Reaction of Amino-acids with *p*-Benzoquinones

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The near-u.v. absorptions which slowly develop in aqueous alcoholic solutions containing amino-acids and *p*-benzoquinones are due to products resulting from disubstitution of the quinone and not to charge-transfer complexes.

THERE have been several reports purporting to describe the formation of charge-transfer complexes in aqueous and aqueous-ethanolic solutions containing an amino-acid and a quinone, particularly tetrachloro-*p*-benzoquinone (chloranil).[1-8] These were based on the slow development of an absorption band, usually in the region 330—370 nm, often accompanied by other absorption in the visible region. Solids were isolated from such solutions which were reported[4] to have i.r. absorption bands at 1730 cm^{-1} (assigned to the carbonyl stretch of an un-ionised carboxy-group) together with weaker carboxy-bands at 1410 and 1250 cm^{-1} and an absence of bands characteristic of $-\overset{+}{N}H_3$ and $-CO_2^-$. It was claimed that these arose because the amino-acid had a non-zwitterionic structure in the complex. The fact that the unchanged amino-acid is present mainly in the zwitterionic form was also used to explain the very slow formation of the proposed complex.[5-7]

There are some exceptional features of this proposal. The most obvious is that for charge-transfer complex formation the rate is extremely low, even if it is accepted[5] that such complex formation involves the non-dipolar structure of the amino-acid so that rates based on the dipolar-structured amino-acid concentrations are effectively reduced by a factor of the order of 10^{-5}. Secondly, if the amino-acids react in the non-dipolar form, they are essentially primary aliphatic amines, and it is well established that these react with *p*-benzoquinones to form 2,5-disubstituted *p*-benzoquinones.[9-15] In fact in 1954 it was shown that under slightly different conditions amino-acids could react with *p*-benzoquinones to form substituted quinones.[16,17] The possibility that the colours observed in such reaction mixtures might be due to this type of compound had been suggested even earlier by Woker and Antener.[18]

There are one or two claims in the literature that the colours which slowly develop on addition of simple aliphatic amines themselves to chloranil in solution are the result of charge-transfer complex formation.[19,20] In fact these products are demonstrably the result of substitution reactions. There are no satisfactory examples of charge-transfer complex formation in solution between aliphatic amines and quinones, although there is some evidence that this type of complex might be formed in certain circumstances by the action of gaseous aliphatic amines on solid chloranil.[21-23]

Because of the recent interest in the possible role of charge-transfer complexes in biosystems we have sought to clarify further the nature of the reaction of amino-acids with *p*-benzoquinones.

RESULTS AND DISCUSSION

When equal volumes of alanine (4×10^{-2}M) in aqueous phosphate buffer (pH 7) and chloranil (2×10^{-3}M) in ethanol are mixed at room temperature, the solution turns bright yellow initially, but with time it becomes purple to mauve. The spectral changes are shown in Figure 1. The absorption maxima at 425 and 450 nm which develop at an early stage may be assigned to the chloranil semiquinone anion. The broad band which also develops fairly quickly in the region 500—600 nm, and which is responsible for the purple colouration, is due partly to the dianion of chloranilic acid (2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone). It is likely that there is also a contribution to the absorption in this region from mono-(1-carboxyethylamino)trichloro-*p*-benzoquinone which is doubtless formed *en route* to (I; R = H, X = Cl), but which could not be isolated. The behaviour of the absorbance with time would imply that the absorption coefficient in this region is greater for the mono- than for the di-substituted compound. In the mono- (II) and 2,5-bis-(dimethylamino)-*p*-benzoquinones (III), both where X = H and where X = Cl, this is in fact the case (Table).

The absorption band at 360 nm which eventually dominates the spectrum is due to 2,5-bis-(1-carboxy-

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[22] P. L. Kronick, H. Scott, and M. M. Labes, *J. Chem. Phys.*, 1964, **40**, 390.
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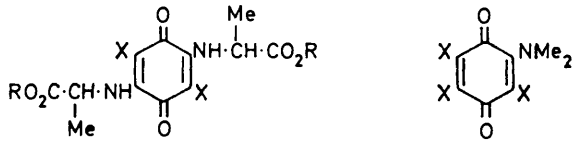
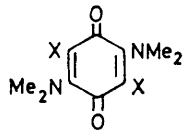
ethylamino)-3,6-dichloro-*p*-benzoquinone (I; R = H, X = Cl). A small contribution to the final absorption in the visible region is due to the weak band of this



(I)

(II)



(III)

species referred to above. This assignment is based on the fact that (I; R = H, X = Cl) may be isolated from the reaction mixture, and its identity established by combustion analysis and i.r. and n.m.r. spectroscopy.

Longest wavelength bands of some *p*-benzoquinones

Quinone	Solvent	λ/nm	ε/ l mol^{-1} cm^{-1}	Ref.
(II; X = H)	MeOH	495	5700	*a*
(III; X = H)	MeOH	513	407	*b*
(II; X = Cl)	Dioxan	545	2300	10
(III; X = Cl)	MeO·CH_2·CH_2·OH	550	340	24

[a] H.-J. Teuber and M. Hasselbach, *Chem. Ber.*, 1959, **92**, 674. [b] J. S. Webb, D. B. Cosulich, J. H. Mowat, J. B. Patrick, R. W. Broschard, W. E. Meyer, R. P. Williams, C. F. Wolf, W. Fulmor, and C. Pidacks, *J. Amer. Chem. Soc.*, 1962, **84**, 3185.

It is assumed that the substitution pattern is the same as with simple aliphatic amines, namely 2,5-. The electronic spectrum [$\lambda_{max.}$ (EtOH) 356 and 527 nm (ε 25,500 and 268 l mol^{-1} cm^{-1})] is consistent with (I; R = H, X = Cl) being a major product in the final

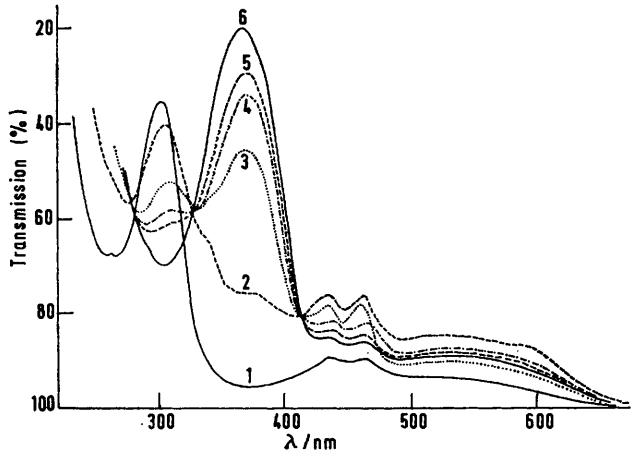


FIGURE 1 Absorption spectrum of (1) chloranil (7×10^{-5}M) in 1:1 v/v aqueous ethanol containing phosphate buffer (pH 7); (2) immediately after adding α-alanine (4×10^{-4}M); (3) after 7 min; (4) after 15 min; (5) after 23 min; (6) after 90 min; all at room temperature, scanning time *ca.* 2 min

solution shown in Figure 1. In this latter solvent (1 : 1 v/v water–ethanol containing phosphate buffer) the absorption maximum of (I; R = H, X = Cl) in the near u.v. is at 361 nm. This material obeys Beer's law as would be anticipated. The small decrease in the absorbance as the temperature is raised is accounted for by the thermal expansion of the solvent. There seems to be no reason why this absorption should be assigned to an intermolecular transition of a charge-transfer complex which, in solution, would be in equilibrium with its molecular compounds. The chloranilic acid is probably formed in a side reaction by a similar nucleophilic attack by hydroxide ion. In support of this suggestion it was noted that buffered aqueous-alcoholic solutions of chloranil in the absence of alanine formed chloranilic acid. In the reaction between alanine and chloranil, the chloranilic acid appears to be formed only in small amounts. It cannot account for the major peak at 360 nm since its absorption maximum in the buffered solvent was shown, in a separate experiment,

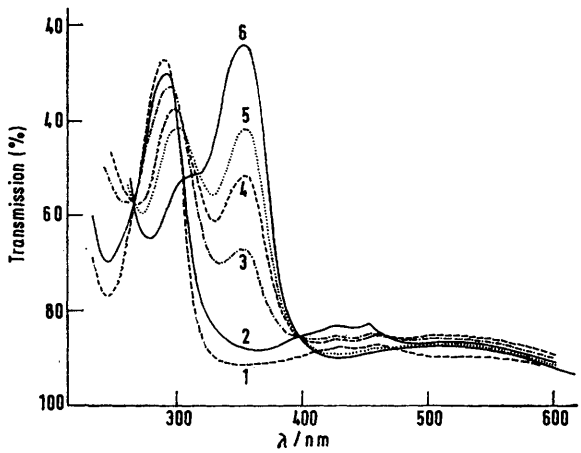


FIGURE 2 Absorption spectrum of (1) chloranil (7×10^{-5}M) in ethanol; (2) immediately after adding α-alanine ethyl ester (5×10^{-5}M); (3) after 5 min; (4) after 11 min; (5) after 16 min; (6) after 45 min; all at room temperature, scanning time *ca.* 2 min

to be below 340 nm. Tetrachlorohydroquinone was also detected in the reaction mixture.

Mixtures of alanine ethyl ester and chloranil in ethanol behave in a similar manner to the alanine–chloranil system described above. Again the chloranil semiquinone anion and the dianion of chloranilic acid are formed at an early stage in the reaction. The absorptions due to these species are followed by the slow development of a band at about 350 nm (Figure 2). The precise position of this band is pH dependent. From the reaction mixture a compound, m.p. 165—166°, identified as 2,5-dichloro-3,6-bis-(1-ethoxycarbonylethylamino)-*p*-benzoquinone (I; R = Et, X = Cl) was isolated. The absorption at *ca.* 350 nm could be assigned to this compound. The similarity of the reactions of alanine and its ester emphasies the simple nature of the substitution product in the former case. Various other analogues, including (I; R = Et, X = F, Br, or H), (IV; R = Et, X = F, Cl, or H), and (V; R = Et), have been obtained under the same conditions.

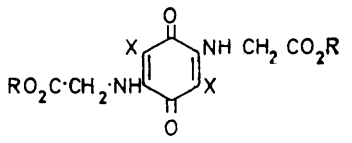
All attempts to hydrolyse these esters to the corresponding acids were unsuccessful. However, a Fischer–Speier esterification of (I; R = H, X = Cl) yielded a product, m.p. 165°, which was identical with (I; R = Et, X = Cl) obtained by the direct action of alanine ethyl ester on chloranil.

A second dichlorobis-(1-ethoxycarbonylethylamino)-*p*-benzoquinone, m.p. 113°, was also obtained. This compound shows only small differences in the i.r. spectrum from the isomer of m.p. 165°. Although in the reaction of *N*-methylaniline with fluoranil, Wallenfels and Draber [24] have shown that substitution in the 2- and 6-positions, as opposed to the 2- and 5-positions, can occur, there is little support for the suggestion that in the present reaction the two compounds are *structural* isomers. Were this the case, the two quinone carbonyl groups of the 2,6-isomer would not be equivalent, and they would be expected to show distinguishable carbonyl stretching frequencies as was observed by Wallenfels and Draber in the case of 2,6-difluoro-3,5-bis-(*N*-methylanilino)-*p*-benzoquinone. Since a racemic mixture of alanine was used in the first place, it is considered likely that the two products are in fact the racemate and the *meso*-compound. This suggestion is supported by the fact that when L-alanine ethyl ester is used, a single diester, m.p. 125—126°, is formed, the i.r. spectrum of which is virtually identical with that of the material of m.p. 113°. We therefore consider that this latter compound is the racemate and that the major product, m.p. 165—166°, is the *meso*-compound.

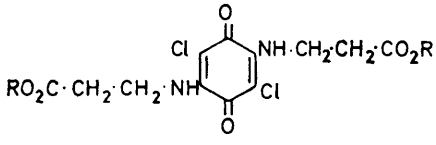
In the reaction of glycine with chloranil in phosphate-buffered aqueous ethanol, the spectral changes in the early stages of the reaction are very similar to those reported for the alanine–chloranil reaction. However, with time the developing band in the near u.v. shifts from 350 to 340 nm. From the solution, 2,5-diamino-3,6-dichloro-*p*-benzoquinone (VI) and not the biscarboxymethylamino-compound (IV; R = H, X = Cl) is obtained. We suggest that the latter is the precursor of (VI). The absorption at 342 nm is consistent with structure (VI). Oxidative deamination in the reaction of amino-acids with *p*-benzoquinone has been reported previously.[17]

The spectral changes on treating α-alanine with unsubstituted *p*-benzoquinone are similar to those for the reaction with chloranil. Again we suggest that the initial increasing absorbance in the visible region, which later declines, is largely due to monosubstitution by the amino-acid. This is because the final disubstituted product can be expected to have a weaker absorption in the visible region than the un-isolable monosubstituted quinone (see Table).

There seems little doubt therefore that, not unexpectedly, the absorptions which develop in solutions containing amino-acids and quinones are the result of substitution reactions. It is, of course, possible that conditions might be found whereby charge-transfer complexing between an amino-acid and a quinone could occur. For example, phenylalanine or tryptophan, preferably in a non-hydroxylic solvent, might show such an effect.



(IV)



(V)

EXPERIMENTAL

^{1}H N.m.r. spectra were measured at 34° using either a Perkin-Elmer R10 spectrometer operating at 60 MHz or a Bruker HX-90 spectrometer operating at 90 MHz. Solutions in $CDCl_3$ were used unless otherwise indicated. U.v. spectra were measured on either a Unicam SP 800 or an Optica CF4R spectrophotometer; for the values cited, ethanolic solutions were used.

meso-2,5-*Dichloro*-3,6-*bis*-(1-*ethoxycarbonylethylamino*)-p-*benzoquinone* (I; R = Et, X = Cl).—DL-α-alanine ethyl ester (1 g) was added dropwise to chloranil (0·5 g) in ethanol (1 l). The solution was left at room temperature in the dark for 2 days, then concentrated at room temperature. A red-brown microcrystalline *powder* (0·75 g), m.p. 165—166°, was obtained by recrystallisation from ethanol (Found: C, 47·8; H, 4·9; Cl, 17·0; N, 6·75. $C_{16}H_{20}Cl_2N_2O_6$ requires C, 47·15; H, 4·95; Cl, 17·4; N, 6·85%); $\nu_{max.}$ 3281, 2980, and 1738 cm^{-1}; $\lambda_{max.}$ 351 and 527 nm (ε 27,200 and 270); δ 1·30 (3H, t, ethyl CH_3), 1·55 (3H, d, CH_3), 4·30 (2H, q, ethyl CH_2), 5·29 (1H, quin, CH), and 7·55br (1H, s, NH) (on shaking with D_2O, the quintet at 5·29 collapses to a quartet).

LL-2,5-*Dichloro*-3,6-*bis*-(1-*ethoxycarbonylethylamino*)-p-*benzoquinone* (I; R = Et, X = Cl).—This was prepared in a similar fashion from L-α-alanine ethyl ester, as orange *needles*, m.p. 125—126° (Found: C, 46·8; H, 4·8; Cl, 17·6; N, 6·8%); $\nu_{max.}$ 3265, 2970, and 1741 cm^{-1}; $\lambda_{max.}$ 351 and 520 nm (ε 17,500 and 320); δ 1·30 (3H, t, ethyl CH_3), 1·58 (3H, d, CH_3), 4·27 (2H, q, ethyl CH_2), 5·19 (1H, quin, CH), and 7·3br (1H, s, NH).

2,5-*Bis*-(1-*ethoxycarbonylethylamino*)-3,6-*difluoro*-p-*benzoquinone* (I; R = Et, X = F).—This was prepared from fluoranil in a similar manner as purple needles (51%) (from toluene), m.p. 174° (Found: C, 51·65; H, 5·2; F, 9·9; N, 7·55. $C_{16}H_{20}F_2N_2O_6$ requires C, 51·3; H, 5·4; F, 10·15; N, 7·5%); $\nu_{max.}$ 3290 and 1738 cm^{-1}; $\lambda_{max.}$ 341 and 524 nm (ε 26,200 and 265).

2,5-*Dibromo*-3,6-*bis*-(1-*ethoxycarbonylethylamino*)-p-*benzoquinone* (I; R = Et, X = Br).—This was prepared from bromanil in a similar manner, as red *crystals* (51%) (from ethanol), m.p. 94° (Found: C, 38·55; H, 4·0; Br, 34·15; N, 5·7. $C_{16}H_{20}Br_2N_2O_6$ requires C, 38·75; H, 4·05; Br, 32·2; N, 5·65%); $\nu_{max.}$ 3228, 1729, and 1750 cm^{-1}; $\lambda_{max.}$ 350 and *ca.* 530 nm (ε 25,200 and *ca.* 200).

[24] K. Wallenfels and W. Draber, *Tetrahedron*, 1964, **20**, 1889.

2,5-*Dichloro*-3,6-*bis*-(2-*ethoxycarbonylethylamino*)-p-*benzoquinone* (V; R = Et).—This was prepared from chloranil and β-alanine ethyl ester in a similar manner and recrystallised from ethanol, then from toluene to give a grey-brown microcrystalline *solid*, m.p. 169° (Found: C, 47·25; H, 5·1; Cl, 17·25; N, 7·0. $C_{16}H_{20}Cl_2N_2O_6$ requires C, 47·2; H, 4·95; Cl, 17·4; N, 6·9%); $\nu_{max.}$ 3260, 3278, and 1720 cm^{-1}.

2,5-*Dichloro*-3,6-*bis*(*ethoxycarbonylmethylamino*)-p-*benzoquinone* (IV; R = Et, X = Cl).—This was prepared in a similar manner. After 2 days an orange solid precipitated. This was recrystallised from ethanol; m.p. 201·5—202·5° (lit.,[16] 199—200°). When this compound (3 g) in dioxan (360 ml) was refluxed with sodium hydrogen carbonate (1·2 g in 40 ml of water) for 10 h, and the solution was then concentrated, 2,5-diamino-3,6-dichloro-*p*-benzoquinone, m.p. 270—275° (from alcohol), was obtained. This was identical with a specimen obtained from the reaction of chloranil with aqueous ammonia, and from glycine–chloranil solutions in buffered aqueous ethanol.

The preparations of 2,5-bis(ethoxycarbonylmethylamino)-*p*-benzoquinone (II; R = Et, X = H) and of 2,5-bis-(1-ethoxycarbonylethylamino)-*p*-benzoquinone (I; R = Et, X = H) are described in the literature.[25]

DL-2,5-*Bis*-(1-*carboxyethylamino*)-3,6-*dichloro*-p-*benzoquinone* (I; R = H, X = Cl).—DL-α-Alanine (3 g) in phosphate buffer (1 l) was added to chloranil (1·75 g) in ethanol (2 l). After a week in the dark, the solution was concentrated at room temperature, the precipitated α-alanine was filtered off, the residue was diluted with ethanol (100 ml), and more precipitated α-alanine was removed. This procedure was repeated several times so that virtually all the excess of α-alanine was removed. The residue was chromatographed twice on a silica gel column (mesh 100—200) with 7 : 2 : 3 chloroform–acetic acid–ethanol as eluant. The violet fraction was separated and concentrated, and the products were recrystallised from ethanol to give pink *crystals*, decomp. 179—180° (Found: C, 41·4; H, 3·9; N, 7·7. $C_{12}H_{12}Cl_2N_2O_6$ requires C, 41·05; H, 3·45; N, 8·0%); $\nu_{max.}$ 3440, 3275, 1707, and 1660 cm^{-1}; $\lambda_{max.}$ 356 and 527 nm (ε 25,500 and 268); δ 1·55 (3H, α-CH_3), 5·29 (1H, quin, CH), and 7·35br (1H, NH). Esterification of this material by the Fischer–Speier method gave 2,5-dichloro-3,6-bis-(1-ethoxycarbonylethylamino)-*p*-benzoquinone, m.p. 165°, identical with that obtained by the reaction of alanine ethyl ester chloranil (*meso*-compound). A second product obtained in this reaction (the corresponding racemate) gave orange *needles* (from ethanol), m.p. 113° (Found: C, 47·3; H, 5·1; N, 6·8. $C_{16}H_{20}Cl_2N_2O_6$ requires C, 47·15; H, 4·95; N, 6·85%); $\nu_{max.}$ 3271, 2920, and 1735 cm^{-1}; $\lambda_{max.}$ 351 and 520 nm (ε 26,600 and 240); δ 1·30 (3H, t, ethyl CH_3), 1·56 (3H, d, CH_3), 4·25 (2H, q, ethyl CH_2), 5·20 (1H, quin, CH), and 7·3br (1H, s, NH).

[4/038 *Received*, 10*th January*, 1974]

[25] E. Fischer and H. Schrader, *Ber.*, 1910, **43**, 525.