Tetrahedron Vol 26, pp. 3833 to 3838 Pergamon Press 1970 Printed in Great Britain

STRUCTURE AND MUTAROTATION OF ASCORBIC ACID OSAZONES*

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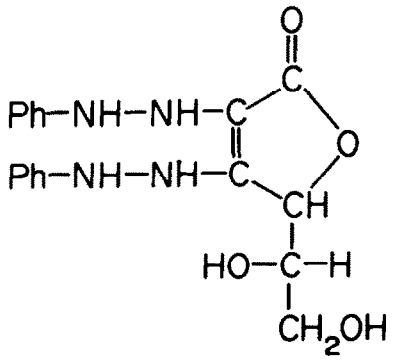
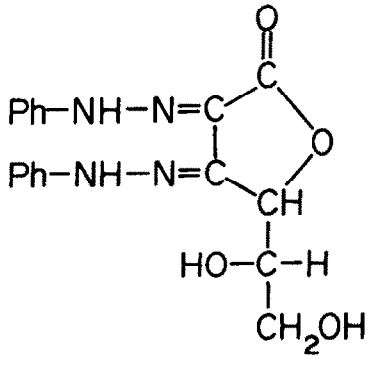
(*Received in the UK* 31 *March* 1970; *accepted for publication* 8 *April* 1970)

Abstract—A study of ascorbic acid osazones by spectrosocopic methods shows that they are γ-lactones, not δ-lactones as claimed recently. It is found that they mutarotate in solution. Mutarotation involves geometric isomerization of the hydrazone moieties and replacement of one chelate system by another. The path for isomerization is discussed.

IN THEIR work on the constitution of ascorbic acid, Hirst *et al.*[1] obtained a variety of phenylhydrazine derivatives containing two moieties of the reagent under different experimental conditions. They recognized the possibilities for structural isomerism and stereoisomerism in these derivatives as well as the difficulty of assigning precise structures to them. For two of these derivatives, a red product (m.p. 187°) and an orange product (m.p. 216°), they suggested the dihydrazide and osazone structures (I and II) respectively. They also found that both *p*-nitrophenylhydrazine and 2,4-dinitrophenylhydrazine gave at least two derivatives each. Although one of the popular



I



II

methods of estimation[2] of ascorbic acid uses the 2,4-dinitrophenylosazone, its existence in more than one form has not been reported in later work. The phenylosazone obtained by Ohle[3] and Antener[4] from dehydroascorbic acid corresponded to the orange derivative reported by Hirst *et al.* Further confirmation of the identity of the osazones of ascorbic acid and dehydroascorbic acid was obtained by Hasselquist.[5] Thus, the present position is that only one form is recognized for any particular

* Communication No. 1445, National Chemical Laboratory, Poona 8, India. The osazones required for this study were prepared at the Central Food Technological Research Institute.

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osazone of ascorbic acid. This would imply that the differences reported by Hirst *et al.* are to be traced either to inadequate purification for some of the samples or to differences in crystal structure. A further possibility, which has been suggested recently,[6] is the existence of these osazones in δ-lactone forms. We have re-examined the structures of these derivatives in the context of our interest[7] in H-bonded and tautomeric systems by spectroscopic methods and found that they display *cis-trans* isomerism and undergo structural changes in solution that are analogous to those found for the mutarotation of sugar osazones.[8,9]

Samples of ascorbic acid phenylosazone, prepared by reaction in aqueous solution and in mixtures of glacial acetic acid and 2-methoxyethanol, were found to be identical. They showed the same m.p. (212°, uncorrected) after crystallization from alcohol and their NMR and IR spectral features were the same. A section of the 60 MHz NMR spectrum of the osazone obtained in DMSO-d_6, namely the region from 5 to 13δ* with which we shall mostly be concerned here, is shown in Fig 1. This shows

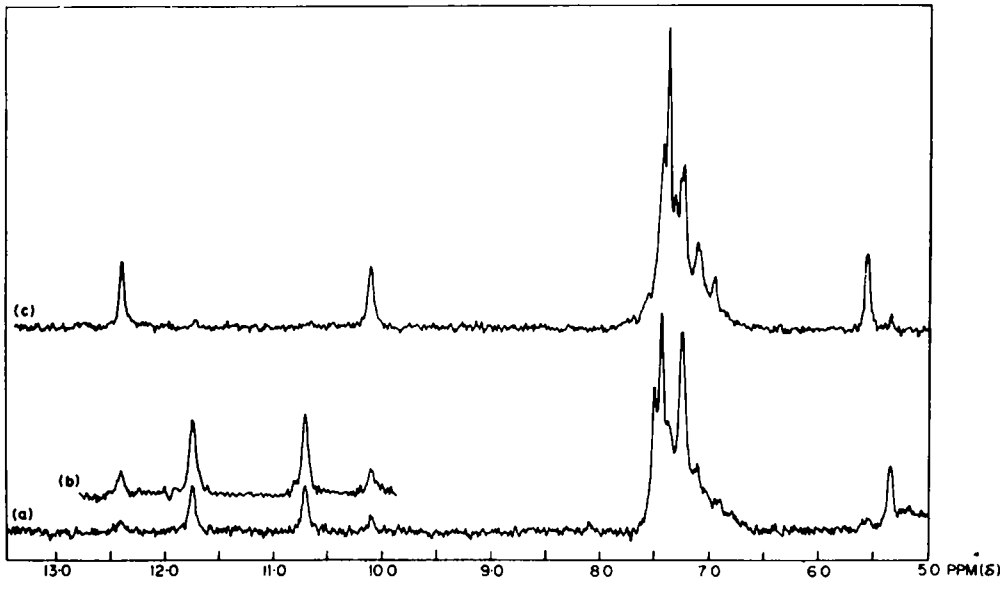


FIG 1. NMR spectrum of ascorbic acid phenylosazone in DMSO-d_6: (a) Initial spectrum, (b) Spectrum after a few minutes, and (c) Spectrum after 6 days

two resonances at 10·85 and 11·86 which may be assigned to imino protons of the hydrazone units. The absorption positions indicate that both protons are H-bonded. The aromatic proton absorptions occur in the range 6·7 to 7·7 and a rather broad singlet assignable to the proton at the γ-C atom (C-4) of the γ-lactone ring of ascorbic acid, is seen at 5·33. Under favourable conditions this absorption could be resolved into a doublet ($J=2$Hz). The absorptions of the C-6 methylene and the C-5 methine protons appear at higher field at about 3·56 and 3·85 respectively.

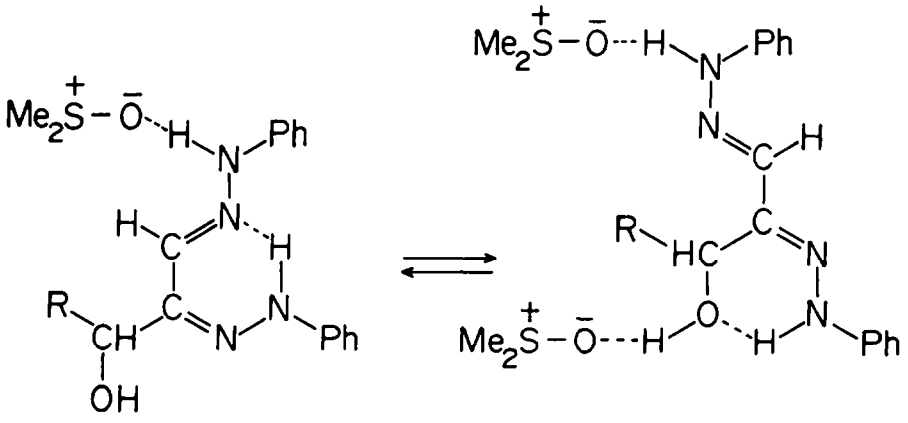
* All chemical shifts are expressed as p.p.m. downfield from tetramethylsilane as internal standard (δ scale).

It has recently been claimed[6] that ascorbic acid phenylosazone is a δ-lactone on the basis of the NMR spectrum of its diacetate, the CO absorption of the osazone at 1740 cm^{-1} and its reluctance to undergo periodate cleavage. The IR absorption was considered to be at too low a frequency for a γ-lactone. In the NMR spectrum of the diacetate, the multiplet absorption of the C-5 proton occurs at slightly lower field than that of the C-4 proton which is a doublet. The authors contended that this would not have been so if the γ-lactone structure is correct. The relative absorption positions of the C-4 proton (5·33, Fig 1) and the C-5 proton (3·85, multiplet) in the osazone spectrum show that the ester linkage is to C-4 and that a δ-lactone structure cannot be considered at all. The lowering of the IR frequency of the lactone CO has its origin in H-bonding, as will be seen later in this paper. The reported failure of the osazone to undergo periodate cleavage is perhaps to be traced to its insolubility in the reaction medium employed. Although Khadem and Ashry[6] obtained their samples by a procedure different from ours, the possibility that they were working with a true δ-lactone appears remote.

A very remarkable change was seen when the NMR spectrum of the osazone was rescanned after a few minutes. The imino proton absorptions at 10·85 and 11·86 diminished in intensity and a new pair of signals began to appear at 10·25 and 12·53. A parallel change was seen for the absorption of the C-4 proton, the new signal appearing slightly downfield at 5·53. On standing for about 6 days, the original set of three signals was replaced almost completely by the new set. These changes are shown in Fig 1. Similar changes were recorded for IR and electronic spectra of the osazone. Also, its optical rotation in solution decreased with time towards an equilibrium value. We may call the basic structural transformation responsible for these changes "mutarotation" by analogy with the behaviour of sugar osazones.

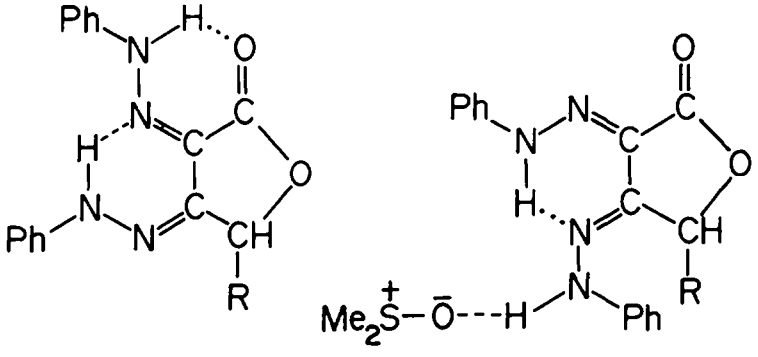
The osazone showed CO IR bands at 1735 and 1765 cm^{-1} in DMSO solution. On standing for a few days, the former band almost disappeared while the latter became intense. In dioxan solution the transformation proceeded to about 60% completion. A fresh solution showed a very strong band at 1745 cm^{-1} and a weak one at 1780 cm^{-1} and, when equilibrium was attained, the bands were of comparable intensity, the latter being stronger. A parallel change in the electronic absorption spectrum accompanied mutarotation. The absorption maxima at 460 and 360 mμ shifted to 440 and 350 mμ respectively. A freshly prepared solution of the osazone had an $(\alpha)_D^{25}$ of + 340° which fell off to + 110° in 72 hr. When the mutarotated osazone was recovered from solution and recrystallized from alcohol, it gave back the original osazone.

In order to clarify the nature of the structural change we have called mutarotation, it is useful to recall the behaviour of sugar osazones.[8,9] NMR spectra of sugar osazones in DMSO solutions (freshly prepared) show imino proton absorptions in the two ranges 12·0 to 12·3 and 10·6 to 10·8. The absorptions in the latter range have been shown to represent the solvent bonded NH groups of the hydrazone units attached to C-1 as in the chelate structure III and those in the former range, at lower field, the NH groups involved in chelation. During mutarotation chelation is broken and for any sugar osazone two new solvent bonded N--H signals appear at higher field. It has been suggested by Chapman *et al.*[9] that these as well as other changes that accompany mutarotation may be rationalized in terms of the transformation of III to IV. The downfield shifts observed for the C-3 proton and the C-3 OH proton on mutarotation have been attributed to intramolecular H-bonding as shown in IV.



III IV

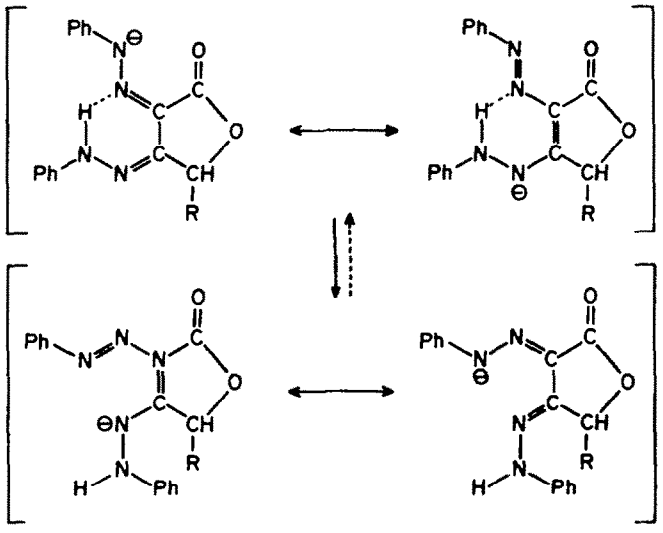
From the foregoing, it is clear that the structural changes in the mutarotation of ascorbic acid phenylosazone are not quite the same as in the mutarotation of sugar osazones. Unlike in the case of sugar osazones, here chelation does not disappear. One chelate system disappears and another appears instead. The spectral data may be rationalized in terms of structure V for the osazone, which appears to be altered to VI in mutarotation. In V the lactone CO is H-bonded intramolecularly to NH and this bonding disappears in VI. The change in CO IR absorption from 1735 to 1765 cm^{-1} in DMSO (and from 1745 to 1780 cm^{-1} in dioxan) is consistent with this formulation. Since imino protons involved in N—H . . . N type chelation absorb at about 12 and since a large upfield shift is involved in the change of H-bonding from this type to the



V VI

N—H . . . O type (solvent bonded or chelated), it is possible to give specific assignments to the two sets of NH absorptions. The absorptions seen initially at 10·85 and 11·86 have to be assigned to NH groups of the C-1 and C-2 hydrazone units respectively, and these are shifted to 12·53 (downfield) and 10·25 (upfield), respectively, on mutarotation. It is thus clear that the configurations of both hydrazone units are altered in the process. This isomerization, which takes place faster in pyridine, is apparently

base catalyzed. The course of the transformation may be tentatively discussed in terms of the type of mechanism suggested by Chapman *et al.* for the mutarotation of sugar osazones although it does not explain the driving force involved. They have suggested that the isomerization is initiated by the abstraction, by the basic solvent, of the imino proton involved in chelation in III. In the anion thus produced the barriers to rotation around C—N bonds are reduced because of reduced mobile bond order resulting from π-electron delocalization, and the species reprotonates after rearrangement to the configuration shown in IV. It is not clear why the alternative scheme involving ionization of the NH group of the C-1 hydrazone unit should be excluded. In such a scheme the isomerization of the anion would involve break up of chelation as well. The case of ascorbic acid osazone would indicate that such a process would also be possible if the geometries of both hydrazone units are changed through the same anion as in the conversion of VII to VIII. Since there are two chelate rings to start with, there



would always be one left behind to be broken during isomerization. The alternative possibility for this case is a two stage isomerization where chelation is broken only by solvent attack and only one hydrazone unit is involved in each stage. The rates of disappearance of the initial N-H signals of the NMR spectrum (or of the appearance of the new signals) are the same. This might be deemed to favour a single stage mechanism. Since mutarotation is not a very fast process in DMSO, one would normally expect a two stage isomerization to be recognizable by the NMR method.

Samples of the phenylosazone prepared by prior oxidation of ascorbic acid with iodine and subsequent treatment with phenylhydrazine showed an extra low field signal at 10·55. In the aromatic proton range also, the superposition of additional absorption bands was evident. The samples were apparently contaminated with a side product which we have not yet identified. The same impurity tended to appear in samples of the osazone prepared by charcoal catalyzed oxidation of ascorbic acid and subsequent treatment with phenylhydrazine.

The behaviour of 2,4-nitrophenylosazone of ascorbic acid was found to be similar to

that of the phenylosazone; but only a small part of it (the former) was isomerized in mutarotation in DMSO solution. The IR carbonyl absorptions corresponding to forms V and VI were at 1745 and 1780 cm^{-1}. The N—H signals corresponding to V appeared at 12·58 and 14·24, and for VI, these absorptions were replaced by another pair at 13·53 and 12·53. The high field absorption of the mutarotated form was not quite resolved from the high field absorption of unmutarotated form, and it appeared as a broadening of the base of the latter on the high field side. The C-4 proton signals were located at 5·54 and 5·70, and they confirmed that the derivative is a γ-lactone.

EXPERIMENTAL

Ascorbic acid phenylosazone

Procedure (*a*): To a 20% aqueous soln. of ascorbic acid (Sarabai Merck Ltd.), 3 mole equiv of phenylhydrazine hydrochloride were added together with NaOAc. The mixture was allowed to stand at room temp for 3 days when a brick-red ppt was formed. This was filtered off and washed several times with water. The ppt was pressed dry and recrystallized from alcohol.

Procedure (*b*). A mixture of ascorbic acid (10 g), glacial AcOH (10 ml) and 2-methoxyethanol (40 ml) was heated to about 80° on a boiling waterbath. Phenylhydrazine (25 ml) was added and heating continued for about 1 hr. The soln became deep red. It was then poured into a litre of water with stirring. The deep red ppt was collected by filtration under suction and washed several times with water. The ppt when pressed dry and recrystallized from EtOH gave the osazone as orange-red needles (yield, 10 g).

The osazone samples obtained by procedures (a) and (b) were identical. They both melted at 212° (dec) and had the same NMR and IR spectral characteristics. (Found: C, 61·13; H, 4·86; N, 16·46; $C_{18}H_{18}N_4O_4$ requires: C, 61·02; H, 5·08; N, 15·83%).

Procedure (*c*). Ascorbic acid was oxidized in aqueous soln with I_2 in KI. The reaction was taken to be complete when a permanent pale-yellow colour appeared. Phenylhydrazine hydrochloride and NaOAc were added without neutralizing the HI formed. The soln was kept at room temp for 3 days. The brick-red ppt which separated was filtered under suction, washed several times with water and recrystallized from EtOH to give brick-red needles, m.p. 216° (dec).

2,4-*Dinitrophenylosazone of ascorbic acid.* Ascorbic acid and 2,4-dinitrophenylhydrazine in the molar ratio 1:3 were refluxed in a large excess of EtOH containing a small quantity of water. Long needles, brick-red in colour, appeared within 8 hr heating under reflux. The product was collected and recrystallized from alcohol–acetone to give brick-red needles, m.p. 280° (dec) (Found: C, 40·32; H, 2·55; N, 20·46. $C_{18}H_{14}N_8O_{12}$ requires: C, 40·30; H, 2·98; N, 20·89%).

Acknowledgement—We are grateful to Dr. M. Srinivasan for his kind interest in the progress of this study.

REFERENCES

[1] R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds and F. Smith, *J. Chem. Soc.* 1270 (1933)
[2] J. H. Roe, *Ann. N.Y. Acad. Sci.* **92**, 277 (1961) and ref therein
[3] H. Ohle, *Ber. Dtsch. Chem. Ges.* **67**; 1750 (1934)
[4] I. Antener, *Helv. Chim. Acta* **20**, 742 (1937)
[5] H. Hasselquist, *Arkiv. Kemi* **4**, 369 (1952); *Chem. Abstr.* **47**, 9267c (1953)
[6] H. El-Khadem and S. H. El-Ashry, *J. Chem. Soc.* (C), 2247 (1968)
[7] B. L. Kaul, P. Madhavan Nair, A. V. Rama Rao and K. Venkataraman, *Tetrahedron Letters* 3897 (1966)
[8] L. Mester and A. Major, *J. Am. Chem. Soc.* **79**, 3232 (1957)
[9] O. L. Chapman, R. W. King, W. J. Welstead, Jr. and T. J. Murphy, *Ibid.* **86**, 4968 (1964)