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THE AUTOXIDATION OF 3-HYDROXYANTHRANILIC ACID IN THE PRESENCE OF AMINO ACIDS

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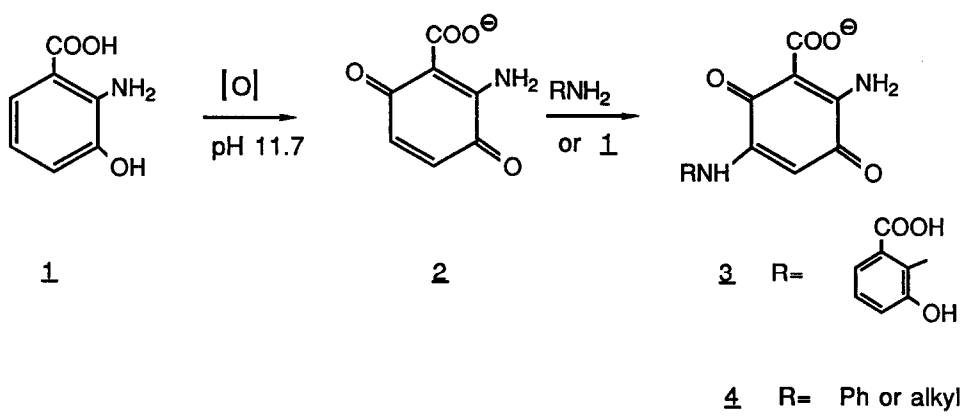
Abstract: The autoxidation of 3-hydroxyanthranilic acid 1 at pH 11.7 in the presence of the amino acids lysine, glycine and alanine gave p-quinone conjugates. The autoxidation of 1 in the presence of proline gave two rearrangement products arising from Strecker degradation of the initially formed proline conjugate. N-α-t-Boc histidine, arginine and cysteine failed to give amino acid conjugates with oxidized 1.

Recently we reported that the autoxidation of 3-hydroxyanthranilic acid 1 at pH 11.7 gave the p-quinone dimer 3.[1] When this autoxidation was carried out in the presence of aniline or aliphatic amines then the corresponding p-quinone adducts 4 could be conveniently obtained and in synthetically useful yields. We suggest these products arise from addition of 1 or amine to the p-quinone intermediate 2 (Scheme 1).

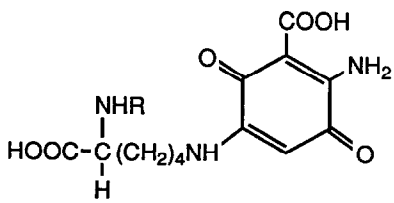


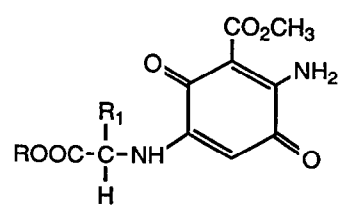
Scheme 1

It is known that 1 is involved in the modification or cross-linking of protein during cocoon formation in Samia cynthia[2] and Bombyx mori[3]. The exact molecular mechanism for this process is not known, however a mechanism involving the modification of protein side chains by an oxidized form of 1 would seem highly likely. To address the problem of protein modification by oxidized 1 we have examined the autoxidation of 1 in the presence of amino acids.

Results and Discussion

When the autoxidation of 1 was carried out at pH 11.7 in the presence of lysine (4 molar equivalents) then the p-quinone adduct 5a could be obtained in 23% yield. This product was identical to that obtained from the previously reported Nα-t-BOC lysine adduct 5b[1] by deprotection of the α- amino group with trifluoroacetic acid. Clearly lysine adds selectively to 2 through the ε- amino group rather than the α- amino group. It is of interest to note that the ε- amino group of lysine residues has often been implicated in the modification or cross-linking of proteins by quinones.





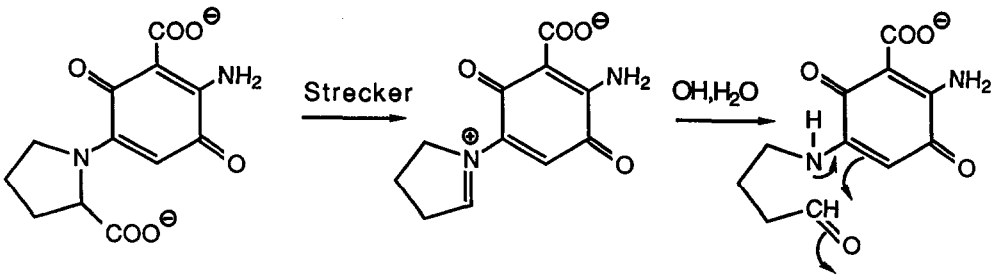
5a R = H

5b R = CO_2t-Bu

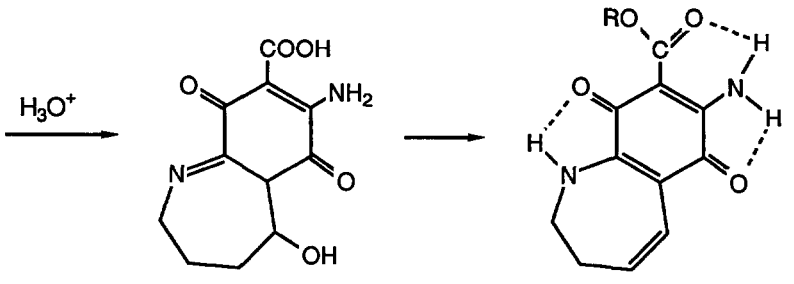
6a R_1 = H, R=CH_3

6b R_1 = CH_3, R = CH_3

The conjugates 6a and 6b were readily obtained in 18% and 30% yield respectively from the autoxidation of 1 in the presence of glycine and alanine respectively, followed by acidification, repeated extraction with ethyl acetate and then methylation with diazomethane. The methylation step allowed for ready separation of 6 from unreacted amino acid. When the above protocol was employed using proline then only the rearrangement products 7e (10%) and 7f (2%) were isolated. The structures of these products are consistent with their ^{1}H and ^{13}C NMR spectral data.



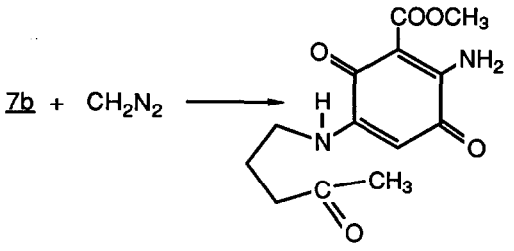
7 7a 7b



7c

7d R = H

7e R = CH_3



7f

Scheme 2

Fig. 1. ^{1}H NMR (400 MHz) spectrum of 7e in $CDCl_3$.

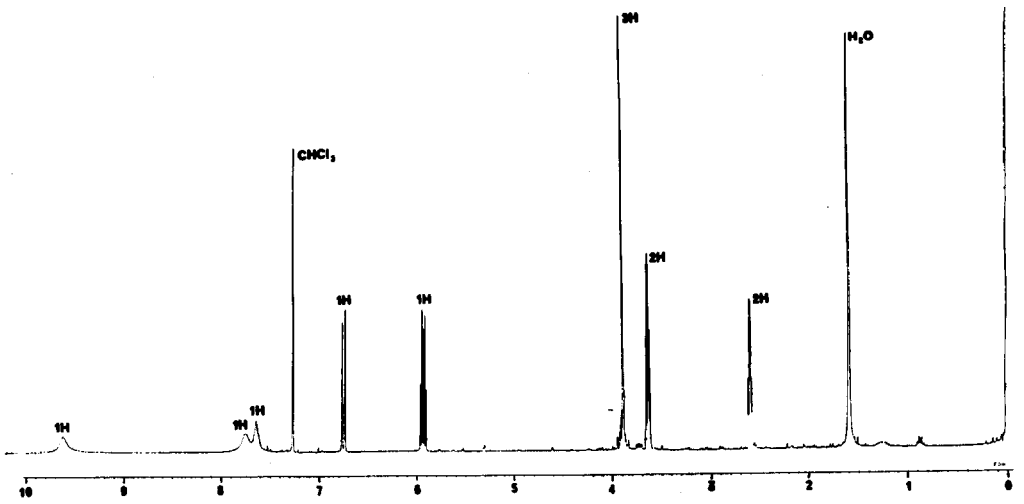
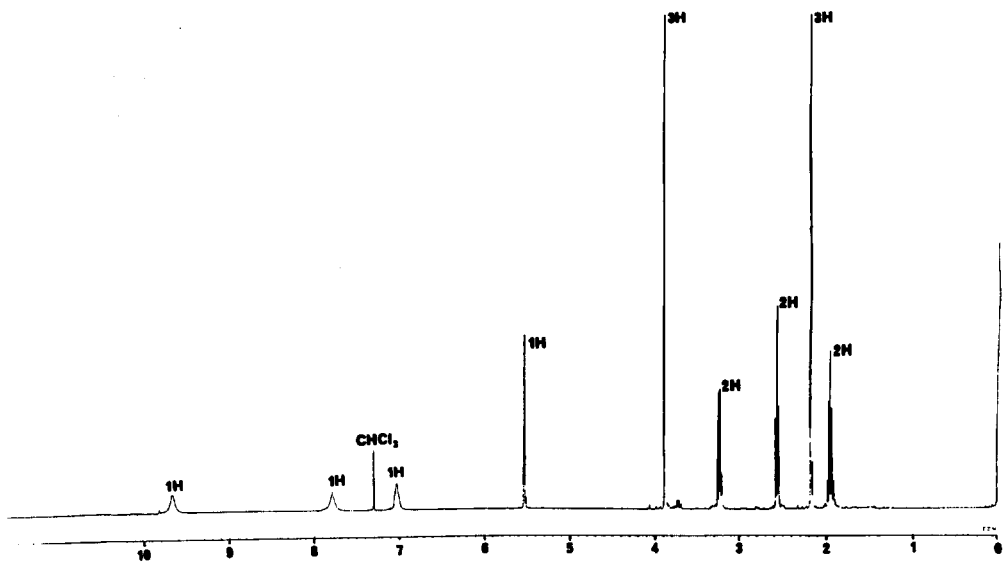


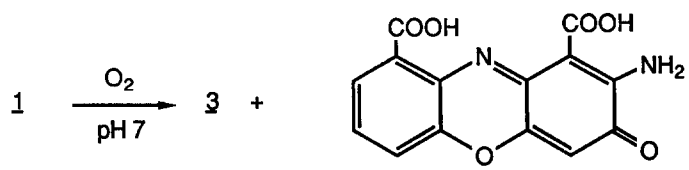
Fig. 2. ^{1}H NMR (400 MHz) spectrum of 7f in $CDCl_3$.



In Figure 1 and 2 are presented the ^1H NMR spectra of 7e and 7f respectively. In Figure 1 the olefinic resonances at δ 6.75 (d t, J = 11.7, 1.1 Hz) and δ 5.92 (d t, J = 11.7, 5.7 Hz) are clearly consistent with the $CH_2CH{=}CH$ moiety of 7e. Noteworthy, is the unusually low chemical shift for H-5, consistent with an olefinic proton in close proximity to an anisotropic carbonyl group. The three distinct low field NH resonances are clearly suggestive of a strongly internally H-bonded system. In Figure 2 the singlet olefinic resonance (δ 5.51) and the resonances for the 5-amino-pentan-2-one side chain of 7f are clearly evident.

In Scheme 2 a mechanistic scheme is proposed to account for the formation of 7e and 7f. We assume that the adduct 7 is formed initially but undergoes a Strecker type degradation[5] to the aldehyde 7b. Upon exposure to acid some of 7b undergoes cyclization of 7d (Scheme 2). Treatment of a mixture of 7b and 7d with diazomethane then gives 7f[6] and 7e respectively. The high pH oxidation of 1 in the presence of N-α-t-BOC-L-histidine, N-α-t-BOC-L-arginine or N-α-t-BOC-L-cysteine failed to produce any amino acid adducts and only the p-quinone dimer 3 (62-75% isolated yield) could be detected. In the latter reaction Nα-t-BOC-L-cysteine was rapidly converted to Nα-t-BOC-L-cystine.

The above-mentioned amino acid adducts could be obtained in very small yields when these autoxidations were performed at pH 7. For example, proline gave 0.3% of 7e upon the usual work-up procedure, whereas lysine gave only trace amounts of 5a. In these reactions 3 and cinnabarinic acid 8[1] were the major products.



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Experimental[7]

General Procedure for the Preparation of 3- Amino Substituted 2,5-dioxo-1,3-cyclohexadiene-1-carboxylic acids 5 and 6.

As a general procedure, 3-hydroxyanthranilic acid (200 mg) was dissolved in a Na_3PO_4 buffer (0.1 M, 100 mL) to which is added the amino acid (80 equivalents). The pH is adjusted to 11.7 with 6M NaOH and oxygen bubbled through the solution for 24 hrs to

maintain oxygen saturation. After this time the red solution is acidified with conc HCl to a pH of 3.0-3.5 and repeatedly extracted with ethylacetate (10 x 50 mL). The organic extracts are combined, dried quickly with $MgSO_4$, filtered, evaporated almost to dryness and dissolved in CH_3OH/Et_2O (25 mL, 1:1). An excess of etheral diazomethane is added and the solution stirred overnight. Removal of solvent gave either solids or oils. These were purified as described below.

Methyl 6-amino-3-(methoxycarbonylmethylamino)-2,5-dioxo-1,3-cyclohexadiene-1-carboxylate 6a.

The solid obtained utilizing glycine as the amino acid was washed with ether to give an orange powder: Yield 62 mg (18%); mp = 188-190°C (darkens at 170°C); ^1H NMR (DMSO-d_6) 3.69 (s, 3H), 3.72 (s, 3H), 4.11 (d, J = 6.4 Hz, 2H), 5.48 (s, 1H), 7.99 (br.t, 1H), 8.78 (br, 1H), 9.32 (br, 1H); FTIR (KBr) 3329.4, 3287, 3203, 1741, 1653, 1558, 1499, 1481, 1437, 1358, 1340, 1301, 1274, 1248, 1228, 1117, 1097, 1030, 1019, 980, 919, 863, 850, 838, 808, 746, 734, 715, 703 cm^{-1}; UV (CH_3OH/DMSO (4%)) 265.6 nm (log ε 4.26), 326.4 (4.58), 467.8 (3.18); Anal. Calcd for $C_{11}H_{12}N_2O_6$: C, 49.3; H, 4.5; N: 10.4%; Found: C, 49.1; H, 4.5; N: 10.2%.

Methyl 6-amino-3-(1-(methoxycarbony)ethylamino)-2,5-dioxo-1,3-cyclohexadiene-1-carboxylate 6b.

The solid obtained utilizing L-Alanine as the amino acid was chromatographed on silica gel using EtOAc as eluent to give an orange solid after evaporation of solvent: Yield 110 mg (30%); mp = 136-138°C (C_6H_6/petroleum spirit 80-100°C); ^1H NMR ($CDCl_3$) 1.53 (d, J = 6.8 Hz, 3H), 3.79 (s, 3H), 3.88 (s, 3H), 4.06 (q.d, J = 7.1 Hz, 6.8 Hz), 5.45 (s, 1H), 7.20 (br.d, J= 7.1 Hz, 1H), 7.63 (br.s, 1H), 9.57 (br.s, 1H). ^{13}C NMR ($CDCl_3$) 17.4, 50.8, 51.6, 52.7, 95.3, 96.5, 49.7, 156.8, 168.6, 171.2, 174.5, 175.5; IR (nujol mull) 3390, 3360, 3325, 1742, 1660, 1608, 1570, 1338, 1307, 1296, 1250, 1227, 843, 820 cm^{-1}; Anal. Calcd for $C_{12}H_{14}N_2O_6$: C, 51.1; H, 5.0; N: 9.9%. Found: C, 51.0; H. 4.7; N: 9.5%.

6-Amino-3(5-amino-5-carboxy-pentylamino)-2,5-dioxo-1,3-cyclohexadiene-1-carboxylic acid 5a.

The N-α-t-BOC-derivative 5b was prepared as previously described.[1] Compound 5b (100 mg) was dissolved in trifluoroacetic acid (7 mL). Bubbling was immediate and after 15 min the TFA was removed under N_2. The red solid was washed with acetone (20

mL) and recrystallized from water to give 46 mg (61%) of 5a as a red solid. mp >250°C (decomposes at 165-175°C); IR (nujol mull) 3500-3200 (br), 3315, 3305, 3195, 1585, 1525, 1205, 1185, 1130 cm^{-1}; UV (H_2O) 267.5 (log e 3.76, 329.0 (4.14), 498.6 (2.81); Anal. Calcd for $C_{13}H_{17}N_3O_6$: C, 50.2; H, 5.5; N: 13.5%. Found C, 49.8; H, 5.2; N: 13.2%.

The above was identical in all respects to 5a prepared by the following method:

3-hydroxyanthranilic acid (300 mg) and L-Lysine (4 equivalents) were dissolved in a Na_3PO_4 buffer (0.1M, 150 mL) and the pH adjusted to 11.7 with 6M NaOH. O_2 is blown through the solution for 24 hrs after which time the pH is lowered to 3.0-3.5 with conc HCl and the solution filtered. The volume of solution is reduced to 50 mL by freeze drying. 1.5 mL portions are applied to pre-conditioned Sep pac cartridges and washed with 2 x 5 mL of water to remove salt and L-Lysine. Flushing with the minimum amount of CH_3OH/H_2O (20%) eluted a red band. Collection of these followed by evaporation of CH_3OH by rotary evaporation then H_2O by freeze drying gave 94 mg (23%) of 5a.

Methyl 6-amino-3(4-oxo-1-pentylamino)-2,5-dioxo-1,3-cyclohexaniene-1-carboxylate 7f and Methyl 6H, 9H, 7-amino 2,3-dihydro-6,9-dioxo benzo [f] 1H-azepine-8-carboxylate 7e.

The oil obtained utilizing proline as the amino acid was chromatographed (silica gel) using EtOAc/hexane (3:1) followed by gradient elution with EtOAc. The initial purple band was collected, blown dry to give 7e as a purple solid. Yield 7 mg (2%). C.I.M.S. 248g/mol; ^1H NMR ($CDCl_3$) 2.59 (t.d.d, J = 5.7, 4.4, 1.1 Hz), 3.62 (t.d, J = 4.4, 5.1 Hz), 3.88 (s, 3H), 5.92 (d.t, J = 11.7, 5.7 Hz, 1H), 6.75 (d.t, J = 11.7, 1.1 Hz, 1H), 7.6 (br.s, 1H), 7.8 (br.s, 1H), 9.6 (br.s, 1H); ^{13}C NMR ($CDCl_3$) 32.2, 45.9, 51.9, 96.0, 107.0, 121.5, 128.8, 148.3, 156.8, 168.7, 174.7, 175.0; IR ($CHCl_3$) 3402, 3290, 1720, 1660, 1570, 1520, 1453, 1438, 1340, 1060, 910 cm^{-1}.

The large orange band gave 37 mg (10%) of 7f upon evaporation of solvent. mp = 158-160°C (ethanol). ^1H NMR ($CDCl_3$) 1.93 (t.t, 6.8, 6.8 Hz, 2H); 2.16 (s, 3H), 2.55 (t, J = 6.8 Hz, 2H), 3.19 (t.d, 6.8, 7.1 Hz, 2H), 3.87 (s, 3H), 5.51 (s, 1H), 6.97 (br.s, 1H), 7.73 (br.s, 1H), 9.65 (br.s, 1H). ^{13}C NMR ($CDCl_3$) 22.1, 29.8, 40.3, 42.2, 51.6, 94.39, 94.43, 151.2, 157.5, 168.6, 174.8, 207.2; UV (EtOH) 208.5 (log e 4.19), 267.5 (3.90), 328.5 (4.27), 479.5 (2.91); Anal. Calcd for $C_{13}H_{16}N_2O_5$: C, 55.4; H, 6.2; N: 9.8%. Found: C, 55.7; H, 5.9; N: 10.0%.

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7. General procedures were as previously described.[1]