The Structure of Dehydro-L-ascorbic Acid Phenylosazone

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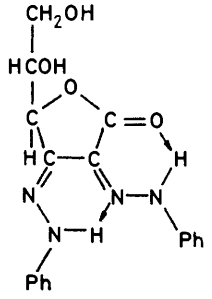
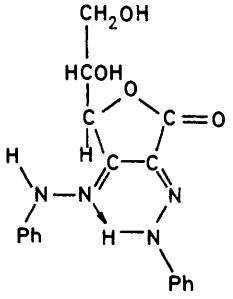
Dehydro-L-ascorbic acid phenylosazone has been examined spectroscopically and found to be 2,3-dideoxy-3-phenylazo-2-phenylhydrazino-L-*threo*-hex-2-enone-1,4-lactone. Some differences in its behaviour compared to D-glucose phenylosazone are explained on the basis of this structure.

ALTHOUGH the behaviour of the phenylhydrazine residues of dehydro-L-ascorbic acid phenylosazone [1] on reduction,[2] oxidation,[3] and acylation [3] is different from that of the phenylhydrazine residues of sugar phenylosazones, such as D-glucose phenylosazone,[4] only one study [5] has been concerned with this aspect of the structure. In that study it was proposed that the osazone has the bi-phenylhydrazone structure (1) mutarotating in solution to (2).



(1)



(2)

In view of the differences in behaviour [2,3] the structure of dehydro-L-ascorbic acid phenylosazone has been investigated to determine the nature of the phenylhydrazine residues.

RESULTS AND DISCUSSION

It is necessary to establish, unequivocably,† the size of the lactone ring in dehydro-L-ascorbic acid phenylosazone. El Khadem and El Ashry originally proposed [3a] a 1,5-lactone ring because treatment with HIO_4 failed to show the presence of an α-glycol group, and because the C=O absorption band at 1 720 cm^{-1} is at too low a frequency for a normal 5-membered lactone ring. However, they later concluded from studies of the behaviour of dehydro-L-ascorbic acid monophenylhydrazone that the osazone contains a 1,4-lactone ring.[3c]

Dehydro-L-ascorbic acid phenylosazone readily forms a benzylidene derivative with no change in the lactone ring size, as shown by the similar absorption frequency (1 720 cm^{-1}) for the C=O group in both compounds. That this is a 5,6-*O*- and not a 4,6-*O*-benzylidene derivative is shown by the fact that it can also be prepared from dehydro-L-ascorbic acid monophenylhydrazone by formation of the benzylidene derivative and reaction of this with phenylhydrazine. Since the monophenylhydrazone has been shown by n.m.r. spectroscopy to have a 1,4-lactone ring [3c] and no change in ring size occurs on benzylidation, as shown by a similar absorption frequency (1 755 cm^{-1}) for the lactone C=O group in both the parent monophenylhydrazone and the benzylidene derivative, dehydro-L-ascorbic acid phenylosazone must have a 1,4-lactone ring.

The principal absorption band in the u.v./visible spectrum of a fresh solution of dehydro-L-ascorbic acid phenylosazone has [5] $\lambda_{max.}$ 462 nm (log ε 4.27) changing on mutarotation to 444 nm (log ε 4.31). Thus both fresh and mutarotated solutions have their principal absorption band at a considerably longer wavelength than do hexose phenylosazones [6] ($\lambda_{max.}$ 390—398 nm, log ε 4.31). Similarly, dehydro-L-ascorbic acid *p*-nitrophenylosazone has $\lambda_{max.}$ 478 nm (log ε 4.44) compared to D-glucose *p*-nitrophenylosazone which has [7] $\lambda_{max.}$ 448 nm (log ε 4.49).

These bathochromic shifts in the principal absorption bands might be due to either the presence of the 5-membered ring or to the presence of the carbonyl group adjacent to the C(2) phenylhydrazine residue. To test these possibilities the 1,2-bis(phenylhydrazone) [8] and the 1,2-bis-(*p*-nitrophenylhydrazone) of mesoxaldehyde have been prepared, together with the phenylosazone and *p*-nitrophenylosazone of cyclopentane-1,2-dione. The u.v. spectra of these compounds (Table) show that whilst

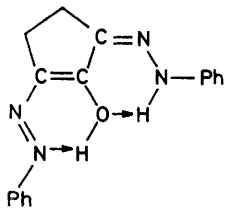
	$\lambda_{max.}$/nm	log $\varepsilon_{max.}$
Dehydro-L-ascorbic acid phenylosazone [5]	462	4.27
D-Glucose phenylosazone [6]	390	4.31
Mesoxaldehyde 1,2-bis(phenylhydrazone) [8]	415	4.30
Cyclopentane-1,2-dione phenylosazone	388	4.25
Dehydro-L-ascorbic acid *p*-nitrophenylosazone	478	4.44
D-Glucose *p*-nitrophenylosazone [7]	448	4.49
Mesoxaldehyde 1,2-bis-(*p*-nitrophenylhydrazone)	450	4.44
Cyclopentane-1,2-dione *p*-nitrophenylosazone	446	4.31

there is a bathochromic shift in the $\lambda_{max.}$ for mesoxaldehyde 1,2-bis(phenylhydrazone) when compared with D-glucose phenylosazone, it is still at considerably shorter wavelength than is $\lambda_{max.}$ of dehydro-L-ascorbic acid phenylosazone. The shift is extremely small for the analogous *p*-nitrophenylhydrazine derivatives and in both cases the presence of a 5-membered ring has a slight hypsochromic effect. These results show that neither the 5-membered ring nor the adjacent carbonyl group can be responsible for the difference in the spectra of the osazones of D-glucose and dehydro-L-ascorbic acid.

In addition to the principal absorption band at 478 nm the spectrum of dehydro-L-ascorbic acid *p*-nitrophenylosazone shows a second band at 370 nm (log ε 4.3). This band is not present in the spectrum of the phenylosazone but is similar to the absorption band due to the NH·C_6H_4·NO_2-*p* group in other compounds.[9,10]

† The author wishes to thank one of the referees for pointing out the uncertainty in the literature concerning the ring size.

The value of $\lambda_{max.}$ for dehydro-L-ascorbic acid phenylosazone is much closer to those of several 2-oxo-1,3-bis(phenylhydrazono)-compounds [11] ($\lambda_{max.}$ 460—488 nm, log ε 4.46—4.58) than it is to those of conventional sugar phenylosazones. These compounds have been shown [11] to have a chelated phenylhydrazono-phenylazo-structure, *e.g.* (3) and although dehydro-L-ascorbic acid cannot form a 2-oxo-1,3-bis(phenylhydrazono)-derivative,[11] the phenylosazone may be considered as a 3-oxo-1,2-bis(phenylhydrazono)-derivative and structure (4)



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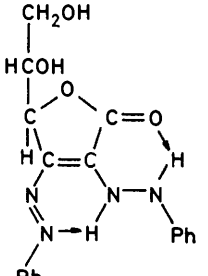


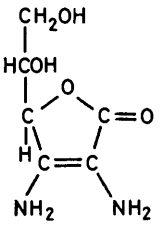
(4)

can be drawn. Both (3) and (4) contain a similar conjugated system that can be represented as X=C–C=C–N=N–C_6H_5. Furthermore, the u.v. spectrum of 2-oxo-1,3-bis-(*p*-nitrophenylhydrazono)indan is similar to that of dehydro-L-ascorbic acid *p*-nitrophenylosazone, with $\lambda_{max.}$ 485 nm (log ε 4.32) together with a second band at 382 nm (log ε 4.35).

The i.r. spectrum of dehydro-L-ascorbic acid phenylosazone shows a strong band at 1 580 cm^{-1} in addition to that at 1 605 cm^{-1} (phenyl ring). A strong absorption band at 1 585—1 570 cm^{-1} has been assigned to a normal aromatic ring vibration intensified by conjugation with an unsaturated group,[12] and more specifically to the phenylazo group.[13] A similar band in the spectra of the 2-oxo-1,3-bis(phenylhydrazono)-compounds has been assigned [11,14] to the phenylazo-group. This supports (4) rather than (1). The conjugation of the carbonyl group in (4) is the reason for its absorption band appearing at a lower frequency than is normal for a 5-membered lactone ring.

While catalytic hydrogenation of D-glucose phenylosazone under neutral conditions yields the saturated 1,2-diamino-1,2-dideoxy-D-mannitol,[4] similar treatment of dehydro-L-ascorbic acid phenylosazone gives [2] the unsaturated 2,3-diamino-2,3-dideoxyhex-2-enone-1,4-lactone (5). This indicates the presence of C(2)–C(3) unsaturation in the parent osazone, as in (4). The difference in oxidative behaviour [3] relative to that of the sugar phenylhydrazones can be explained by the presence of the C(2) phenylhydrazine group. The first step in the reaction is presumably oxidation of the –NH–NH– group of (4) to an azo-group (6) followed by double-bond rearrangement and cyclisation to give (7).

N.m.r. studies show that during mutarotation of the unacetylated osazone one of the chelate rings opens up, whilst the u.v. spectrum shows a decrease in $\lambda_{max.}$ and an increase in $\varepsilon_{max.}$. On working up the mutarotated solution only the starting material is recovered. These changes were attributed [5] to isomerization between (1) and (2) but they can also be explained as due to isomerisation between (4) and (8). The spectral changes that



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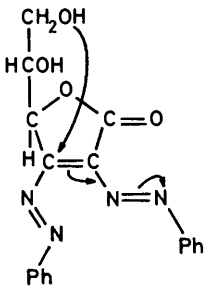

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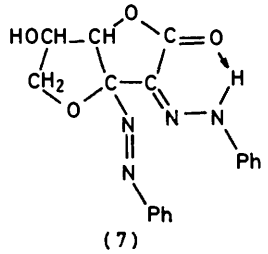

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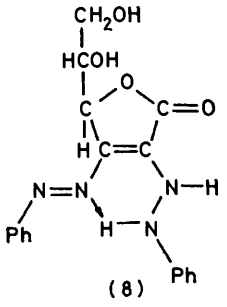

(8)

occur on mutarotation, and the fact that only starting material is recovered on working up the mutarotated solution, are similar to those reported for the mutarotation of D-glucose phenylosazone.[15] This latter process involves the breaking of a chelate ring, followed by a conformational change to a more transoid structure.[16,17] Thus, the proposed structural change for dehydro-L-ascorbic acid phenylosazone is consistent with the change undergone by sugar osazones.

Thus, the spectroscopic data and the chemical behaviour of dehydro-L-ascorbic acid phenylosazone show it to be 2,3-dideoxy-3-phenylazo-2-phenylhydrazino-L-*threo*-hex-2-enone-1,4-lactone (4).

EXPERIMENTAL

U.v. spectra were recorded using a Unicam SP spectrophotometer using spectroscopically pure methanol as solvent.

Cyclopentane-1,2-dione Phenylhydrazone.—This was prepared from cyclopentanone (8.85 ml) by the method given in the literature for cyclohexane-1,2-dione phenylhydrazone,[18] yield 8.9 g, m.p. 193 °C (Found: C, 70.3; H, 6.35; N, 14.75. $C_{11}H_{12}N_2O$ requires C, 70.21; H, 6.38; N, 14.89%).

Cyclopentane-1,2-dione p-*Nitrophenylhydrazone.*—Cyclopentanone (8.85 ml) was treated as described in the literature [18] for cyclohexanone and after adjusting the pH to 7 the aqueous extract was treated with diazotised *p*-nitroaniline (12 g) and set aside overnight at room temperature. The solid which formed was filtered off and recrystallised from methanol to give yellow crystals of cyclopentane-1,2-dione *p*-nitrophenylhydrazone, m.p. 249—250 °C (Found: C, 56.7; H, 4.7; N, 17.95. $C_{11}H_{11}N_3O_3$ requires C, 56.65; H, 4.7; N, 18.0%).

Cyclopentane-1,2-*dione Phenylosazone*.—Cyclopentane-1,2-dione phenylhydrazone (1 g) was dissolved in the minimum quantity of boiling methanol and phenylhydrazine (1 ml) added. After refluxing the solution for 30 min followed by cooling, cyclopentane-1,2-dione phenylosazone crystallised out. This was recrystallised from methanol, m.p. 140 °C (Found: C, 73.3; H, 6.4; N, 20.1. $C_{17}H_{18}N_4$ requires C, 73.4; H, 6.5; N, 20.1%).

Cyclopentane-1,2-*dione* p-*Nitrophenylosazone*.—Cyclopentane-1,2-dione *p*-nitrophenylhydrazone (1 g) was dissolved in boiling methanol and *p*-nitrophenylhydrazine (1 g) in methanol added. After refluxing for 30 min the mixture was cooled and the precipitate filtered off and recrystallised from methanol to give dark red crystals of cyclopentane-1,2-dione *p*-nitrophenylosazone, m.p. 272—273 °C (Found: C, 55.5; H, 4.4; N, 22.7. $C_{17}H_{16}N_6O_4$ requires C, 55.4; H, 4.35; N, 22.8%).

Mesoxaldehyde 1,2-*Bis*-(p-*nitrophenylhydrazone*).— D-Glucose *p*-nitrophenylosazone (1 g) was suspended in aqueous methanol and sodium periodate (2 g) added. After 240 h at room temperature the suspension was filtered and the solid recrystallised from methanol to give dark red crystals of mesoxaldehyde 1,2-bis-(*p*-nitrophenylhydrazone), m.p. 224—226 °C (Found: C, 52.75; H, 3.6; N, 24.8. $C_{15}H_{12}N_6O_5$ requires C, 52.9; H, 3.5; N, 24.7%).

2-*Oxo*-1,3-*bis*-(p-*nitrophenylhydrazono*)*indan*.— Ninhydrin (indan-1,2,3-trione monohydrate) (1 g) was treated with *p*-nitrophenylhydrazine (2.5 g) in 75% aqueous acetic acid. The precipitate that formed was filtered off and recrystallised from aqueous dimethylformamide to give dark red crystals of 2-oxo-1,3-bis-(*p*-nitrophenylhydrazono)indan, m.p. 313—315 °C (Found: C, 58.4; H, 3.4; N, 19.5. $C_{21}H_{14}N_6O_5$ requires C, 58.6; H, 3.25; N, 19.55%).

5,6-O-*Benzylidenedehydro*-L-*ascorbic Acid Monophenylhydrazone*.—Dehydro-L-ascorbic acid monophenylhydrazone [3c] (1 g) was suspended in benzaldehyde (15 ml) and finely ground fused zinc chloride (2 g) added. The mixture was shaken for 15—20 min, during which time the phenylhydrazone dissolved. The mixture was poured onto crushed ice (500 g) and, after the ice had melted and the water decanted, was taken up in methanol and poured onto a fresh portion of crushed ice. After 96 h the solid was filtered off and recrystallised from methanol to give yellow crystals of 5,6-*O*-benzylidenedehydro-L-ascorbic acid monophenylhydrazone, m.p. 142.5 °C (Found: C, 64.8; H, 4.6; N, 7.8. $C_{19}H_{16}N_2O_5$ requires C, 64.8; H, 4.55; N, 7.95%), $\nu_{max.}$ (Nujol) 1 755 (lactone C=O) and 1 695 (keto C=O) cm^{-1}.

5,6-O-*Benzylidenedehydro*-L-*ascorbic Acid Phenylosazone*.—5,6-*O*-Benzylidenedehydro-L-ascorbic acid monophenylhydrazone (1 g) was dissolved in methanol (25 ml) and phenylhydrazine (0.5 ml) and glacial acetic acid (0.1 ml) were added. The solution was refluxed for 30 min and on cooling, a precipitate formed. This was filtered off and recrystallised from methanol to give red crystals of 5,6-*O*-benzylidenedehydro-L-ascorbic acid phenylosazone, m.p. 206—207 °C (Found: C, 67.95; H, 4.9; N, 12.8. $C_{25}N_{22}N_4O_4$ requires C, 67.9; H, 5.0; N, 12.7%), $\nu_{max.}$ (Nujol) 1 720 (lactone C=O) cm^{-1}.

Treatment of dehydro-L-ascorbic acid phenylosazone with benzaldehyde and fused zinc chloride gave an identical product.

[8/130 *Received*, 26*th January*, 1978]

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