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STRUCTURE OF THE REACTION PRODUCT OF 4-HYDROXY-2,3-DIOXO-4-PHENYLBUTANOIC ACID 1,4-LACTONE WITH *o*-PHENYLENEDIAMINE*

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ABSTRACT

Examination of the structure of the yellow product (**5a**), obtained by treating 4-hydroxy-2,3-dioxo-4-phenylbutanoic acid 1,4-lactone (4-phenyl-2,3-dioxobutyro-1,4-lactone) with two moles of *o*-phenylenediamine, by high-resolution ^{1}H-, ^{13}C-, and ^{15}N-n.m.r. spectroscopy, as well as by electron-impact mass spectrometry, confirmed without ambiguity the structure of the product as the quinoxaline amide **5a**. When 4-hydroxy-2,3-dioxo-4-phenylbutanoic acid 1,4-lactone is treated with *o*-phenylenediamine, the Schiff base is first formed, which is then converted into a quinoxaline lactone (**6a**). The excess of *o*-phenylenediamine then converted the quinoxaline lactone (**6a**) into the yellow product (**5a**).

INTRODUCTION

In 1934 Erlbach and Ohle[1] found that dehydro-D- and -L-ascorbic acids (**1b** and **1c**), reacted with two moles of *o*-phenylenediamine to yield yellow products, to which they assigned structures **2b** and **2c** respectively. Later, Ohle and Gross[2], abandoned structures **2b** and **2c**, in favor of structures **3b** and **3c**. Dahn and Lawendel[3], after initially adopted structure **2a** for the analogous derivative obtained from 4-hydroxy-2,3-dioxo-4-phenylbutanoic acid 1,4-lactone (4-phenyl-2,3-dioxobutyro-1,4-lactone, **1a**), abandoned[4] this structure in favor of structure

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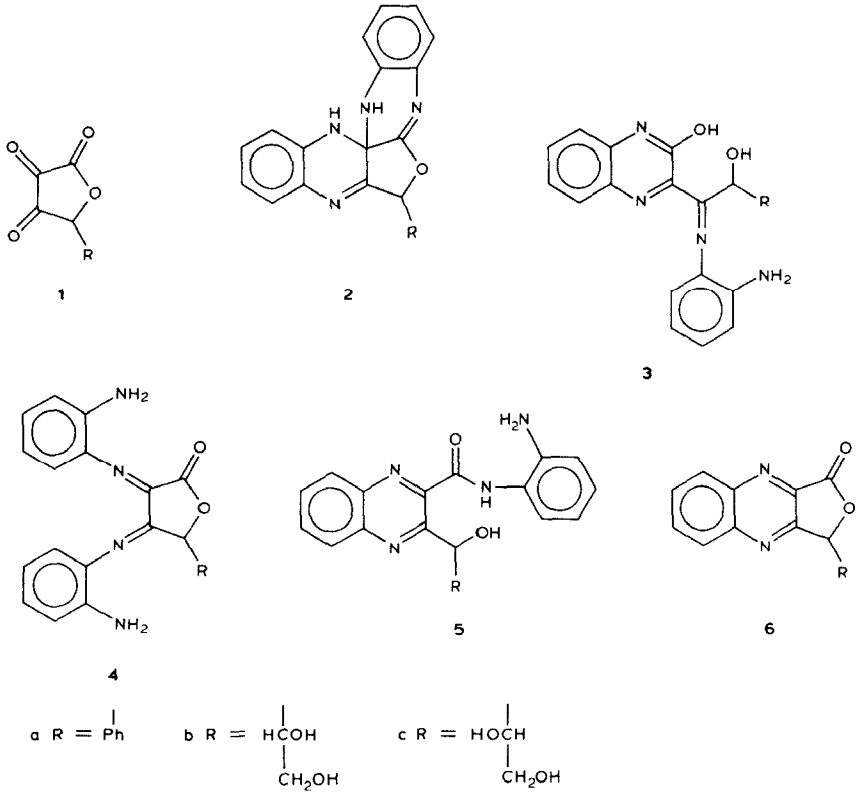

5a. In the interim, Hasselquist[5] proposed a lactone structure (**4a**), which was also used by El Khadem *et al.*[6].

In their study that led to structures **5a** and **5c**, Dahn and Moll[4] found that the aforementioned yellow products prepared from **1a** and **1c** were very similar in their u.v. and i.r. spectra and in their chemical behavior. Both showed the presence of a primary aromatic amino group; upon treatment with acid, they lost one mole of *o*-phenylenediamine giving a quinoxaline lactone (**6a** and **6c** respectively). The structure of compound **6a** was confirmed by studying its fragmentation pattern in electron-impact mass spectrometry[6]. As all of the structures (**2–5**) assigned to the yellow products were developed before the advent of high-resolution nuclear magnetic resonance spectroscopy (n.m.r.) and mass spectrometry (m.s.), it was decided to reexamine the structures **2a–5a** proposed for the phenyl analog and assign structures from the high-resolution n.m.r. and mass spectra.

RESULTS AND DISCUSSION

Examination of the structure of the yellow product (**5a**), obtained by treating 4-hydroxy-2,3-dioxo-4-phenylbutanoic acid 1,4-lactone with two molecular equivalents of *o*-phenylenediamine, by high-resolution ^{1}H-, ^{13}C-, and ^{15}N-n.m.r. spectros-

copy, and by electron-impact mass spectrometry, as outlined below unambiguously confirmed structure **5a**, first suggested by Dahn and Moll[4].

Closer study of the course of the reaction by thin-layer chromatography (t.l.c.) and high-performance liquid chromatography (l.c.) revealed, however, that the reaction proceeds in somewhat different way than previously believed. Thus, when 4-hydroxy-2,3-dioxo-4-phenylbutanoic acid 1,4-lactone was treated with *o*-phenylenediamine, a Schiff's base was formed first, which was slowly converted into the quinoxaline lactone (**6a**) and not the yellow *o*-aminoanilide (**5a**) as first thought. The latter derivative was formed with an excess of *o*-phenylenediamine, which caused the quinoxaline lactone (**6a**) to undergo nucleophilic attack at its carbonyl carbon atom resulting in the opening of the ring to afford the yellow product (**5a**). The last reaction usually occurs at a higher pH (4–5); at low pH (2–4) the reaction stops at the lactone stage (**6a**).

^{1}H-N.m.r. study. — The ^{1}H-n.m.r. spectrum (see Fig. 1b) of a solution of the yellow product **5a** in dimethyl sulfoxide-d_6 (Me_2SO-d_6) displays a total proton integral of 18, which is grossly consistent with the molecular formula ($C_{22}H_{18}N_4O_2$) of structures **3a, 4a,** and **5a**, but not with that of structure **2a**. The one-proton singlet at δ 10.086, two-proton singlet at δ 4.971, and one-proton doublet at δ 6.308 disappear on dilution of the solution in Me_2SO-d_6, with deuterium oxide (see Fig. 1a), thereby indicating the presence of three types of exchangeable protons. Concomitantly, the one-proton doublet at δ 6.573 collapses to a singlet (see

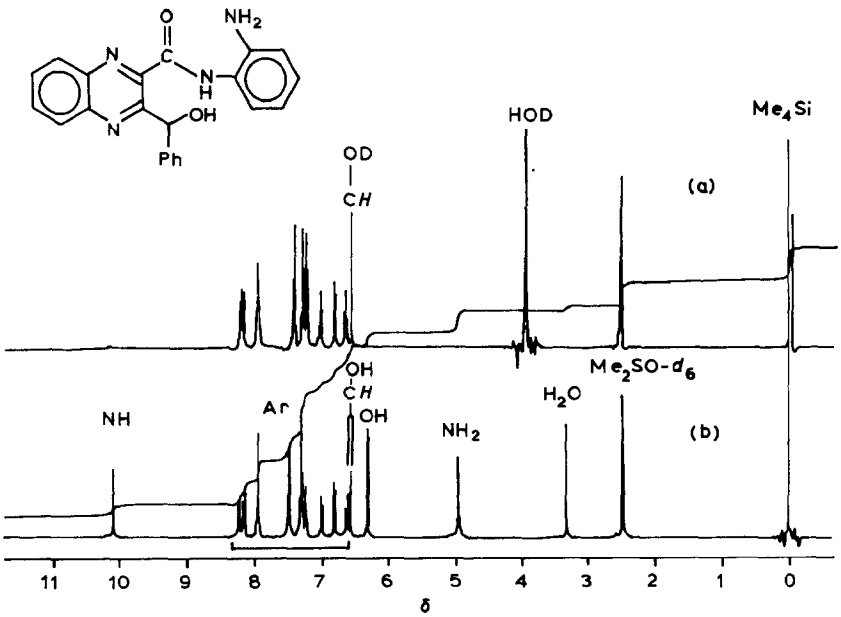

Fig. 1. ^{1}H-N.m.r. spectra, 16 scans each, of solutions of **5a** in Me_2SO-d_6 at 400 MHz: (a) with four drops of deuterium oxide added and (b) in Me_2SO-d_6 alone.

Fig. 1a), thus demonstrating that this proton is coupled to a single, exchangeable proton. The presence of this coupling constant in the spectrum of unexchanged **5a** was confirmed by a homonuclear spin-decoupling experiment in which selective irradiation at the frequency of the doublet at δ 6.308 caused the doublet at δ 6.573 to collapse to a singlet. These observations are consistent with structures **3a** and **5a**, but not with structure **4a**.

^{13}C-N.m.r. study. — The proton decoupled ^{13}C-n.m.r. spectrum (see Fig. 2a) of a solution of **5a** in Me_2SO-d_6 displays a total of 20 resolved ^{13}C resonances.

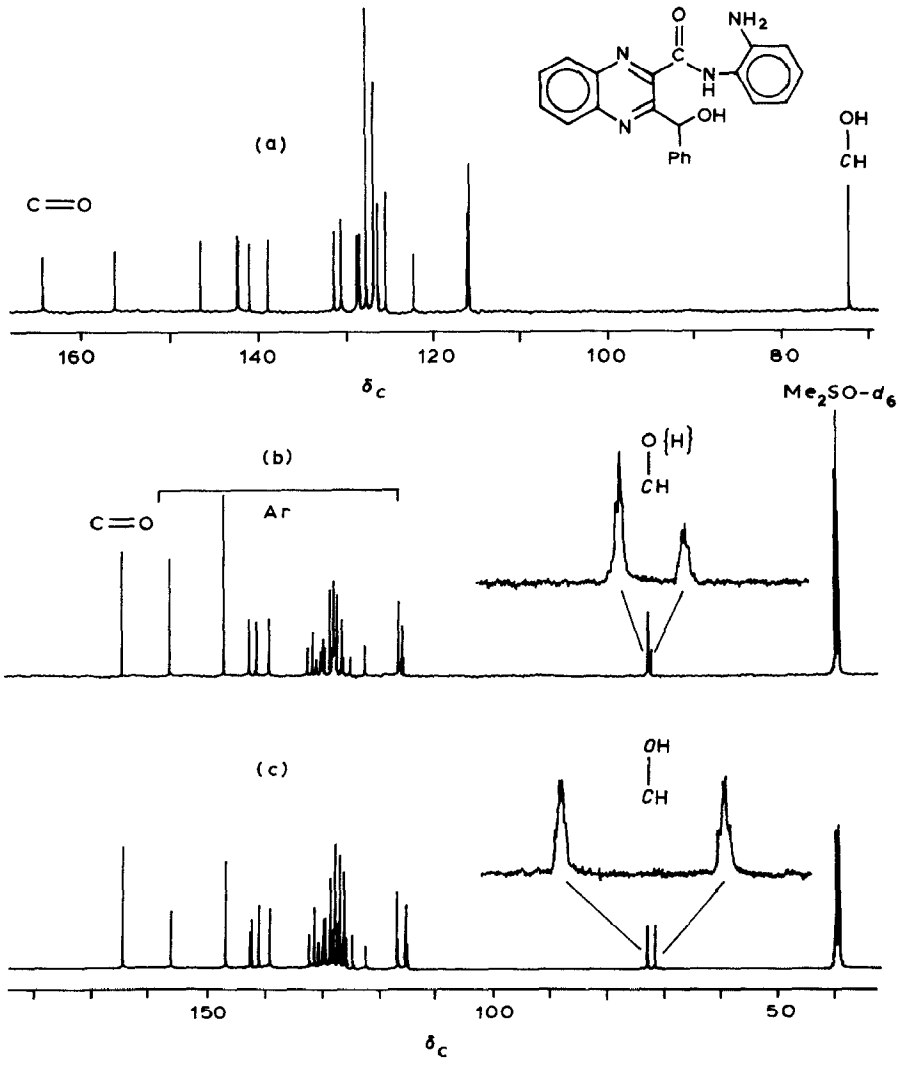

Fig. 2. ^{13}C-N.m.r. spectra of a solution of **5a** in Me_2SO-d_6 at 100.6 MHz: (a) 400 scans, proton decoupled, (b) 8,556 scans, with selective irradiation of the hydroxyl proton, and (c) 8,408 scans, proton coupled with n.O.e. present.

However, this spectrum is consistent with a total carbon count of at least 22, because two of the resonances have increased intensities apparently arising from accidental chemical-shift equivalences in two pairs of ^{13}C nuclei. The resonance at δ_C 164.4 was assigned to a carbonyl ^{13}C nucleus and the signals in the range δ_C 116.0–156.3 to aromatic ^{13}C nuclei, thus leaving the signal at δ_C 72.3 as a unique ^{13}C resonance at higher field. The hydroxymethine function is a key feature of structures **3a** and **5a**, and a ^{13}C-n.m.r. experiment was designed to confirm the presence of this structural feature. It was surmised that the hydroxyl protons of **3a** and **5a** would each display a small coupling constant with the methine ^{13}C nucleus two bonds distant. The proton-coupled ^{13}C-n.m.r. spectrum (see Fig. 2c) of **5a** displays the methine ^{13}C signal at δ_C 72.3 as a doublet of quartets, the large spacing (146.7 Hz) being due to coupling of the methine ^{13}C nucleus with its attached proton and the small spacings (~3.3 Hz) to coupling of the methine ^{13}C with *three* more remote protons. On selective irradiation of the exchangeable proton at δ_H 6.308, the methine ^{13}C signal collapsed to a doublet of triplets (see Fig. 2b), thereby indicating the removal of a small coupling between the exchangeable (hydroxyl) proton and the methine ^{13}C nucleus. The results of this experiment are also consistent with structures **3a** and **5a**.

^{15}N-N.m.r. study. — Definitive evidence for structure **5a** was obtained by ^{15}N-n.m.r. spectroscopy. The proton decoupled, natural abundance ^{15}N-n.m.r. spectrum (see Fig. 3a) of a solution of **5a** in Me_2SO-d_6 [recorded under conditions designed to secure the nuclear Overhauser effect (n.O.e.), if present] shows four distinct ^{15}N resonances. The two positive singlets at δ_N −49.6 and −51.5 may be assigned to aromatic-ring nitrogen nuclei that do not bear attached protons, whereas the negative singlet at δ_N −249.0 may be assigned to an amide nitrogen nucleus, on the basis of its chemical shift. By elimination, the negative singlet at δ_N −319.6 is assigned to the anilino nitrogen nucleus. A spectrum very similar to that shown in Fig. 3a was obtained from a solution of **5a** in pyridine-d_5.

The aforementioned ^{15}N assignments are unambiguously confirmed by the proton-coupled ^{15}N-n.m.r. spectrum (also recorded with n.O.e., see Fig. 3b) of a solution of **5a** in Me_2SO-d_6, which displays positive singlets for the aromatic ring nitrogen nuclei, together with a negative doublet and negative triplet at δ_N −249.0 and −319.6, respectively. The multiplicities observed for the latter two signals allow the doublet to be definitively assigned to an amide NH nitrogen nucleus and the triplet to an anilino NH_2 group. The spacing $^1J_{NH}$ 91.6 Hz in the doublet is appropriate for sp^2 hybridization of an amide nitrogen atom, and the spacing $^1J_{NH}$ 80.7 Hz in the triplet is similar to the value $^1J_{NH}$ 82.6 Hz reported[7] for aniline in Me_2SO-d_6. The negative n.O.e.s observed for the doublet and triplet are consistent with the presence of two nitrogen nuclei having one or more attached protons. Structures **2a**–**5a** each contain nitrogen functions of differing multiplicity, and, therefore, these structures are clearly differentiated by the proton-coupled ^{15}N-n.m.r. spectrum. Only structure **5a** could be expected to generate an NH doublet together with an NH_2 triplet.

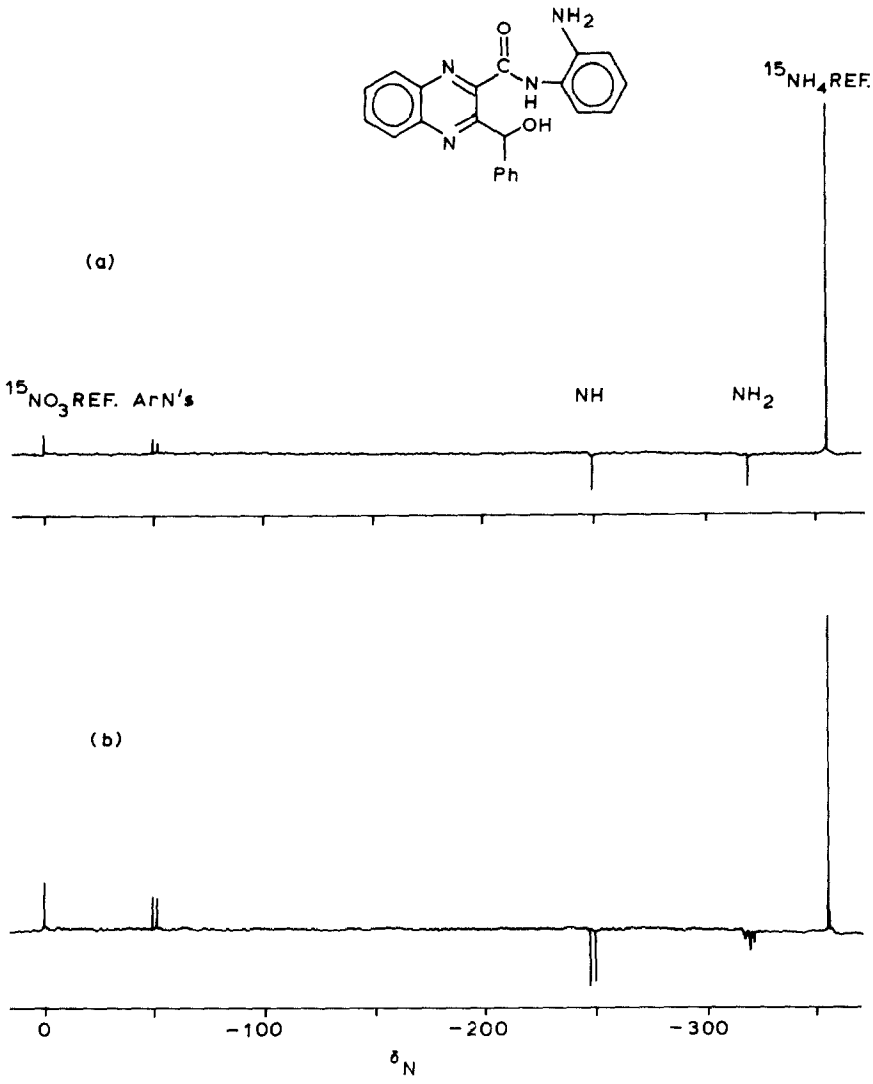

Fig. 3. ^{15}N-N.m.r. spectra of a solution of **5a** in Me_2SO-d_6 at 40.5 MHz: (a) 1,924 scans, proton decoupled with n.O.e., and (b) 8,900 scans, proton coupled with n.O.e.

The proton-coupled ^{15}N-n.m.r. spectrum of a solution of **5a** in pyridine-d_5 displays the aromatic-ring nitrogen resonances as positive singlets, the NH signal as a negative doublet ($^1J_{NH}$ 91.7 Hz), and the NH_2 signal as a broadened, negative singlet. The collapse of the spin coupling in the NH_2 signal indicates that the pyridine-d_5 solvent promotes rapid chemical exchange of the NH_2 protons, thus emphasizing the desirability, for purposes of structural analysis, of using a solvent in which the exchange of all NH protons is slow on the n.m.r. time-scale.

Mass spectrometry study. — The electron-impact mass spectrum of the anilide

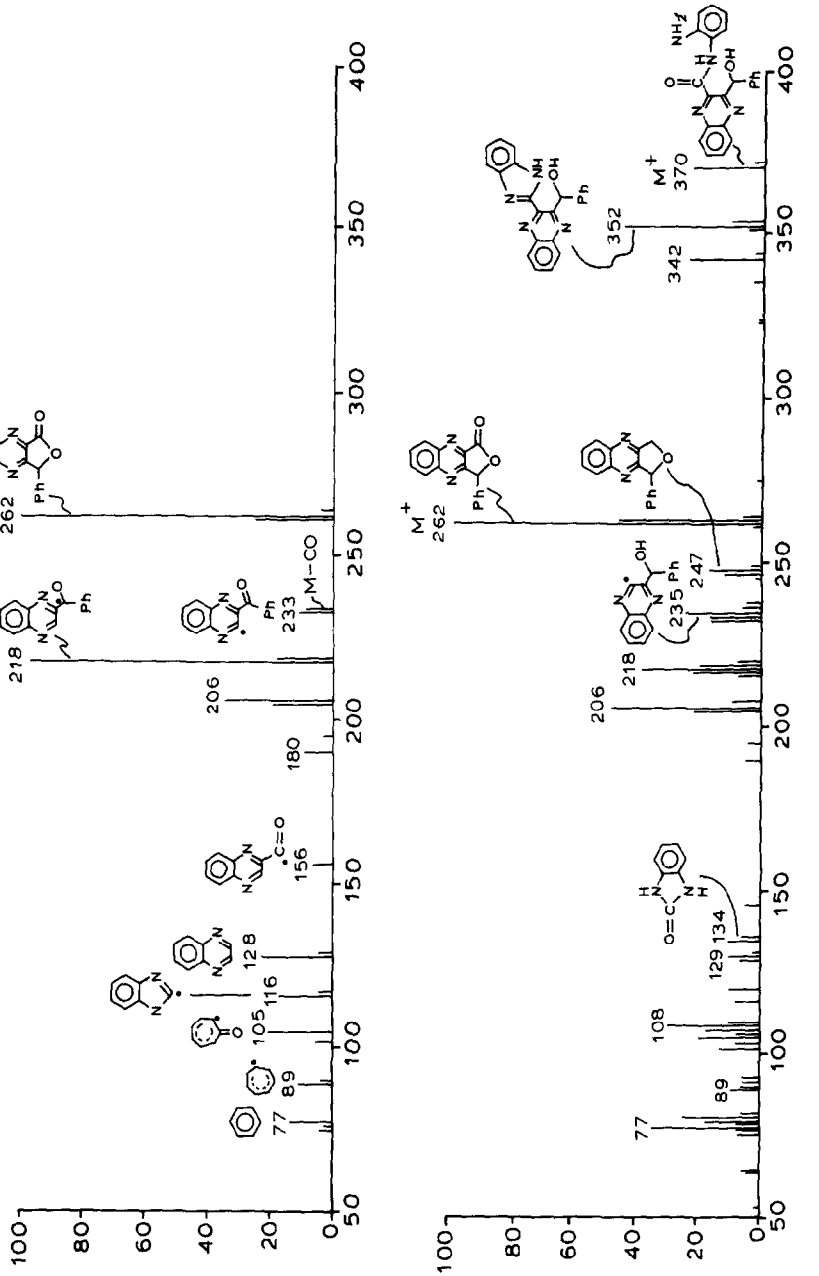

Fig. 4. Electron-impact mass spectra: (a) quinoxaline lactone derivative **6a** and (b) amide derivative **5a**.

5a is dominated by formation of the ion of the parent quinoxaline lactone (**6a**). This becomes evident when the spectra of both compounds are compared (see Fig. 4). The yellow product (**5a**) showed a base peak at *m/z* 262, which corresponds to the loss of *o*-phenylenediamine and the formation of the quinoxaline lactone (**6a**). The spectrum of **5a** also shows a significant molecular peak at *m/z* 370, followed by fragments at *m/z* 352 and 342 resulting from the loss of water and CO, respectively. Except for peaks at *m/z* 247 and 235, as well as a few fragments (for example, *m/z* 108 and 134) originating from *o*-phenylenediamine, all of the peaks observed below *m/z* 262 have their equivalents in the spectrum of the quinoxaline lactone (**6a**).

In conclusion, we have shown that the reaction of *o*-phenylenediamine with 4-substituted-4-hydroxy-2,3-dioxobutanoic acid 1,4-lactones (as **1a**), initially affords the quinoxaline lactone (as **6a**), which then can undergo reaction at the carbonyl carbon with an excess of *o*-phenylenediamine. This ring opening yields yellow quinoxaline amides (as **5a**). One may safely adopt this statement for the reactions of *o*-phenylenediamine with dehydro-D- and -L-ascorbic acids equally, because of the close similarity found between products **5a** and **5c**.

EXPERIMENTAL

General. — Thin-layer chromatography was performed on precoated Eastman Kodak* silica gel plates with fluorescent indicator (254 nm), and eluted with toluene–ethyl acetate (4:1 v/v); **5a**, R_F 0.28; **6a**, R_F 0.78. High-performance liquid chromatography was performed with a Waters analytical instrument* with a 30 × 0.4 cm μPorasil column, a flow rate of 3.0 mL/min, and a column back-pressure of 9.6 MPa. Two elution solvents, hexane (*A*) and 20% acetonitrile in dichloromethane (*B*), were used, with a linear solvent-gradient starting at 20% solvent (*B*) and ending at 100% solvent (*B*) after five min. The separated components were detected with a differential u.v. detector set at 254 nm; compound **6a** was eluted at 3.2 min and compound **5a** at 5.8 min.

N.m.r. spectroscopy. — The ^{1}H-, ^{13}C-, and ^{15}N-n.m.r. spectra were recorded in the pulse-Fourier transform mode at 400.1, 100.6, and 40.5 MHz, respectively, using a Bruker Instruments model WM-400 spectrometer*. The ^{1}H- and ^{13}C-n.m.r. spectra were obtained from a solution of **5a** (20 mg and 53 mg, respectively) in Me_2SO-d_6 (0.5 mL) contained in 5-mm n.m.r. sample tubes. ^{15}N-n.m.r. spectra were recorded for solutions of **5a** (0.5 g) in Me_2SO-d_6 (3.5 mL) and **5a** (0.42 g) in pyridine-d_5 (3.5 mL), contained in 15-mm sample tubes. All n.m.r. data were acquired by means of the Bruker DISNMRP program.

^{1}H-n.m.r. spectra were recorded by use of a 45° pulse (width 7 μs), a 16,384-

*Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendations by the National Bureau of Standards, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

point data set, a spectral width of 5 kHz, and a total relaxation delay of 4 s. Proton decoupled ^{13}C-n.m.r. spectra were acquired by use of a 75° pulse (width 13 μs), a 16,384-point data-set, a spectral width of 20 kHz, a total relaxation delay of 6 s, and two-level proton decoupling with a low-power:high-power duty cycle ratio of 13.7:1.

Proton-coupled and selectively proton-decoupled ^{13}C-n.m.r. spectra were acquired under high-resolution conditions using a 32,768-point data set that was resolution enhanced, and then zero fitted to 65,536 points prior to Fourier transform. Resolution enhancement was performed by Gaussian, exponential filtering of the free-induction decay signal, using a line broadening of −1 Hz and a Gaussian broadening fraction of 0.7. The proton coupled ^{13}C-n.m.r. spectra were obtained with the n.O.e. present, by means of a gated decoupling sequence.

^{15}N-N.m.r. spectra were acquired by use of a 90° pulse (width 41 μs) and a 16,384-point data-set. Proton-decoupled ^{15}N-n.m.r. spectra were measured at a spectral width of 29.4 kHz using two-level proton decoupling with a low-power:high-power duty-cycle ratio of 21.5:1, and a total relaxation-delay of 6.3 s. Proton-coupled ^{15}N-n.m.r. spectra were obtained with the n.O.e. present and at a spectral width of 20 kHz, by use of a gated decoupling sequence with a low-power:zero-power duty-cycle ratio of 13.7:1.

The ^{1}H and ^{13}C chemical shifts were measured with respect to tetramethylsilane used as the internal reference and ^{15}N chemical shifts were referred to the $^{15}NO_3^-$ resonance of an external, concentric capillary of an aqueous, saturated solution of $^{15}NH_4{}^{15}NO_3$ (95.3 atom % ^{15}N).

Compound **5a** displayed the following n.m.r. data: ^{1}H-n.m.r. δ_H 10.086 (1 H, NH), 8.214 (m, 1 H, Ar), 7.941 (m, 2 H, Ar), 7.477 (d, 2 H, *J* 7.3 Hz, Ar), 7.303 (m, 3 H, Ar), 7.227 (t, 1 H, *J* 7.3 Hz, Ar), 7.003 (qi, 1 H, *J* 7.6, 1.4 Hz, Ar), 6.809 (q, 1 H, *J* 8.0, 0.9 Hz, Ar), 6.636 (qi, 1 H, *J* 7.6, 1.0 Hz, Ar), 6.573 (d, 1 H, *J* 6.4 Hz, C*H*OH), 6.308 (d, 1 H, *J* 6.5 Hz, OH), and 4.971 (2 H, NH_2); ^{13}C-n.m.r. δ_C 164.4 (C=O), 156.3, 146.8, 142.7, 142.4, 141.1, 139.1, 131.6, 130.7, 128.9, 128.6, 127.9 (2 C), 127.1 (2 C), 127.0, 126.6, 125.6, 122.4, 116.2, 116.0 (20 Ar), and 72.3 (*C*HOH); ^{15}N-n.m.r. (Me_2SOd_6) δ_N −49.6, −51.5 (2 Ar ring N), −249.0 (d, 1 N, J_{NH} 91.6 Hz, NH), −319.6 (t, 1 N, J_{NH} 80.7 Hz, NH_2); ^{15}N-n.m.r. (pyridine-d_5) δ_N −51.4, −52.1 (2 Ar ring N), −251.2 (d, 1 N, J_{NH} 91.7 Hz, NH), and −321.9 (1 N, NH_2). Signal multiplicities are indicated by: no symbol, singlet; m, multiplet; d, doublet; t, triplet; qu, quintet resembling a triplet; q, quartet.

Mass-spectrometry study. — The mass spectrum of compound **6a** was recorded with an Hewlett–Packard 5780 gas chromatograph–mass spectrometer in the direct-insertion mode. The sample volatilized at 200°, ionized with 70 keV, and was recorded from 50 to 450 *m/z*: *m/z* 371.2 (5.25, M + 1), 370.2 (21.36, M^+), 353.2 (10.91), 352.2 (43.2), 351.2 (7.09), 343.1 (5.9), 342.1 (23.67), 335.2 (6.26), 264.1 (7.8), 263.1 (45.22), 262.1 (100, base), 247.1 (17.62), 246.2 (13.35), 237.1 (6.43), 236.1 (7.32), 235.2 (25.54), 234.2 (16.29), 233.2 (17.41), 220.2 (6.52), 219.2 (19.31), 218.2 (38.13), 217.1 (21.54), 216.2 (6.26), 207.2 (9.73), 206.2 (46.46),

205.2 (21.42), 190.1 (6.37), 134.1 (9.79), 129.1 (9.76), 128.1 (5.43), 119.1 (9.1), 116.1 (6.37), 109.1 (8.42), 108.1 (27.86), 107.1 (18.6), 106.1 (6.43), 105.1 (19.31), 103.1 (6.14), 102.1 (13.62), 90.1 (5.72), 89.1 (8.81), 80.2 (24.92), 79.1 (17.65), 78.1 (6.64), 77.1 (34.39), 76.1 (7.68), 75.1 (6.17), 65.1 (5.1), 53.1 (6.55), 52.1 (6.2), and 51.1 (10.94).

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