Addition of Primary Aliphatic Amines to 1,2-Benzoquinone. The Absence of Reaction between a Secondary Amide and 1,2-Benzoquinone

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The addition of methylamine or ethylamine to 1,2-benzoquinone results in two isomeric major products, a 4,5-diamino-1,2-benzoquinone monoimine and a 2,5-diamino-1,4-benzoquinone monoimine. Addition of n-propylamine or n-butylamine to 1,2-benzoquinone affords the 2,5-diamino-1,4-benzoquinone monoimine. No reaction was detected between *N*-methylacetamide and 1,2-benzoquinone.

REACTIONS between oxidising polyphenols and proteins are thought to be partly responsible for the so-called ' enzymic browning ' in plants,[1] and have been found to result in a reduction in the nutritional value of plant protein concentrates which are used to supplement animal feedstuffs.[2] The proposed course of the reaction [1] involves initial enzymic oxidation of the polyphenol, usually an *o*-hydroxyphenol, to an intermediate *o*-quinone, which can then react non-enzymically with several groups within the protein. However it has not been shown conclusively that the reactive intermediate is an *o*-quinone; it could equally be a semiquinone radical or even a species analogous to the colourless *o*-benzoquinone, first reported by Willstätter.[3]

A possible approach to a study of this problem is to examine model reactions between crystalline *o*-quinones and compounds which represent the reactive groups within the protein molecule. The structures of the products may then be compared with those actually formed in the deterioration of foodstuffs by enzymic browning.[2] This paper describes a study of the reactions of 1,2-benzoquinone with methylamine, ethylamine, n-propylamine, and n-butylamine in methanol. The primary aliphatic amines are intended as models for lysine, one of the nutritionally most important amino-acids, and one which has been strongly implicated in protein–quinone coupling reactions. A little is known concerning the reactions of crystalline *o*-quinones with aliphatic amines,[4-10] although a thorough study of their reactions with aromatic amines has been made.[11]

We have also tested a proposal [12] that oxidising polyphenols can combine with the peptide linkage. This proposal was invoked to explain the observation that *N*-acetylcasein treated with the caffeic acid–*o*-diphenoloxidase system had a lower biological value than control *N*-acetylcasein, despite the amino-acid contents of the two samples being similar. The reaction of 1,2-benzoquinone with *N*-methylacetamide was examined to test whether this proposed combination involves formal chemical bonds.

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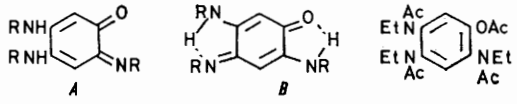
RESULTS AND DISCUSSION

The reactions of 1,2-benzoquinone with methylamine, ethylamine, n-propylamine, and n-butylamine in methanol are extremely complex. T.l.c. indicates the formation of over fifty compounds in each reaction, the majority in less than 1% yield, as well as considerable amounts of polymer. In consequence only the major products were studied. Methylamine gives compounds (1) and (2), ethylamine (3) and (4), n-propylamine (5),



A *B*

(2) R = Me
(4) R = Et

(1) R = Me
(3) R = Et
(5) R = Pr^n
(6) R = Bu^n

(7)

and n-butylamine (6). The violet compounds (2) and (4) are unstable with respect to polymerisation.

The properties of these products (Experimental section) indicate that all are formed by the reaction of 3 molecules of amine with one of quinone. The n.m.r. spectra ($CDCl_3$) of compounds (1)—(6) each show two one-proton singlets in the region τ 4.6—4.8, assigned to protons attached to the quinonoid ring. Their sharp singlet nature suggests that the protons lie *para* to one another. This implies that two molecules of amine have added to the 1,2-quinone in conjugate manner at positions 4 and 5, whilst the third has attacked the carbonyl group, forming a Schiff's base.

In the n.m.r. spectrum of compound 1, two of the *N*-methyl signals occur almost coincidentally at τ 7.15 and 7.17, whilst the third is at lower field, at τ 6.65. In the spectrum of (2), these three signals occur much closer together, at τ 6.74, 6.83, and 7.03. In the spectra of (3) and (4), the signals in the region τ 6—7 are assigned to the N·CH_2 groups. The spectrum of (4) shows a poorly resolved six-proton multiplet, centred at τ 6.52.

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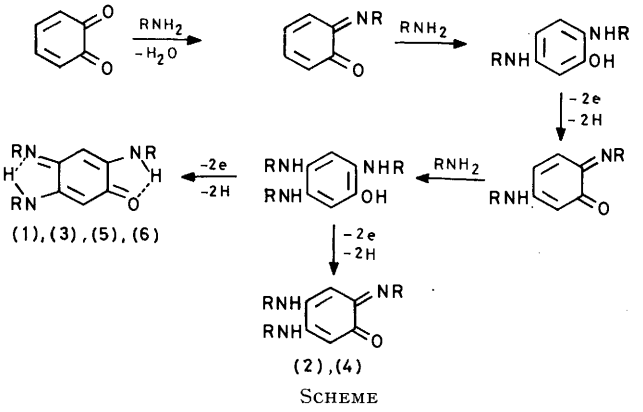
That of (3) in this region shows two overlapping quartets, integrating for 4 protons, centred at τ 6.83, with a third quartet, integrating for 2 protons, at lower field (τ 6.47). The spectra of (5) and (6) are similar to that of (3) in the region τ 6—7, in that in both one of the N·CH_2 groups is deshielded relative to the other two.



SCHEME

These results suggest that compounds (1), (3), (5), and (6) have a common general structure, and that (2) and (4) share a different general structure. The n.m.r. and mass spectral and analytical data are consistent with the two general structures (*A*) and (*B*).

The effect of the shift reagent $Eu(fod)_3$ on the n.m.r. spectra of compounds (1) and (2) was studied. The shifts of all signals in the spectrum of (2) are greater than those of the corresponding signals in the spectrum of (1), in some cases being twice as large. Models of (*A*) and (*B*) show that in both structures access to the amino nitrogen atoms is subject to considerable steric hindrance, whereas the carbonyl group is relatively unhindered. It seems reasonable therefore that the europium atom of the shift reagent will complex to a carbonyl lone pair in both (1) and (2). In structure (*B*), intramolecular hydrogen bonding is likely to reduce the availability of the carbonyl lone pair. Therefore for this structure, the europium atom is unlikely to approach the carbonyl oxygen as closely as it will for structure (*A*). Since the observed shift for a given proton is inversely proportional to the cube of the proton–metal distance,[13] the signals of the protons of structure (*A*) should suffer a greater shift than those of the protons of structure (*B*). This suggests that structure (*A*) should be assigned to compounds (2) (R = Me) and (4) (R = Et), and structure (*B*) to (1) (R = Me) and (3) (R = Et), (5) (R = Pr^n) and (6) (R = Bu^n).

The structures assigned to compounds (2) and (4) are tautomers of structures (1) and (3), respectively. In basic solution (2) and (4) were converted into (1) and (3), respectively; conversely under acidic conditions (1) and (3) were converted into (2) and (4). As further proof of the tautomeric nature of (3) and (4), reductive acetylation of both compounds in each case gave a single product. The two products had identical g.l.c. and reversed phase h.p.l.c. retention times and identical mass spectra, in accord with the probable structure (7).

There is further evidence to support our structural assignments. Compounds (2) and (4) have lower R_F values (t.l.c.) than their isomers (1) and (3) (see Experimental section). This concurs with the observation [14] that molecules that form intramolecular hydrogen bonds are less strongly adsorbed on silica gel or hydrated alumina than isomeric compounds without this property. The i.r. spectra of (1) and (2) show ν_{CO} 1 650 cm^{-1} for the *o*-quinonoid, and 1 630 cm^{-1} for the *p*-quinonoid compound. The u.v.–visible spectra of compounds (1)—(6) show marked similarity, but the large bathochromic shift of the longest-wavelength band of (2) and (4) in relation to the other compounds is typical of *o*-quinonoid isomers.[15,16]

Thus although the isolation of individual tautomers is surprising in view of the fact that proton transfer between heteroatoms is usually fast, and therefore equilibration of (1) and (2) would be expected, there is good evidence to support the structures we have assigned.

Tedder [11] has studied the addition of aromatic amines to 1,2-benzoquinone. In methanol, he obtained the 4,5-dianilino-1,2-benzoquinones, and only in ether did he obtain the triadducts; these were the 2,5-dianilino-1,4-benzoquinone monoanils, equivalent to our compounds (1), (3), (5), and (6). There was no evidence to suggest the presence of the *ortho*-isomers. The explanation put forward for this solvent effect was that acetal formation takes place when the *o*-quinone is dissolved in methanol. Nucleophilic addition is favoured at the carbonyl group. Therefore in methanol solution, acetalisation takes place, and the amine can only add in conjugate fashion. Increased nucleophilic reactivity does not always follow increased basicity. However for groups with the same attacking atom, one generally does follow the other. Thus aliphatic amines, with pK_a *ca.* 10.7, are likely to be better nucleophiles than aniline, pK_a 4.6. It is probably this increase in nucleophilicity which accounts for the formation of the diaminobenzoquinone monoimines from the aliphatic amines in methanol, by enabling the aliphatic amine to compete successfully with methanol for initial attack at the carbonyl group. A proposed mode of formation of compounds (1)—(6) is given (Scheme).

To test the proposal regarding the reactivity of the peptide bond towards oxidising polyphenols,[12] the interaction of 1,2-benzoquinone with *N*-methylacetamide in methanol was studied. Column chromatography of the reaction mixture gave back *N*-methylacetamide almost quantitatively. The mass spectra of the reaction mixture, and of the mixture resulting from 1,2-benzoquinone and *NN*-dimethylacetamide, which should not react with each other, were virtually identical. It therefore seems that there is no reaction between *N*-

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methylacetamide and 1,2-benzoquinone. This suggests that the proposed combination of oxidising polyphenols with the peptide linkage does not involve formal chemical bonds. This does not rule out the possibility that such combination takes place *via* hydrogen-bonding or other attractive non-bonded interaction.

EXPERIMENTAL

1,2-Benzoquinone was prepared by oxidation of pyrocatechol by tetrachloro-1,2-benzoquinone in dry ether at −25 °C. It was stored at −78 °C and used without further purification.

Reaction of 1,2-*Benzoquinone with Aliphatic Amines.*—A solution of the amine (0.056 mol) in methanol (120 ml), containing a small amount of anhydrous sodium sulphate as desiccant, was stirred at room temperature. Solid 1,2-benzoquinone (0.028 mol) was added. An immediate exothermic reaction took place, the solution turning dark brown. The reaction vessel was protected with a calcium chloride guard tube, and the mixture stirred at room temperature overnight, then concentrated under reduced pressure to 20 ml. The resultant dark brown viscous liquid was applied to a large column of activated alumina. Elution was commenced with diethyl ether. Fractions (100 ml) of the highly coloured eluate were collected. When the colour intensity of the eluate began to diminish, increasing proportions of chloroform and then methanol, were introduced into the eluant. Dark-brown polymeric material was retained on the column.

Fractions from the column were concentrated, and spread on 20 × 20 cm² plates coated with a 0.5 mm layer of Kieselguhr (*ca.* 30 mg of solid applied per plate). Plates containing fractions which had been eluted from the column with ether or chloroform were developed with 95 : 5 chloroform–acetone. Those containing fractions eluted with methanol were developed with 90 : 10 chloroform–methanol. T.l.c. showed the presence of at least fifty highly coloured products from each reaction.

Individual bands were scraped from the plates, and the appropriate bands combined. The coloured product was washed from the Kieselguhr with chloroform, and the solution concentrated and weighed. Nearly all the products were present in <1% yield. Only the major products were examined.

Methylamine gave 2,5-*bismethylamino*-1,4-*benzoquinone methylimine* (1) (11%),* red-orange crystals (from CCl_4), m.p. 126—128°; R_F † 0.09; $\nu_{max.}$ (KBr) 3 319, 3 280, 1 582, 1 507, 1 420, 1 337, 1 064, and 844 cm^{-1}; $\lambda_{max.}$ (EtOH) 216 (log ε 4.46), 266 (3.80), 337 (4.47), and 475 nm (2.89); τ ($CDCl_3$) 3.90br (2 H), 4.66 (1 H, s), 4.76 (1 H, s), 6.65 (3 H, s), 7.15 (3 H, s), and 7.17 (3 H, s); *m/e* 179 (M^+) (Found: C, 60.25; H, 7.05; N, 23.0. $C_9H_{13}N_3O$ requires C, 60.3; H, 7.3; N, 23.45%); and 4,5-*bismethylamino*-1,2-*benzoquinone methylimine* (2) (8%), violet crystals (from MeOH), m.p. 130—150° (decomp.); R_F 0.01; $\nu_{max.}$ (KBr) 3 480, 3 250, 3 140, 1 575, 1 540, 1 504, 1 418, and 1 313 cm^{-1}; $\lambda_{max.}$ (EtOH) 218 (log ε 4.34), 266 (3.76), 342 (4.37), and 502 nm (2.72); τ ($CDCl_3$) 4.66 (1 H, s), 4.76 (1 H, s), 6.74 (3 H, s), 6.83 (3 H, s), and 7.03 (3 H, s) (Found: M^+, 179.1071. $C_9H_{13}N_3O$ requires *M*, 179.1058).

Ethylamine gave the 1,4-*quinone imine* (3) (10%), red-orange crystals (from CCl_4–hexane), m.p. 76.5—78°; R_F 0.22; $\nu_{max.}$ (KBr) 3 321, 3 304, 1 592, 1 505, 1 360, 1 211, and 782 cm^{-1}; $\lambda_{max.}$ (EtOH) 217 (log ε 4.34), 266 (3.75), 339 (4.40), and 477 nm (2.89); τ ($CDCl_3$) 4.30br (2 H), 4.68 (1 H, s), 4.77 (1 H, s), 6.47 (2 H, q), 6.83br (4 H, q), and 8.73 (9 H, m); *m/e* 221 (M^+) (Found: C, 64.4; H, 8.25; N, 18.65. $C_{12}H_{19}N_3O$ requires C, 65.15; H, 8.65; N, 19.0); and the 1,2-*quinone imine* (4) (7%), violet crystals (from Me_2CO), m.p. 164—165°; R_F 0.02; $\nu_{max.}$ (KBr) 3 300, 3 222, 3 101, 1 594, 1 376, and 1 316 cm^{-1}; $\lambda_{max.}$ (EtOH) 218 (log ε 4.26), 266 (3.67), 342 (4.29), and 510 nm (2.61); τ ($CDCl_3$) 4.63 (1 H, s), 4.70 (1 H, s), 6.52 (6 H, m), and 8.52 (9 H, m) (Found: M^+, 221.1512. $C_{12}H_{19}N_3O$ requires *M*, 221.1528).

n-Propylamine gave the 1,4-*quinone imine* (5) (10%), red-orange crystals (from hexane), m.p. 72.5—74°; R_F 0.42; $\nu_{max.}$ (KBr) 3 323, 1 635, 1 595, 1 505, 1 338, and 824 cm^{-1}; $\lambda_{max.}$ (EtOH) 217 (log ε 4.22), 266 (3.60), 340 (4.26), and 472 nm (2.79); τ ($CDCl_3$) 4.20br (2 H), 4.67 (1 H, s), 4.76 (1 H, s), 6.57 (2 H, t), 6.88br (4 H, t), 8.31br (6 H, sext), and 9.07 (9 H, t of t); *m/e* 263 (M^+) (Found: C, 68.1; H, 9.3; N, 15.45. $C_{15}H_{25}N_3O$ requires C, 68.4; H, 9.55; N, 15.95%).

n-Butylamine gave the 1,4-*quinone imine* (6) (10%), red-orange crystals (from pentane), m.p. 24—26°; R_F 0.54; $\nu_{max.}$ (KBr) 3 364, 3 314, 1 590, 1 505, 1 465, 1 338, 1 200, and 821 cm^{-1}; $\lambda_{max.}$ 217 (log ε 4.36), 266 (3.76), 341 (4.40), and 471 nm (2.97); τ ($CDCl_3$) 4.25br (2 H), 4.70 (1 H, s), 4.79 (1 H, s), 6.55 (2 H, t), 6.92 (4 H, d of t), 8.50 (12 H, m), and 9.07 (9 H, t of t); *m/e* 305 (M^+) (Found: C, 70.75; H, 10.15; N, 14.0. $C_{18}H_{31}N_3O$ requires C, 70.8; H, 10.25; N, 13.75%).

Reductive Acetylation of Compounds (3) *and* (4).—Compound (3) or (4) (1—2 mg) was suspended in pure acetic anhydride (1 ml), and dry zinc powder (0.1 g) and anhydrous sodium acetate (20 mg) were added. The mixture was warmed gently until the colour of the quinone had largely disappeared, and then boiled for 1 min. Glacial acetic acid (1 ml) was added and the mixture boiled again. The hot solution was decanted from the residue, and sufficient water added to hydrolyse the acetic anhydride. The solution was made alkaline and extracted with dichloromethane (3 × 1 ml), and the extract dried (Na_2SO_4). The products of both reactions were analysed by g.l.c. and by h.p.l.c. Both gave one g.l.c. or h.p.l.c. peak, with identical retention times. [G.l.c. was carried out on a Pye 104 gas chromatograph with a glass column (5 ft × ¼ in o.d.) of 3% OV-1 on Diatomite CLQ, 100—120 mesh, and a flame ionisation detector, operated isothermally at 250 °C; nitrogen flow rate 50 ml min^{-1}. H.p.l.c. was carried out using a Pye LC20 separator equipped with a 250 × 4 mm Partisil ODS column and a UV detector operating at 266 nm; eluent was 30 : 70 EtOH–H_2O; flow rate 1.20 ml min^{-1}; pressure 91 ± 1 atm.] G.l.c.–mass spectrometry showed *m/e* 391 (M^+).

* Calculations of yields are based on the initial amount of 1,2-benzoquinone.

† R_F Values refer to t.l.c. on 0.5 mm thick silica plates, with 95% (v/v) chloroform–acetone as eluant.

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