end of the titration the amount of oxalic acid present in solution was estimated in the usual way as calcium oxalate (yield, 95% of the theoretical quantity for 1 mol. of oxalic acid). Exhaustive blank experiments at each stage of the above procedure proved that there were no interfering factors. The above results are in exact accord with the view that the first oxidation product is a 2 : 3-diketohexonic acid which is oxidised to oxalic acid and trihydroxybutyric acid. The isolation and characterisation of the latter substance are described below. Precisely similar results were obtained when chlorine water, followed by alkaline hypochlorite, was used as oxidising agent.

In another experiment the hydriodic acid formed during the titration with iodine was eliminated by carrying out the titration in the presence of an excess of calcium carbonate (0·100 g. of substance required 11·4 c.c. of *N*/10-iodine. Calc., 11·4 c.c.) We established also that the

titration may be carried out in the presence of an excess of citric acid without alteration of the end-point. An estimation was also made of the quantity of hydriodic acid liberated during the titration. Ascorbic acid (0·0737 g.) was dissolved in water and titrated with an alcoholic solution of iodine (8·0 c.c. of 0·1022 *N*-iodine. Calc., 8·2 c.c.). The presence of water is essential. A slight excess of aqueous silver nitrate was then added to the cold solution. It was easy to filter off the precipitated silver iodide before any interaction took place between the oxidation product and the silver nitrate (Found : 0·2016 g. AgI. Calc. for 2 mols. HI : AgI, 0·1920 g.).

When aqueous solutions of ascorbic acid were titrated with potassium permanganate the reagent was decolorised almost instantaneously until the equivalent of about 1·3 atoms of oxygen had been added. A slower reaction followed, which ended somewhat indefinitely when permanganate equivalent to 2O had been used up. Thereafter the reaction proceeded still more slowly without definite end-point. The arrest at the point corresponding to 1·2O is caused by slight overlapping of the first and the second stage of the oxidation, the primary oxidation product being readily attacked by permanganate. Oxalic acid and carbon dioxide were detected as oxidation products.

FIG. 1.

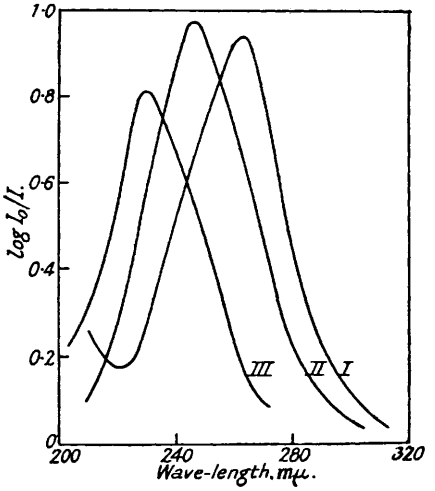


FIG. 2.

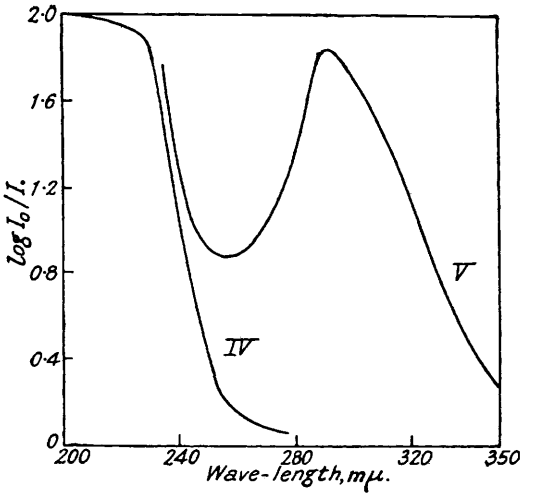


FIG. 1.—I. *Ascorbic acid or its sodium salt in water* (2 *mg. per* 100 *c.c.*); $l = 1$ *cm.* II. *Ascorbic acid in alcohol or acid aqueous solution* (2 *mg. per* 100 *c.c.*); $l = 1$ *cm.* III. *Dimethyl ascorbic acid in alcohol* (2·5 *mg. per* 100 *c.c.*); $l = 1$ *cm.*

FIG. 2.—IV. *Oxidation solution of ascorbic acid immediately after preparation* (330 *mg. per* 100 *c.c.*); $l = 1$ *cm.* V. *Oxidation solution of ascorbic acid at equilibrium* (330 *mg. per* 100 *c.c.*); $l = 1$ *cm.*

Absorption Spectrum of Ascorbic Acid.—The observations were obtained by use of a Hilger sector photometer and a Hilger quartz spectrograph. For most of the work cells of 1 cm. length were employed. In aqueous solution ascorbic acid is characterised by a single very intense band with its head at 260—265 mμ. The molecular extinction coefficient is approximately 7000 for solutions containing about 2 mg. per 100 c.c. In stronger solutions (*ca.* 50 mg. per 100 c.c.) wide deviations from Beer's law are encountered. But for concentrations ranging between 0·5 and 2·5 mg. per 100 c.c. Beer's law holds with sufficient exactitude to permit of the use of spectrophotometric measurements for quantitative estimations of concentration. The intensity of the band diminishes rapidly, falling to half value in a few hours (decomposition of ascorbic acid by oxidation).

The band is much more persistent in acidified aqueous solutions ($p_H < 3$). The head is then at λ 245 mμ (ε approx. 7500 for *c* 0·002%). In ethyl alcohol it occurs at λ 245 mμ (ε approx. 7500, for *c* 0·002%). In methyl alcohol the head of the band is at λ 263 mμ (ε 7500 for *c* 0·002%). In this solvent concentrated solutions show wide divergences from Beer's law; *e.g.*, at λ 280 mμ ε is 800 for solutions with *c* 0·02%, 2000 for *c* 0·005%, and 4400 for *c* 0·002%. The sodium salt of ascorbic acid shows in aqueous solution a band at λ 265 mμ, the character of which, including intensity, is exactly similar to that of ascorbic acid in water.

These absorption bands closely resemble those shown by acetylpyruvic acid ($\varepsilon^{c.\ 2\ mg.\ per\ 100\ c.c.}_{\lambda\ 285}$

4000 in water) and dihydroxymaleic acid ($\varepsilon^{c.\ 2\ mg.\ per\ 100\ c.c.}_{\lambda\ 290}$ 6700 in water). Similar but weaker bands are shown by lævulic acid in water ($\varepsilon^{c.\ 0{\cdot}6\ g.\ per\ 100\ c.c.}_{\lambda\ 283}$ 24), glucosone in alkali ($\varepsilon^{c.\ 134\ mg.\ per\ 100\ c.c.}_{\lambda\ 310}$ 13), and acetone in alcohol ($\varepsilon^{c.\ 0{\cdot}1\ g.\ per\ 100\ c.c.}_{\lambda\ 265}$ 15). Selective absorption is not shown by ordinary sugars and sugar derivatives, either of the pyranose or the furanose type, neither is it displayed by hexuronic acids, *e.g.*, galacturonic acid, glycuronic acid, nor by 2-ketogluconic acid. Such substances are highly transparent in solution and display only weak continuous absorption in the ultra-violet, with ε less than 5 at λ 260 mμ. Details of these observations will be given in a separate communication.

Properties of the First Oxidation Product of Ascorbic Acid.—The newly formed first oxidation product has $[\alpha]_{5780}$ + 56°. This value diminishes when the solution is kept. $[\alpha]_{5780}$ + 46° (2 hrs.); 38° (4 hrs.); 30° (6 hrs.); 25° (8 hrs.); 21° (10 hrs.); 6° (20 hrs.); ± 0° (28 hrs.); − 3° (40 hrs.); − 6° (70 hrs., constant value). These observations were made in the presence of the mineral acid formed during the oxidation. When this mineral acid was exactly neutralised, the mutarotation followed a similar course but at a much slower speed. $[\alpha]_{5780}$ + 56°

FIG. 3.

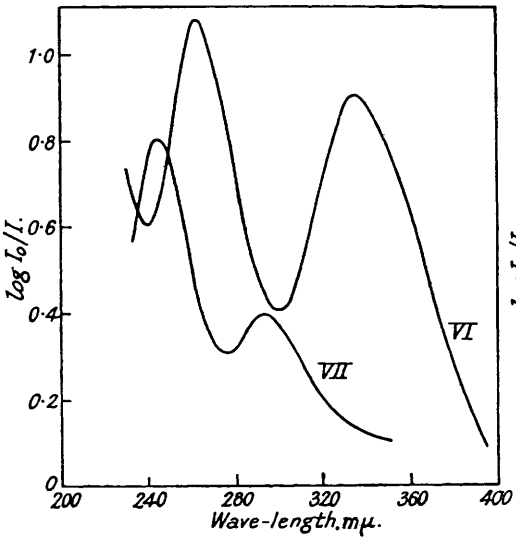


FIG. 4.

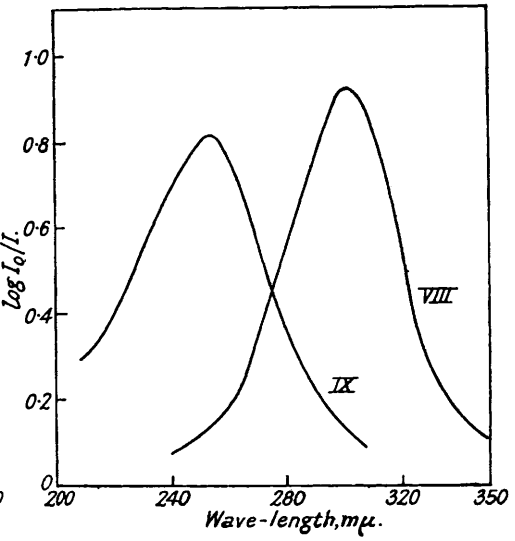


FIG. 3.—VI. *Oxidation solution of ascorbic acid made alkaline* (23·3 *mg. per* 100 *c.c.*); $l = 1$ *cm.* VII. *Oxidation solution of ascorbic acid made alkaline and immediately acidified* (14 *mg. per* 100 *c.c.*); $l = 1$ *cm.*

FIG. 4.—VIII. *Dihydroxymaleic acid in alcohol* (1·8 *mg. per* 100 *c.c.*); $l = 1$ *cm.* IX. *Methyl dimethoxymaleate in alcohol* (2·7 *mg. per* 100 *c.c.*); $l = 1$ *cm.*

(initial value); 44° (10 hrs.); 38° (20 hrs.); 32° (30 hrs.); 27° (40 hrs.); 19° (60 hrs.); 13° (80 hrs.); 8° (100 hrs.); ± 0° (140 hrs.); − 4° (170 hrs.); − 7° (200 hrs.); − 9° (250 hrs.); − 12° (300 hrs., constant value). The equilibrium solutions can be preserved indefinitely without change in properties. Simultaneously with the decrease in rotation there occurs an increase in the acidity of the solution. At the equilibrium position titration with alkali shows that about 85% of the organic material exists as free acid and only about 15% as lactone. For 0·1000 g. of ascorbic acid a total of 5·6 c.c. of N/10-alkali was required for the organic acid, and of this 4·8 c.c. were used immediately (free acid) and the remainder slowly (lactone). When the titration is carried out quickly on a freshly oxidised solution, only a negligible proportion of the organic substance is titrated as free acid. The equilibrium solution contains no oxalic acid.

The rotation of the sodium salt of the oxidation product depends markedly on the p_H value of the solution. For example, when 1·5 c.c. of N/10-sodium hydroxide were added to a neutral solution (5 c.c.) of the oxidation product from 0·017 g. of ascorbic acid the rotation changed immediately from − 26° to − 100°. This value is probably not the maximum, since rapid mutarotation was observed. $[\alpha]_{5780}$ − 93° (1 min. after addition); − 69° (5 mins.); − 56° (7 mins.); − 46° (10 mins.); − 41° (12 mins.); − 36° (14 mins.); − 32° (16 mins.); − 29° (18 mins.); − 26° (20 mins.); − 24° (26 mins., constant for a few minutes). At this stage no oxalic acid was present in the solution. After acidification the rotation was − 22° and remained

constant indefinitely. When this solution, the alkaline solution with $[\alpha]_{5780}$ — 100°, either of the equilibrium solutions mentioned above ($[\alpha]_{5780}$ — 6° and — 12° respectively), or the neutral sodium salt of the oxidised substance was treated with phenylhydrazine in acetic acid, in every case the same yellow phenylhydrazine derivative, m. p. 210°, was obtained in good yield (see below for details concerning phenylhydrazine derivatives). An entirely different orange phenylhydrazine derivative, m. p. 216°, is given by the newly formed oxidised ascorbic acid.

Alkaline solutions of oxidised ascorbic acid decompose in the presence of oxygen with formation of oxalic acid and other products. For this reason the observations recorded above for alkaline solutions were carried out in an inert atmosphere, but slow decomposition of the oxidation product, again with formation of oxalic acid, took place even under these conditions.

Solutions of oxidised ascorbic acid which had been made slightly alkaline and then acidified always took up iodine corresponding to about 5% of the total quantity of ascorbic acid submitted to oxidation.

Corresponding with these changes there occur changes in the absorption spectrum of the oxidised ascorbic acid. On account of the transparency of chlorides in solution all spectrophotometric work on the oxidised substance was carried out after oxidation by chlorine. The newly formed oxidation product displays no selective absorption and is remarkably transparent in the ultra-violet region even when examined in concentrated solution, general absorption commencing at λ 240 mμ with solutions containing 300 mg. per 100 c.c. (*l*, 1 cm.). No trace of the intense band due to ascorbic acid is present. As the solution of the oxidised substance approaches the equilibrium condition, a weak band at λ 290 mμ makes its appearance, reaching its maximum intensity when the mutarotation has ceased and the solution has reached equilibrium (see Fig. 2). The condition of the oxidised substance can be judged equally well by absorption measurements, rotation measurements or by titration of the free acid present.

The slightly alkaline solutions of the oxidised substance display two moderately intense bands with heads at λ 265 mμ and λ 340 mμ respectively (see Fig. 3). On acidification of the solution these bands move to λ 245 mμ and λ 295 mμ. A band at λ 245 mμ having the same intensity would be given by an amount of ascorbic acid equal to 5% of that submitted to oxidation. In the alkaline solution the band at λ 265 mμ is fleeting and disappears as decomposition sets in. The band at λ 245 mμ is persistent in acid solution.

After oxidation of ascorbic acid by chlorine water the product gave only a trace of furfuraldehyde when heated with 12% hydrochloric acid.

Regeneration of Ascorbic Acid from the Oxidised Substance.—(a) From freshly oxidised ascorbic acid : Ascorbic acid (0·176 g.) was oxidised in aqueous solution by addition of an alcoholic solution of iodine (0·254 g.). The colourless solution was then evaporated to dryness in a vacuum at room temperature. Separation of iodine commenced when the solution had evaporated to a thick syrup. The solid mixture of crystalline ascorbic acid and iodine was placed in a current of air. The iodine soon disappeared by sublimation, leaving ascorbic acid as a cream-coloured powder identical in all respects with an authentic sample (yield, quantitative). The crude material before recrystallisation had m. p. 180° and iodine titration and spectrophotometric examination showed that it was at least 90% pure.

With freshly oxidised material the reduction may be carried out also by hydrogen sulphide. In this case it is possible to follow the reaction spectrophotometrically. Ascorbic acid was oxidised by the calculated quantity of chlorine water. A photometric test showed that no trace of the ascorbic acid band remained. Hydrogen sulphide was then passed through the solution for 15 minutes. The dissolved sulphide was removed by addition of the minimum quantity of lead acetate, and the lead removed by addition of a little oxalic acid. A portion of the solution appropriately diluted showed the ascorbic acid band at λ 245 mμ. The yield of regenerated ascorbic acid calculated from the spectrophotometric observations was 90—95% of the theoretical. The iodometric titration value was in exact agreement with this.

(b) From the equilibrium solution of the oxidised product : In this case hydrogen sulphide was ineffective. The maximum yield of regenerated product, when the experiment was carried out as previously described, was 6%. The reduction may still be carried out by hydriodic acid. The procedure was exactly as given above except that the solution containing the oxidised product was allowed to reach equilibrium before the evaporation was commenced. An alcoholic iodine solution was used as oxidising agent and the solution finally obtained contained 15% of alcohol by volume ($[\alpha]_D$ + 60°). The presence of the alcohol greatly retarded the mutarotation, equilibrium ($[\alpha]_D$ — 6°) being reached in about 200 hours. Reduction of the oxidation product and separation of iodine took place as before, with the exception that in this case some brown amorphous by-product accompanied the regenerated ascorbic acid. The yield

of the latter judged by (a) iodometric titration, (b) spectrophotometric examination, (c) quantitative *X*-ray examination—all carried out on the crude product—was 75—80%. One recrystallisation from acetone gave pure ascorbic acid.

Phenylhydrazine Derivatives.—I. *Derivatives obtained from ascorbic acid.* (a) With phenylhydrazine : An aqueous solution of ascorbic acid was heated for 30 minutes at 90° with phenylhydrazine (3 mols.) until the solution became red. The water-bath was then allowed to cool slowly. The *product* separated as deep red needles, which when washed with dilute acetic acid, water, and dried in a vacuum had m. p. 187° (decomp.). It was insoluble in water, moderately easily soluble in dilute aqueous sodium hydroxide, giving an orange solution from which the original osazone was precipitated on acidification (Found : C, 60·8; H, 5·6; N, 15·7. $C_{18}H_{20}O_4N_4$ requires C, 60·7; H, 5·6; N, 15·7%. $C_{18}H_{18}O_4N_4$ requires C, 61·0; H, 5·1; N, 15·8%).

If the red solution mentioned above is cooled rapidly, the product obtained is mainly amorphous, m. p. 204° (decomp.), but has the same elementary composition (Found : C, 60·8; H, 5·6; N, 15·7%).

(b) With *p*-nitrophenylhydrazine : The procedure was the same as in (a) and the crystalline *product* obtained was bright red, m. p. 259° (decomp.). M. p. 262° (decomp.) after recrystallisation from alcohol (Found : C, 48·3; H, 4·2; N, 18·8. $C_{18}H_{18}O_8N_6$ requires C, 48·4; H, 4·0; N, 18·8%. $C_{18}H_{16}O_8N_6$ requires C, 48·6; H, 3·6; N, 18·9%).

(c) With *p*-bromophenylhydrazine : The crystalline compound obtained was dark red, m. p. 170° (decomp.) (Found : C, 42·3; H, 3·4; N, 10·4. Calc. for $C_{18}H_{18}O_4N_4Br_2$: C, 42·05; H, 3·5; N, 10·9%. Calc. for $C_{18}H_{16}O_4N_4Br_2$: C, 42·2; H, 3·1; N, 10·9%). This substance was also obtained as a monohydrate.

(d) With 2 : 4-dinitrophenylhydrazine : A methyl-alcoholic solution of ascorbic acid was boiled for 3 hours with a slight excess of 2 : 4-dinitrophenylhydrazine. When the dark red solution was cooled, brownish-red needles separated, m. p. 282° (decomp.) (Found : C, 40·45; H, 2·8; N, 21·4. Calc. for $C_{18}H_{16}O_{12}N_8$: C, 40·3; H, 3·0; N, 20·9%. Calc. for $C_{18}H_{14}O_{12}N_8$: C, 40·4; H, 2·6; N, 21·0%).

II. *Derivatives obtained from oxidised ascorbic acid.* (a) With phenylhydrazine : (i) Ascorbic acid was dissolved in water and oxidised with *N*/10-iodine. The solution was made slightly alkaline with *N*/10-sodium hydroxide and then immediately acidified with acetic acid. It was warmed for 15 minutes at 70° with a slight excess of phenylhydrazine in acetic acid. An orange-yellow precipitate separated. This was washed with dilute acetic acid and water and dried in a vacuum. M. p. 187° (decomp.). After recrystallisation from absolute alcohol it was obtained as yellow needles, m. p. 210° (decomp.) (Found : C, 60·9; H, 5·4; N, 15·6%).

(ii) Ascorbic acid was oxidised with iodine in aqueous solution. A slight excess of phenylhydrazine in acetic acid was added immediately. The solution was warmed for 15 minutes at 70°. A light red compound separated which after recrystallisation from alcohol gave orange silky needles, m. p. 216° (decomp.) (Found : C, 60·8; H, 5·1; N, 15·7%).

(b) With *p*-nitrophenylhydrazine : (i) An oxidised solution of ascorbic acid was used which had been rendered slightly alkaline and then immediately acidified with acetic acid. The product obtained was light red, m. p. 255° (decomp.). Recrystallisation from alcohol–acetone gave light red needles, m. p. 260° (decomp.) (Found : C, 48·5; H, 4·4; N, 18·8%).

(ii) A solution of freshly oxidised ascorbic acid was used. The mineral acid was not removed. The product on recrystallisation from alcohol formed yellow needles, m. p. 246° (decomp.) (Found : C, 48·2; H, 3·9; N, 17·8%).

(c) With *p*-bromophenylhydrazine : (i) The compound obtained after neutralisation of the oxidised ascorbic acid was yellowish-red, darkened at 160°, and melted at 220° (decomp.) (Found : C, 40·6; H, 3·7; N, 10·5. Calc. for $C_{18}H_{16}O_4N_4Br_2,H_2O$: C, 40·7; H, 3·4; N, 10·6%).

(ii) When freshly oxidised ascorbic acid was used, a bright red compound was isolated which after recrystallisation from alcohol gave yellow needles, m. p. 208° (decomp.) (Found : C, 42·3; H, 3·5; N, 8·15%). Repetition of the nitrogen estimation gave N, 8·1%. This substance and also the corresponding derivative with *p*-nitrophenylhydrazine gave values for the nitrogen content which are abnormal when compared with those given by the other derivatives.

(d) With 2 : 4-dinitrophenylhydrazine : (i) An aqueous solution of ascorbic acid was oxidised with iodine. The solution was made slightly alkaline with sodium hydroxide and immediately acidified with hydrochloric acid. A slight excess of 2 : 4-dinitrophenylhydrazine in 2*N*-hydrochloric acid was added, and the solution warmed for 30 minutes at 70°. A light red compound was obtained, m. p. 268° (decomp.) (Found : C, 40·4; H, 2·95; N, 20·7%).

(ii) Ascorbic acid was oxidised with iodine in aqueous solution, and the product treated immediately with 2 : 4-dinitrophenylhydrazine in 2*N*-hydrochloric acid. The mixture was warmed for 30 minutes at 70°. The product, recrystallised from alcohol–acetone, had m. p. 280° (decomp.) (Found : C, 40·95; H, 2·7; N, 20·4%).

The above derivatives can be obtained with equal facility after oxidation of ascorbic acid by chlorine water.

o-*Tolylenediamine and Ascorbic Acid.*—Ascorbic acid was treated with *o*-tolylenediamine (2 mols.) in aqueous solution. The mixture was warmed at 40° for 10 minutes and then kept at room temperature. After 4 days a yellow precipitate formed. This separated from aqueous solution as a yellow amorphous powder which after recrystallisation from aqueous alcohol was obtained as pale yellow needles, darkening at 110°, m. p. 115° (decomp.) (Found : C, 59·4; H, 6·2; N, 12·7%).

Oxidation of Ascorbic Acid by Potassium Permanganate. Isolation of Methyl Trimethyl Threonate.—I. *Oxidation.* Ascorbic acid (6 g.) (from paprica) was dissolved in water (60 c.c.). 5*N*-Sulphuric acid (30 c.c.) was added, and a normal aqueous solution of potassium permanganate slowly added at room temperature. The oxidation was instantaneous until the equivalent of about one and half atoms of oxygen had been added, after which the reaction proceeded more slowly. More sulphuric acid was now added and oxidation continued. After the addition of the equivalent of two atoms of oxygen the reaction was slow and evolution of carbon dioxide took place as the permanganate solution was added. Some manganese dioxide was deposited, but disappeared on shaking, the solution becoming colourless. In all 205 c.c. of *N*-potassium permanganate (equivalent to 3 atoms of oxygen) and 40 c.c. of 5*N*-sulphuric acid were used.

The solution was now left over-night, the clear liquid made alkaline by addition of concentrated aqueous potassium hydroxide, and the precipitated manganese hydroxide filtered off after treatment with charcoal. The brown liquid was concentrated in a vacuum at 40° to 30 c.c., and the potassium sulphate which crystallised was filtered off. The alkaline solution was now non-reducing.

II. *Methylation of the product.* After the addition of a little acetone this solution was methylated in the usual manner by 50% aqueous potassium hydroxide (95 c.c.) and methyl sulphate (57 c.c.). The solution was cooled over-night and, after removal of the precipitated potassium sulphate, acidified with hydrochloric acid. After concentration and filtration methyl alcohol was added, and the solution concentrated under diminished pressure at 40°. This operation was repeated several times, the precipitated inorganic salts being filtered off from time to time. Finally a yellow syrup containing much inorganic material was obtained.

The syrup was boiled for 4 hours with 3% methyl-alcoholic hydrogen chloride and neutralised (silver carbonate). On evaporation a yellow syrup contaminated with inorganic matter was obtained. Extraction with warm chloroform and evaporation of the solvent gave a mobile syrup (3·0 g.), $n_D^{15°}$ 1·4460. On fractional distillation this gave (a) 0·64 g., b. p. 125°/25 mm. (bath temp.), $n_D^{15°}$ 1·4332, $[\alpha]_{5780}^{18°}$ + 25° in methyl alcohol (*c*, 3·6) (Found : C, 47·3; H, 7·9; CO_2Me, 35%); (b) 0·87 g., b. p. 140—145°/25 mm. (bath temp.), $n_D^{15°}$ 1·4386. The remainder (1·4 g.) was incompletely methylated and did not distil. This distillation results in loss of yield, but is necessary to remove all inorganic matter from the partly methylated ester, which requires further methylation. Previous experience had shown us that the above sequence of operations results in the formation of potassium methyl sulphate during the esterification and if this is not removed serious complications are encountered later on owing to its transformation into methyl sulphate. Fractions (a) and (b) were combined and remethylated with methyl iodide and silver oxide. The product gave on distillation *methyl trimethyl* l-*threonate* as a colourless mobile liquid (1·11 g.), b. p. 120°/13 mm. (bath temp.), $n_D^{15°}$ 1·4275, $[\alpha]_{5780}^{18°}$ + 49° in methyl alcohol (*c*, 2·9), + 31° in water (*c*, 1·3), $d^{15°}$ 1·090 (Found : C, 49·9; H, 8·4; OMe, 63·4; CO_2Me, 32·4. $C_8H_{16}O_5$ requires C, 50·0; H, 8·3; OMe, 64·6; CO_2Me, 30·8%).

Methyl trimethyl *l*-threonate (0·1 g.) was dissolved in dry methyl alcohol (3 c.c.) saturated with ammonia at 0°. After 24 hours at 15° the solvent was evaporated in a desiccator, leaving a colourless syrup which soon crystallised. Recrystallisation from light petroleum (b. p. 40—60°) gave *trimethyl* l-*threonamide* as shining colourless hexagonal plates (yield, almost quantitative), m. p. 78°. $[\alpha]_{5780}^{20°}$ + 44° in water (*c*, 1·0), + 68° in methyl alcohol (*c*, 0·8). A mixed m. p. with trimethyl *d*-erythronamide, m. p. 57°, showed a large depression (Found : C, 47·7; H, 8·5; N, 8·2; OMe, 50·8; *M*, by *X*-ray analysis, 177. $C_7H_{15}O_4N$ requires C, 47·5; H, 8·5; N, 7·9; OMe, 52·5%; *M*, 175).

Oxidation of Ascorbic Acid from Adrenal Glands.—A specimen of ascorbic acid from suprarenal glands (specimen b; see first paragraph) was oxidised with gaseous oxygen in faintly alkaline

solution with a trace of copper as catalyst until one extra carboxyl group per molecule of ascorbic acid had been formed. The oxidation was completed by acid potassium permanganate (2O) and the product, which was worked up as before, was identified in the form of trimethyl *l*-threonamide, m. p. 78°, identical with the above. Mixed m. p. 78°. $[\alpha]_D^{15^\circ}$ + 67° in methyl alcohol (*c*, 0·3) (Found : C, 47·5; H, 8·9; N, 8·2; OMe, 52·6%).

Oxidation of Ascorbic Acid by Iodine and Alkaline Hypoiodite.—To a solution of ascorbic acid (6 g.) in water (30 c.c.), 100 c.c. of an iodine solution (19 g. of crystalline iodine and 16 g. of potassium iodide in 200 c.c. of water) were added until a permanent brown colour was obtained. More iodine solution (20 c.c.) was added, followed by 20% potassium hydroxide solution (10—15 c.c.) until the brown colour was almost discharged. Alternate additions of the reagents were continued in such a way that a slight excess of iodine over alkali always prevailed, so that the last addition of alkali (making 80 c.c. in all) produced a colourless solution. The mixture was maintained at 0° and after 45 minutes it was cautiously acidified with 5*N*-sulphuric acid, the excess of iodine being removed by sulphur dioxide. The amount of iodoform produced was negligible.

Isolation and esterification of the oxidation products. Aeration removed sulphur dioxide and the solution was made neutral with silver carbonate. Shaking with silver sulphate (60 g.) for 3 hours removed all iodides and oxalates, and the liquid was filtered, treated with a slight excess of potassium carbonate to remove silver sulphate, again filtered, and concentrated to 120 c.c. at 40°/20 mm. The potassium sulphate which separated was removed, and the filtrate and washings were united and treated with methyl sulphate (60 c.c.) and 40% potassium hydroxide solution (144 c.c.) in ten portions during 90 minutes, the methylation being conducted in the presence of acetone at 50°. The alkaline solution was cooled and neutralised with 5*N*-sulphuric acid, potassium sulphate removed, and the solution concentrated to 200 c.c. Sulphuric acid was then added until the solution was definitely acid, followed by an equal bulk of methyl alcohol. After standing, the inorganic salt was removed and the acid solution concentrated at 35°/20 mm. to 200 c.c. By neutralisation with barium carbonate, filtration and concentration the partly methylated organic acids were obtained as barium salts mixed with potassium sulphate. This solution was treated with a slight excess of sulphuric acid, filtered, and concentrated, the residue being extracted repeatedly with warm ethyl alcohol. The extract was concentrated and neutralised in aqueous solution with barium carbonate, filtered, and treated with a slight deficiency of sulphuric acid so that some barium salt remained undecomposed. After centrifuging, the solution was taken to dryness under diminished pressure and the residue dried.

Esterification and methylation. The mixture of organic acid and barium salts so obtained was treated with 2½% methyl-alcoholic hydrogen chloride during 6 hours at 70°. The acid was neutralised with silver carbonate, and the alcohol removed. The syrupy esters were incompletely soluble in methyl iodide, therefore two methylations with silver oxide and methyl iodide were conducted in the presence of methyl alcohol at 45° for 5 hours. The product was isolated in the usual way and a third methylation followed, methyl iodide and silver oxide alone being used. A mobile syrup (2·1 g.) was isolated which was subjected to fractional distillation : (A) 0·3 g., b. p. 120—130°/15 mm., $n_D^{15^\circ}$ 1·4252; (B) 1·1 g., b. p. 130—140°/15 mm., $n_D^{15^\circ}$ 1·4291; (C) 0·2 g., b. p. 140—145°/15 mm., $n_D^{15^\circ}$ 1·4325; (D) 0·1 g., b. p. 145—170°/15 mm., $n_D^{15^\circ}$ 1·4370; residue 0·4 g. (all temperatures are bath-temperatures). Fraction B, being incompletely methylated, was subjected to a further two Purdie methylations, followed by distillation at 135°/15 mm. to give a mobile ester showing $[\alpha]_{5780}^{18^\circ}$ + 49° (*c*, 1 in methyl alcohol), $n_D^{16^\circ}$ 1·4270 (Found : C, 49·5; H, 7·9; OMe, 62·4. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·3; OMe, 64·8%).

Isolation of trimethoxy l-*threonamide.* The syrup (0·3 g.) was treated with methyl-alcoholic ammonia at 0° for 2 days. On removal of solvent partial crystallisation set in, which was completed on the addition of ether. The crystals were removed, washed, and recrystallised from ethyl alcohol (0·02 g.). M. p. above 255° (decomp.) (Found : OMe, 36·4. Calc. for dimethoxysuccinamide : OMe, 35·2%).

The ethereal extract crystallised in needles which on extraction with boiling light petroleum (b. p. 40—60°) gave the characteristic plates of trimethyl *l*-threonamide (0·06 g.), m. p. 75—76° alone or in admixture with an authentic specimen. $[\alpha]_{5780}$ + 44° in water (*c*, 1) (Found : C, 47·9; H, 8·4; OMe, 50·75. Calc. for $C_7H_{15}O_4N$: C, 47·5; H, 8·5; OMe, 52·5%).

The Oxidation of Ascorbic Acid with Hypochlorite : Conversion of the Oxidation Product into d-*Tartaric Acid.*—Ascorbic acid (10 g.) in water (50 c.c.) was oxidised by the addition of small portions of an alkaline hypochlorite solution (100 c.c. of 15% potassium hydroxide solution containing 8·4 g. of available chlorine) and alternately with 15% potassium hydroxide solution

(140 c.c.) so that a slight excess of chlorine was present over the theoretical quantity (7·8 g.). After the mixture had stood for 30 minutes at 15° and after acidification with sulphuric acid, a trace of sulphur dioxide was sufficient to remove the excess of chlorine. Treatment with silver carbonate, silver sulphate (30 g.), and potassium carbonate in the manner described in the first part yielded a solution containing the potassium salts of the oxidation products together with potassium sulphate, which was evaporated to dryness at 40°/15 mm. The powdered residue was treated with 5*N*-sulphuric acid (30 c.c.) and shaken with absolute alcohol (1 l.) for 2 hours. The residue was re-extracted in the same way after acidification, and the combined alcoholic filtrates were neutralised with barium hydroxide solution and allowed to stand. The clear filtrate was evaporated to dryness, forming a glass, the mixture of barium sulphate and insoluble barium salts was leached with hot water, and the solution of barium salts obtained was mixed with the residue from the alcoholic filtrates. Cautious addition of sulphuric acid followed until only a trace of barium remained in solution. Barium sulphate was removed and the solution was taken to dryness under diminished pressure (yield, 7 g.).

Oxidation with nitric acid. (I) The thick syrup (3·7 g.) obtained by this treatment was oxidised with nitric acid (*d* 1·2; 8 c.c.) at 40—45° for 24 hours. The solution was diluted with water, and water was distilled off continuously for 3 hours. Methyl alcohol was then added and the solution concentrated to a syrup, which was esterified with 3% methyl-alcoholic hydrogen chloride (150 c.c.) for 6 hours at 70°. The esters were isolated after neutralisation with silver carbonate and two methylations with methyl iodide and silver oxide followed, to yield 3·35 g. of a mobile syrup.

Fractional distillation of the esters. After heating at 100° for 3 hours at 15 mm. to remove methyl oxalate, the residue was distilled at 15 mm.

	Bath temp.	Yield, g.	$n_D^{18°}$.	$[\alpha]_{5780}^{19°}$ in MeOH (*c*, 1).
A	100—130°	0·5	1·4180	−14°
B	145—160	1·6	1·4339	+52
C	160—200	0·4	1·4465	+31

Fraction B was mainly methyl *d*-dimethoxysuccinate (Found: C, 47·1; H, 7·0; OMe, 56·3; CO_2Me, 55·2%. Calc. for $C_8H_{14}O_6$: C, 46·6; H, 6·9; OMe, 60·2; CO_2Me, 57·3%).

Isolation of d-*dimethoxysuccinamide.* Fraction B (0·16 g.) was treated with ammonia in dry methyl alcohol. On standing over-night, large crystals separated (0·1 g.), $[\alpha]_{5780}^{19°}$ + 98° (*c*, 1) in water. M. p. 285—290° (decomp.), alone or in admixture with an authentic specimen. A second crop of small crystals had m. p. 285° (decomp.), $[\alpha]_{5783}$ + 89°.

Isolation of d-*dimethoxysuccinomethylamide.* 0·1 G. of fraction B was treated in methyl alcohol (5 c.c.) with methylamine. On concentration feathery crystals of the methylamide separated, m. p. 206° alone or mixed with an authentic specimen prepared from *d*-dimethoxysuccinic ester. $[\alpha]_{5780}^{19°}$ + 137° in water (*c*, 1), m. p. 190° mixed with an authentic sample of *i*-dimethoxysuccinomethylamide.

Oxidation with nitric acid. (II) A syrup (3 g.) prepared as described by means of alkaline potassium hypochlorite was oxidised with nitric acid as before, esterified, methylated, and distilled at 15 mm.

	Bath temp.	Yield, g.	$n_D^{18°}$.	$[\alpha]_{5780}^{25°}$ in MeOH (*c*, 1).
A	100—135°	0·5	1·4200	+ 3°
B	145—155	0·3	1·4340	+50
C	155—165	0·6	1·4355	+55

All three fractions were treated with dry methyl-alcoholic ammonia. A gave oxamide, and *d*-dimethoxysuccinamide, m. p. 280° (decomp.); B gave *d*-dimethoxysuccinamide, m. p. 280° (decomp.); C gave *d*-dimethoxysuccinamide, $[\alpha]_{5780}^{19°}$ + 97° in water (*c*, 1). The total yield of crystalline amide was 0·7 g. Repeated crystallisations after the first crop had been removed yielded no evidence of the presence of any inactive dimethoxysuccinamide.

The Preparation of Dimethyl Ascorbic Acid.—Ascorbic acid (4 g.), dissolved in dry methyl alcohol (30 c.c.), was mixed with dry ether (30 c.c.) and treated at − 5° with diazomethane generated from nitrosomethylurethane (32 c.c.) and 25% methyl-alcoholic potassium hydroxide (48 c.c.). After being kept over-night, the yellow solution on concentration yielded a neutral syrup which ultimately crystallised. Dimethyl ascorbic acid was not oxidised by iodine in neutral or acid solution. It did not reduce Fehling's solution except on prolonged boiling. $[\alpha]_{5780}^{18°}$ + 27° in water (*c*, 1·5) (Found: OMe, 31·0. Calc. for $C_8H_{12}O_6$: OMe, 30·4%).

In alcohol and in water dimethyl ascorbic acid showed an intense absorption band with its head at λ 230 mμ (ε 7000, for an ethyl-alcoholic solution; *c*, 2·5 mg. per 100 c.c.) (see Fig. 1).

Dimethyl ascorbic acid reacts very readily in the cold with $N/10$-sodium hydroxide, one equivalent of alkali being used. The titration resembles that of an acid lactone and is complete in a few minutes at 15°. During the reaction the rotation changes from $[\alpha]_{5780}$ + 27° to $[\alpha]_{5780}$ − 12°. The same change takes place with hot aqueous sodium hydroxide and the product is not affected by continued heating at 90° in $N/10$-alkali. Concentrated alkali effects profound decomposition. The sodium salt does not display selective absorption in the ultra-violet region. On acidification of solutions of the sodium salt ($[\alpha]_{5780}$ − 12°) the rotation remains negative ($[\alpha]_{5780}$ − 6°, constant value). The sodium salt is produced without loss of either of the methoxyl groups of dimethyl ascorbic acid. A weighed quantity (about 100 mg.) of dimethyl ascorbic acid was placed in a small distillation flask together with an excess of $N/10$-sodium hydroxide. The side limb of the flask entered a trap (to retain any liquid which might creep over) and the exit from the trap was a glass tube which led directly into the hydriodic acid in the bulb of a micro-Zeisel apparatus. A current of nitrogen was passed through the apparatus. The flask and trap were kept at 100° with all exposed parts well lagged to prevent undue condensation. The Zeisel apparatus was operated in the usual way. The amount of methyl alcohol which distilled over in the experiments with dimethyl ascorbic acid was negligible (Found : OMe < 2% : experiment continued for 3 hours). During control experiments with methyl dimethoxysuccinate, over 70% of the methyl alcohol liberated on hydrolysis of the ester groups collected in the Zeisel apparatus and was converted into methyl iodide within 2 hours. Confirmation of the above results was obtained by converting dimethyl ascorbic acid into the barium salt by treatment with hot barium hydroxide {Found : OMe, 19·3. $[C_6H_7O_5(OMe)_2]_2Ba$ requires OMe, 21·3%}.

Dimethyl ascorbic acid remained unaltered when heated for 30 minutes at 65° with $N/10$-sulphuric acid. When dimethyl ascorbic acid was heated in a sealed tube at 35° for 12 hours in methyl alcohol saturated with ammonia at 0°, the crystalline substance described by Micheel and Kraft (*loc. cit.*) was formed in small yield and separated on evaporation of most of the solvent. After recrystallisation from methyl alcohol it had m. p. 124°, $[\alpha]_{5780}$ − 24° in 50% aqueous methyl alcohol (*c*, 2·1), in agreement with Micheel and Kraft's observations. We found, however, that this material gave analytical figures in agreement with the formula $C_9H_{19}O_7N$ and not $C_8H_{15}O_6N$ as reported by these authors. Their value for nitrogen is in exact agreement with ours and is much lower than the calculated value for their formula. The substance contains one molecule of combined methyl alcohol, which is lost at 100°. (The methyl alcohol eliminated by heating at 100° was swept forward by a current of carbon dioxide into a micro-Zeisel apparatus and weighed as silver iodide in the usual way. Found : MeOH, 11·6. Calc. for $C_8H_{15}O_6N$,MeOH : MeOH, 12·6%.) The two methyl groups of dimethyl ascorbic acid are retained in the product, which is amidic in character, giving off ammonia on treatment with alkali and reacting as an amide with sodium hypochlorite. It does not show selective absorption in the ultra-violet region (observations carried as far as 200 mμ). It showed unexpected resistance to oxidation by ozone in aqueous acetic acid [Found : C, 42·7; H, 7·6; N (amidic) 5·7; OMe, 35·5. $C_9H_{19}O_7N$ requires C, 42·7; H, 7·5; N, 5·5; OMe, 36·7%].

Tetramethyl Ascorbic Acid.—Dimethyl ascorbic acid (4·5 g.) was treated with silver oxide and methyl iodide in the presence of methyl alcohol at 45° for 5 hours. Three such operations (no methyl alcohol being necessary for the last two) yielded on distillation at 150°/0·06 mm. a syrup (5·0 g.) having $n_D^{17°}$ 1·4690. This syrup gave the correct analytical figures for tetramethyl ascorbic acid. $[\alpha]_{5780}^{20°}$ 0° in water, + 2° in 30% methyl alcohol–water (*c*, 6·0); + 8° in 50% methyl alcohol–water (*c*, 0·8) (Found : C, 51·9; H, 7·2; OMe, 53·1. Calc. for $C_{10}H_{16}O_6$: C, 51·7; H, 6·95; OMe, 53·4%). Tetramethyl ascorbic acid is much less stable towards alkali than the above dimethyl derivative.

Other preparations were carried out in which the yield of the fully methylated product on distillation was not so high owing to the presence of a resinous residue, although it never fell below 50%. The distillates, however, appeared to be identical in all cases except for slight variations in the refractive index ($n_D^{17°}$ 1·4680—1·4690). This may be due to the existence of different enolic modifications. No trace of oxidation was observed during the methylation.

The Ozonisation of Tetramethyl Ascorbic Acid.—(I) *Preparation of a neutral ester.* Tetramethyl ascorbic acid (1·9 g.), dissolved in acetic acid (30 c.c.) and water (6 c.c.), was ozonised during 90 minutes. Removal of solvent was effected at 35—40°/20 mm., methyl alcohol being added in the later stages. A syrup was obtained, a small portion of which was purified for analysis by solution in chloroform and treatment with sodium bicarbonate solution (Found : C, 46·4; H, 6·4; OMe, 44·0. Calc. for $C_{10}H_{16}O_8$: C, 45·5; H, 6·1; OMe, 46·2%).

Hydrolysis of the ester and esterification of the products. The syrup obtained from the previous experiment was dissolved in acetone (10 c.c.) and treated with 0·27*N*-barium hydroxide (120 c.c.). Barium oxalate was immediately precipitated, and removed after warming to 50° and standing for 45 minutes. The solution was then neutralised with *N*-sulphuric acid so that barium still remained in solution; the barium sulphate was removed in a centrifuge, and the solution taken to dryness at 50°/20 mm. The mixture of barium salt and organic acid was esterified during 6 hours with 70 c.c. of 5% methyl-alcoholic hydrogen chloride. The esters were isolated after neutralisation with barium carbonate and extracted from the barium chloride residues with ether (1·2 g.) and fractionally distilled, giving (a) 0·75 g., b. p. 140—150°/20 mm. (bath temp.), $n_D^{16°}$ 1·4395, $[\alpha]_{5780}^{20°}$ + 6° in water (*c*, 1·0) (Found : C, 47·5; H, 8·0; OMe, 49·5; CO_2Me, 35·5. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 52·2; CO_2Me, 33·1%), and (b) 0·1 g., b. p. 150—180°/20 mm. (bath temp.), $n_D^{16°}$ 1·4540. Residue 0·3 g. By treatment of fraction (a) with methyl-alcoholic ammonia an amide was obtained which crystallised spontaneously on removal of the solvent and was readily recrystallised from ethyl acetate (or water), m. p. 113° and mixed with 2 : 4-dimethyl *d*-erythronamide (m. p. 106°), m. p. 85°. $[\alpha]_{5780}^{20°}$ − 34° in water (*c*, 1) (Found : C, 44·4; H, 8·0; OMe, 36·1. Calc. for $C_6H_{13}O_4N$: C, 44·2; H, 8·0; OMe, 38·0%). The amount of crystalline material was, however, not more than 15—20% of the total. After removal of the crystals the syrupy amide which constituted the bulk of the material had $[\alpha]_{5780}^{20°}$ + 29° in water (*c*, 4).

0·025 G. of the above crystalline amide, dissolved in water (0·4 c.c.) and cooled in ice, was treated with 0·11 c.c. of the standard sodium hypochlorite solution described by Weerman (*Rec. trav. chim.*, 1917, **36**, 16). The hypochlorite disappeared more rapidly than in the case of gluconamide and when none remained saturated solutions of semicarbazide hydrochloride and sodium acetate were added. After one minute a copious precipitate of hydrazodicarbonamide came down which had m. p. 255° alone or admixed with an authentic specimen prepared from gluconamide. Control experiments with acetamide and gluconamide emphasised the accuracy of the observations.

The syrup from which the crystals had been removed also gave a strong positive Weerman reaction, the yield of hydrazodicarbonamide being such that almost the whole of it must necessarily have been derived from the 3 : 4-dimethyl *l*-threonamide.

(II). Tetramethyl ascorbic acid (4 g.) was ozonised for 2 hours in acetic acid (60 c.c.) and water (15 c.c.). The subsequent treatment was exactly as described under (I), the ozonisation product being hydrolysed with barium hydroxide and esterified. The amount of oxalate liberated was determined by titration and amounted to only two-thirds of the theoretical quantity (220 c.c. *N*/10-$KMnO_4$). This may have been due to polymerisation during ozonisation or the presence of a different form of the tetramethyl compound due to enolisation. Fractional distillation of the esters gave : (a) 1·67 g., b. p. 140—160°/15 mm. (bath temp.), $n_D^{17°}$ 1·4400, $[\alpha]_{5780}^{20°}$ + 5° in methyl alcohol (*c*, 2·0), − 4° in water (*c*, 1·0); (b) 0·9 g., b. p. 160—200°/15 mm. (bath temp.), $n_D^{17°}$ 1·4730; residue 0·4 g.

Isolation of 3 : 4-*dimethyl* l-*erythronamide.* 0·6 G. of fraction (a) was treated with methyl-alcoholic ammonia. On removal of the solvent partial crystallisation occurred and the amide described above, 3 : 4-dimethyl *l*-erythronamide, m. p. 112°, was isolated. The syrup remaining had $[\alpha]_{5780}^{20°}$ + 25° and both the syrup and the crystalline material gave positive Weerman tests both by the isolation of hydrazodicarbonamide and benzaldehyde semicarbazone.

Conversion of methyl 3 : 4-*dimethyl* l-*threonate into trimethyl* l-*threonamide.* The distillate [fraction (a) above] (1 g.) was methylated three times with methyl iodide and silver oxide and the resulting syrup was distilled, giving 0·8 g., b. p. 135—140°/20 mm. (bath temp.), $n_D^{16°}$ 1·4280, $[\alpha]_{5780}^{18°}$ + 37° in methyl alcohol (*c*, 1·5) (Found : C, 49·5; H, 8·2; OMe, 61·8. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·3; OMe, 64·6%).

0·2 G. of this ester yielded on treatment with methyl-alcoholic ammonia the characteristic amide previously described (0·12 g.), m. p. 77° after one recrystallisation and mixed m. p. 76—77° with an authentic specimen, $[\alpha]_{5780}^{20°}$ + 44° in water (*c*, 1). (XVII) is therefore the main constituent of the degradation products.

(III) *Hydrolysis with ammonia.* Tetramethyl ascorbic acid (1·5 g.) was ozonised in acetic acid (30 c.c.) and water (7 c.c.) during 2½ hours. Solvent was removed at 40—50°/20 mm. and the syrup was dissolved in chloroform, the solution being washed with dilute sodium bicarbonate solution and water. Removal of chloroform yielded a syrup, which was treated with methyl-alcoholic ammonia. Oxamide was precipitated almost immediately (0·27 g. or 54% of the theoretical). The solution was concentrated to a syrup having $[\alpha]_{5780}^{20°}$ + 14° in water. Estimation of the amidic nitrogen indicated that 46% of amide was present, but no crystallisation was

observed. The syrup gave a positive Weerman test, the hydrazodicarbonamide isolated having m. p. 257°.

The syrupy amide (0·9 g.) was heated with 8 c.c. of *N*-sodium hydroxide for 1 hour, the alkali neutralised with hydrochloric acid, and the neutral solution taken to dryness. The residue was esterified with 5% methyl-alcoholic hydrogen chloride (50 c.c.) for 6 hours, after which neutralisation with silver carbonate, filtration and concentration were followed by fractional distillation, giving (a) 0·2 g., b. p. 140—155°/25 mm. (bath temp.), $n_D^{15°}$ 1·4410, $[\alpha]_{5780}^{21°}$ + 11° in methyl alcohol; (b) 0·05 g., b. p. 155—180°/25 mm. (bath temp.), $n_D^{15°}$ 1·4500, $[\alpha]_{5780}^{21°}$ + 7° in methyl alcohol; residue 0·4 g.

On treatment with methyl-alcoholic ammonia fraction (a) gave an amide, $[\alpha]_{5780}^{20°}$ + 11°, which crystallised partly on nucleation with 3 : 4-dimethyl *l*-erythronamide, the crystals having m. p. 113°.

The results obtained by this method therefore are similar to those obtained by methods (I) and (II).

The yield of oxamide produced in duplicate experiments varied from 90 to 50% in the same way as the yield of barium oxalate varied when barium hydroxide was employed to hydrolyse the neutral ester. It is only possible to account for these variations by supposing that different varieties of the tautomeric forms possible by enolisation are present in the fully methylated derivative.

A trimethyl derivative of ascorbic acid can be prepared by methylation with methyl sulphate and alkali in an atmosphere of nitrogen. This substance is markedly different from the neutral dimethyl derivative prepared by the action of diazomethane, since the trimethyl derivative is definitely acidic in character.

Properties of Acetylpyruvic Acid.—This substance was prepared by Claisen and Stylos' method (*Ber.*, 1887, **20**, 2188). It did not react with iodine in aqueous acid solution and in alkaline solution it hydrolysed rapidly, giving oxalic acid and acetone (contrast with ascorbic acid). In aqueous solution it gives a strong absorption band at λ 285 mμ; ε 4000 (*c*, 2 mg. per 100 c.c.).

Properties of Dihydroxymaleic Acid.—In aqueous solution dihydroxymaleic acid has a strong band at λ 290 mμ, ε = 6700 (*c*, 2·4 mg. per 100 c.c.) and at λ 300 mμ in alcohol, ε 7500 (*c*, 1·8 mg. per 100 c.c.). The substance reacts with aqueous iodine (2 atomic proportions), giving dihydroxytartaric acid, 2 mols. of hydrogen iodide being liberated in the process (0·092 g. required 9·8 c.c. of *N*/10-iodine, and 10 c.c. of *N*/10-NaOH were required to neutralise the hydriodic acid liberated. Calc. for $C_4H_4O_6,2H_2O$: 10 c.c. of iodine and 10 c.c. of NaOH). The same oxidation can be carried out with chlorine water. Spectrophotometric examination of the oxidised solution showed that the band had disappeared entirely, the product showing no selective absorption (compare ascorbic acid). When the solution (after oxidation with iodine) was evaporated to dryness in a vacuum desiccator, reduction took place with liberation of iodine. The iodine was removed by sublimation in a current of air. The product (yield, quantitative) had all the properties of the original dihydroxymaleic acid. Alcoholic iodine solutions have no action on dihydroxymaleic acid in the absence of water. Anhydrous dihydroxymaleic acid reacted rapidly with diazomethane in ether containing some methyl alcohol, giving methyl dihydroxymaleate, which reacted more slowly, giving the enolic dimethyl ether (Found: OMe, 53·3%, after one treatment with diazomethane). Methyl dihydroxymaleate (prepared by Fenton's method, J., 1894, **65**, 905) reacted with aqueous iodine in the same manner as the free acid. The fully methylated substance was most conveniently obtained by one treatment of the acid with diazomethane, followed by methylation with silver oxide and methyl iodide.

Methyl dimethoxymaleate was a mobile liquid, b. p. 85°/0·03 mm., $n_D^{15°}$ 1·4525 (Found: OMe, 58·0. $C_8H_{12}O_6$ requires OMe, 60·8%). It did not react with neutral or acid iodine and had negligible reducing power even in alkaline solution. In alcoholic solution it showed an intense absorption band at λ 255 mμ, ε 6000 (*c*, 2·7 mg. per 100 c.c.).

The work now described was rendered possible by the kindness of Prof. A. Szent-Györgyi, who generously provided the ascorbic acid, and to him and his collaborator, Dr. Svirbely, we desire to express our thanks and appreciation. Some of the material was prepared in the Biochemical Laboratory of the University of Cambridge. Another supply came from the Mayo Clinic, Rochester, New York, and the remainder from the Medical Chemistry Department of the University of Szeged. It was by the wish of Prof. Szent-Györgyi that the chemical investigation of ascorbic acid was undertaken in Professor W. N. Haworth's laboratories and we wish to

record our gratitude to Professor Haworth for providing facilities for the work and for his constant encouragement and continued interest in its progress. We are indebted also to Mr. E. G. Cox, whose crystallographic and *X*-ray investigations have been of the greatest assistance to us at various stages of the work.

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NOTE.

The Nitration of Disulphonanilides. By F. BELL and R. COHEN.

ALTHOUGH the di-*m*-nitrobenzenesulphonyl derivatives of aniline and *o*- and *p*-toluidine are unacted upon by concentrated nitric acid, yet with the fuming acid they undergo mononitration. The nitro-group enters the *m*- and the *p*-position in aniline, the 2 and 4 positions in *o*-toluidine, and the 2 position in *p*-toluidine (Me = 1). These results are comparable with those of Brady, Quick, and Welling (J., 1925, 127, 2264) from the nitration of the corresponding phthalanils.

The following disulphonanilides were prepared by allowing the amine and *m*-nitrobenzenesulphonyl chloride (2½ mols.) to interact in pyridine solution for several days. The products were repeatedly boiled with acetic acid until all the more soluble sulphonanilide had been eliminated. *Di*-m-*nitrobenzenesulphon-anilide,* needles, m. p. 189° (Found : N, 9·0. $C_{18}H_{13}O_8N_3S_2$ requires N, 9·1%), -p-*toluidide,* m. p. 199° (Found : N, 8·7. $C_{19}H_{15}O_8N_3S_2$ requires N, 8·8%), and -o-*toluidide,* m. p. 226° (Found : N, 8·8%).

Nitration of Di-m-*nitrobenzenesulphonanilide.*—10 G. were added slowly to fuming nitric acid (30 c.c.), and the resultant solution poured on ice. The precipitate (11 g., m. p. 195—205°) could not be purified by boiling with acetic acid or by fractional precipitation from pyridine by means of ethyl alcohol. 5 G. were left with sulphuric acid for several hours and the mixture poured into water. The product on extraction with acetic acid left di-*m*-nitrobenzenesulphon-*m'*-nitroanilide, m. p. 235° (0·8 g.) (J., 1930, 1077), and *m*-nitrobenzenesulphon-*p'*-nitroanilide, m. p. 180° (J., 1929, 2788), was isolated from the filtrate. Both were identified by comparison with authentic specimens, and the former also by scission with warm piperidine, *m*-nitrobenzenesulphonylpiperidine, m. p. 124°, and *m*-nitrobenzenesulphon-*m'*-nitroanilide, m. p. 151°, being obtained.

Nitration of Di-m-*nitrobenzenesulphon*-p-*toluidide.*—3 G. were added to nitric acid (10 c.c.) and the solution poured on ice. The precipitate (3·3 g., m. p. *ca.* 205°) after boiling with acetic acid gave pure *di*-m-*nitrobenzenesulphon*-2'-*nitro*-p-*toluidide,* m. p. 208°. (Found : N, 10·8. $C_{19}H_{14}O_{10}N_4S_2$ requires N, 10·7%). It was left with sulphuric acid for several hours, and the clear solution poured into water. The precipitate after crystallisation from acetic acid formed prisms, m. p. 136°, alone or mixed with an authentic specimen of m-*nitrobenzenesulphon*-2'-*nitro*-p-*toluidide* (Found : N, 12·6. $C_{13}H_{11}O_6N_3S$ requires N, 12·5%).

Nitration of Di-m-*nitrobenzenesulphon*-o-*toluidide.*—3 G. as above gave a product readily separable by acetic acid into a less soluble, m. p. 221°, and a more soluble part, needles, m. p. *ca.* 95°, or after heating to remove acetic acid, m. p. 185°. The less soluble part on hydrolysis with sulphuric acid gave 4-nitro-*o*-toluidine; it must therefore be *di*-m-*nitrobenzenesulphon*-4'-*nitro*-o-*toluidide* (Found : N, 10·9%). The other, which was not identical with the already-described 3'- and 5'-nitro-compounds (J., 1930, 1077), must be *di*-m-*nitrobenzenesulphon*-6'-*nitro*-o-*toluidide* (Found : N, 10·9%). By solution in piperidine it was severed to give m-*nitrobenzenesulphon*-6'-*nitro*-o-*toluidide* (Found : N, 12·4%), which crystallised from acetic acid in needles, m. p. 148°.—BATTERSEA POLYTECHNIC, S.W. 11. [*Received, July 25th,* 1933.]
