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Note

A nitrogen-15 n.m.r. study of some dehydro-L-ascorbic bis(phenylhydrazone) derivatives*

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Dehydro-L-ascorbic acid bis(phenylhydrazone) undergoes some unique reactions not exhibited by the saccharide osazones. Thus, on mild oxidation, it yields a bicyclic, azo derivative[1–4] **3**, instead of forming an osotriazole[5]. Similarly, on treatment with bases, its lactone ring is opened, and then recyclized on acidification to yield a pyrazolinedione monophenylhydrazone[4,6] (**6**).

Recently, the structure of the bicyclic, oxidation product **3** was put in doubt. The mass spectra of **3** and its acetate **4** were each found to display a molecular ion having a mass that was two units greater than expected[7], and it seemed as though no oxidation had taken place, but merely a cyclization to an intermediate such as **2**. Although the ^{1}H-n.m.r. spectra of compounds **3** and **4** each showed only one imino proton[1], instead of the three expected for structure **2**, the mass-spectrometric evidence prompted us to examine more closely the structure of compound **4** by ^{15}N-n.m.r. spectroscopy. Were structure **2** correct, it would be expected that the signals would be observed of three σ-bonded, nitrogen nuclei at high field, each split by coupling with a directly bonded proton, in addition to one uncoupled, C=N signal at low field. On the other hand, structure **3** or its acetate **4** would be expected to afford the signal of only one σ-bonded, nitrogen nucleus, split by coupling with a directly bonded proton, together with three nitrogen-15 signals at lower field; of these signals, two would arise from the -N=N- group, and would appear at a lower field than the C=N signal.

The proton-decoupled, natural-abundance, ^{15}N-n.m.r. spectrum (see Fig. 1a) of a solution of the acetylated oxidation product (**4**) of dehydro-L-ascorbic acid bis(phenylhydrazone) in chloroform-*d* [containing 0.05M chromium(III) (acetylacetonate)$_3$ as a relaxation reagent] displayed four signals, at 147.5, 139.8, —14.2,

*The n.m.r. spectra were recorded at the high-field n.m.r. facility of the National Measurement Laboratory.

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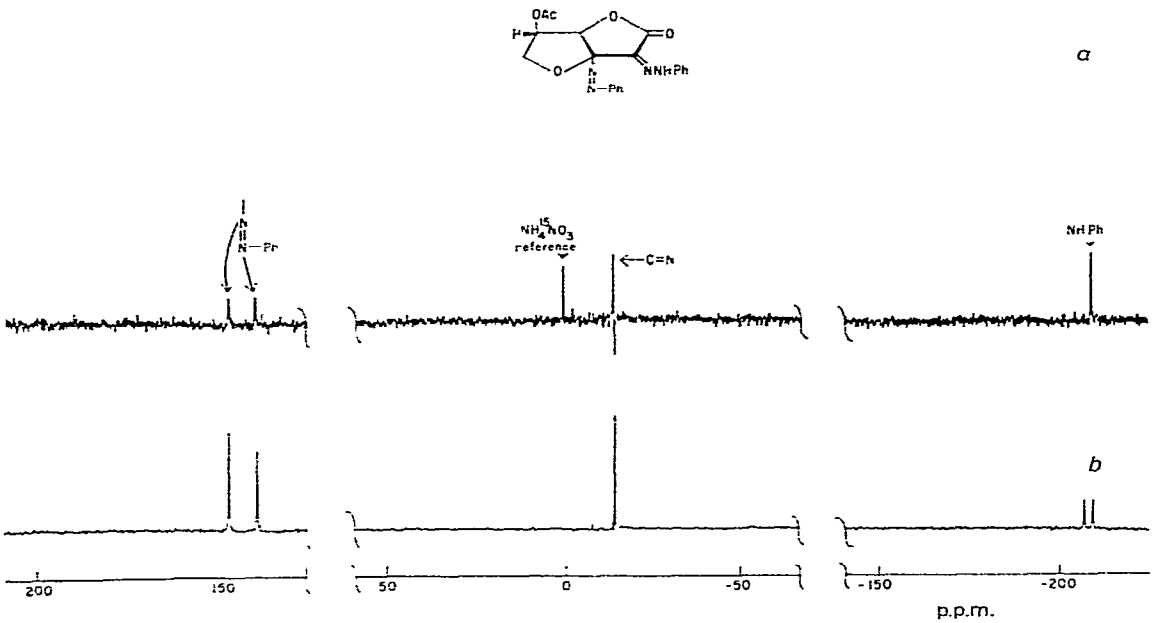

Fig. 1. Natural-abundance, ^{15}N-n.m.r. spectra at 40.5 MHz of oxidation product (3) of L-ascorbic acid bis(phenylhydrazone) in chloroform-*d* containing 0.05M chromium(III) (acetylacetonate)$_3$. [Key: *a* (upper spectrum), proton-decoupled, 10.807 scans; *b* (lower spectrum), proton-coupled, 115.040 scans.]

and —208.5 p.p.m., as expected of structure **4**. The ^{15}N chemical-shifts of the two resonances at low field (147.5 and 139.8 p.p.m.) are characteristic of azo nitrogen nuclei, whereas the resonance at —14.2 p.p.m. is appropriate for the tertiary nitrogen nucleus of a phenylhydrazone. The ^{15}N resonance at —208.5 p.p.m. is characteristic of the secondary nitrogen nucleus of phenylhydrazones (compare benzaldehyde phenylhydrazone[8], —50 and —233 p.p.m.).

The presence of only one imino proton in the structure of compound **4** was confirmed by its proton-coupled, ^{15}N-n.m.r. spectrum (see Fig. 1b), which displayed the three ^{15}N signals at lowest field as singlets, but the signal at highest field (—208.5 p.p.m.) as a doublet ($^1J^{15}_{NH}$ 95.2 Hz). The magnitude of the nitrogen-15–proton coupling over one bond is similar to that of that simple phenylhydrazone derivatives[9]. These data confirm structure **3** for the oxidation product of dehydro-L-ascorbic acid bis(phenylhydrazone), and structure **4** for its acetate.

The cyclization product of dehydro-L-ascorbic acid bis(phenylhydrazone) was first described by Ohle[6], who assigned the product the 1-phenyl-4-(phenylazo)-2-pyrazolin-5-one structure **8**. However, more recently, this product was found to show an imino proton signal in its ^{1}H-n.m.r. spectrum, which favored[4] the tautomeric hydrazone structure **6**. Here, too, a ^{15}N-n.m.r. spectrum was expected to be useful for distinguishing between structures **6**, **8**, and the enol structure **9**. Thus, structure **6** would exhibit two signals at high field, due to σ-bonded, nitrogen nuclei, one of which

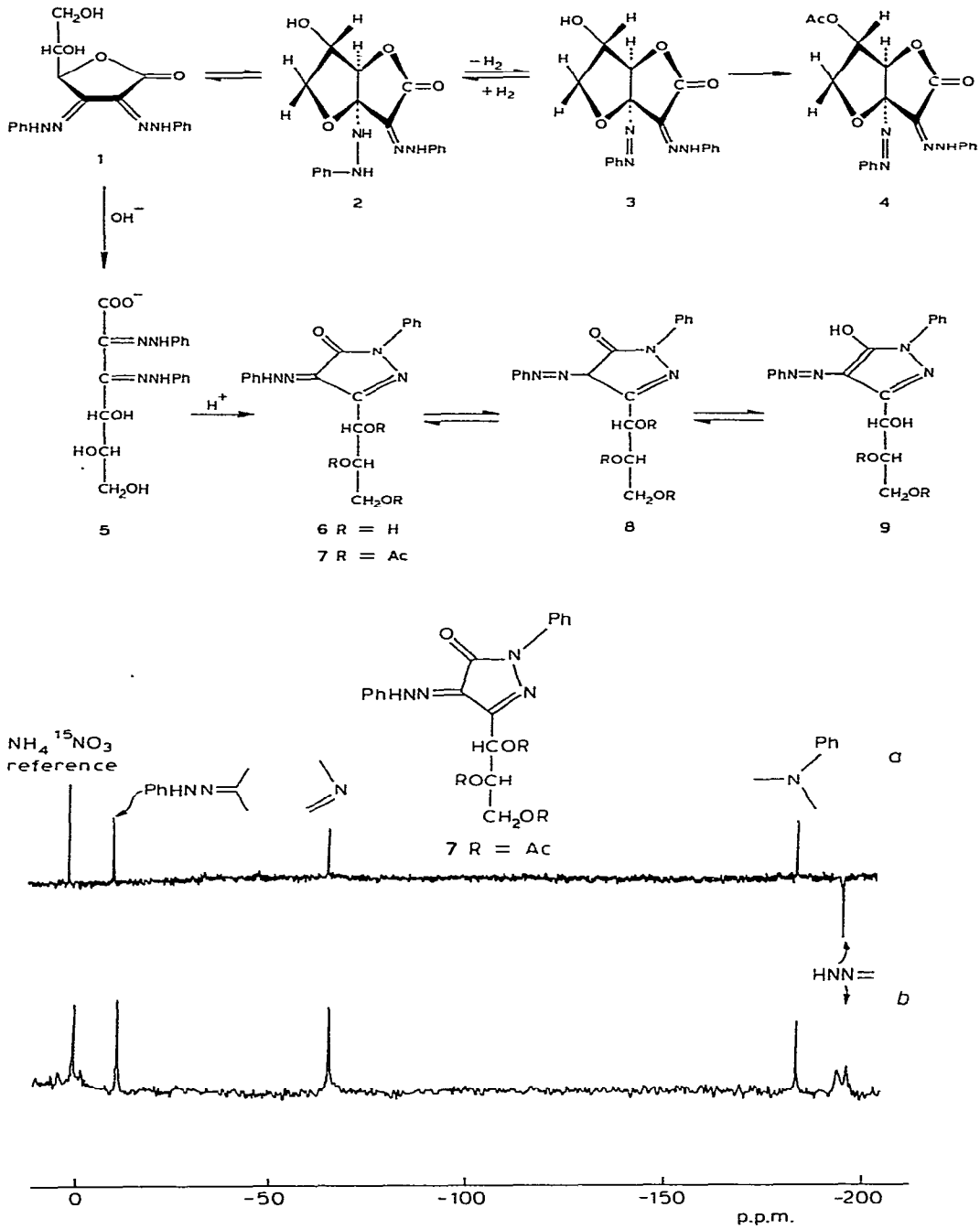

Fig. 2. Natural-abundance, ^{15}N-n.m.r. spectra of the acetylated cyclization product (7) of dehydro-L-ascorbic acid bis(phenylhydrazone) in chloroform-*d* containing 0.05M chromium(III) (acetylacetonate)$_3$, at 40.5 MHz. [Key: *a* (upper spectrum), proton-decoupled, 91,960 scans; *b* (lower spectrum), proton-decoupled, 208,500 scans.]

would be coupled to a proton, as well as two deshielded, C=N nitrogen signals not split by coupling with protons. Structure **8** would afford only one tertiary-nitrogen signal, unsplit by coupling with protons, and also three nitrogen signals at lower field, due to the -N=N- and C=N groups. The enolic form **9** would be expected to give a ^{15}N-n.m.r. spectrum quite similar to that of structure **8**.

When the ^{15}N-n.m.r. spectrum of the acetylated cyclization product of dehydro-L-ascorbic acid bis(phenylhydrazone) was measured it gave results that were in complete agreement with structure **7**. Thus, in the presence of 0.05M chromium(III) (acetylacetonate)$_3$, the proton-decoupled, natural-abundance, ^{15}N-n.m.r. spectrum (see Fig. 2a) of a solution of **7** in chloroform-*d* consists of four singlets at −11.3, −65.2, −184.2, and −195.9 p.p.m. By analogy with the ^{15}N-n.m.r. spectrum of compound **4**, the ^{15}N resonance at −11.3 p.p.m. may be assigned to the C=N nitrogen nucleus of the exocyclic, phenylhydrazono group of **7**. Hence, the singlet at −65.2 p.p.m. is assigned to the chemically similar, C=N nitrogen nucleus within the five-membered ring.

The proton-coupled, ^{15}N-n.m.r. spectrum of **7** (see Fig. 2b) displays three singlets at lower field, and a wide doublet ($^1J^{15}_{NH}$ 93 Hz) at highest field that indicates the presence in **7** of a saturated nitrogen atom having a directly bonded, hydrogen atom. Therefore, the signal at −195.9 p.p.m. was assigned to the NH nitrogen nucleus of **7**, and, by elimination, the singlet at −184.2 p.p.m. was assigned to the saturated, tertiary nitrogen nucleus. The latter ^{15}N chemical-shift is in excellent agreement with that (−184 p.p.m., recalculated) of N-1 of 3-methyl-1-phenyl-2-pyrazolin-5-one, which has been shown[10] to exist in chloroform solution as a single tautomer having a ketonic structure similar to that of **7**.

The value of $^1J^{15}_{NH}$ reported here for **7** is only approximate, because the doublet for the NH nitrogen-nucleus (see Fig. 2b) is broadened by chemical exchange of the NH protons. In an attempt to decrease the rate of this exchange process, ^{15}N-n.m.r. spectra were also obtained for **7** in dimethyl sulfoxide-d_6 solution[11]. However, under these conditions, the NH nitrogen-resonance observed in the absence of proton irradiation was a very broad singlet that indicated an *increased* rate of NH proton-exchange in this solvent. As 3-methyl-1-phenyl-2-pyrazolin-5-one is known to exist as a mixture of keto and enol tautomers in dimethyl sulfoxide solution[10], it seems possible that the unexpectedly high rate of NH proton-exchange in the solution of **7** is due to exchange with minor concentrations of other tautomers, such as **8** and **9**. Nevertheless, the ^{15}N chemical-shifts (−12.6, −67.3, −184.7, and −193.5 p.p.m.) of **7** in dimethyl sulfoxide-d_6 solution containing 0.05M chromium(III) (acetylacetonate)$_3$ were found to be very similar to those of chloroform-*d* solutions under the same conditions, and no other species were detected.

The negative, nuclear Overhauser effect (n.O.e.) displayed by the resonance at highest field in the proton-decoupled, ^{15}N-n.m.r. spectrum of **7** (see Fig. 2a) is also consistent with assignment of this resonance to a nitrogen nucleus attached directly to a proton. It is interesting that this n.O.e. was not removed at the concen-

tration of relaxation reagent used, in contrast to the example of compound **4** (see Fig. 1a).

In conclusion, the bicyclic, oxidation product of dehydro-L-ascorbic acid bis(phenylhydrazone) possesses structure **3**, and its cyclization product has structure **6**.

EXPERIMENTAL*

The dehydro-L-ascorbic acid derivatives (compounds **3** and **6**) were prepared by procedures previously described[1,12].

^{15}N-N.m.r. spectra were recorded in the pulse-Fourier-transform mode at 40.5 MHz by means of a Bruker Instruments spectrometer, model WM-400. Solutions of compounds **4** (0.62 g) and **7** (0.30 g), each in chloroform-*d* (3.0 mL) containing 0.05M chromium(III) (acetylacetonate)$_3$, were examined in 15-mm, n.m.r. sample-tubes by using a 45° pulse (22 μs), a minimum, pulse-repetition time of 0.82 s, a spectral width of 20 kHz, a 32,768-point data-set, quadrature detection, and field : frequency stabilization on the deuterium nuclei of the solvent. Proton-decoupled, ^{15}N-n.m.r. spectra were obtained with non-gated, broad-band irradiation at 400 MHz. However, proton-coupled spectra were obtained with the decoupler turned off. Nitrogen-15 chemical-shifts are reported with respect to the nitrate resonance of an external capillary of aqueous 1.05M $NH_4{}^{15}NO_3$.

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