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A STUDY OF THE REACTIONS OF α-AMINOACIDS WITH A MODEL AMINOCHROME[1]

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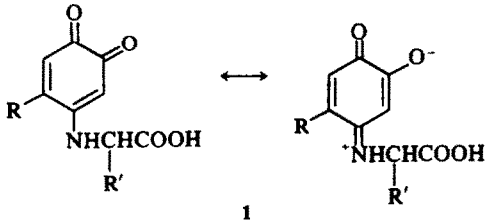
Abstract—Chemical and spectroscopic evidence leads to the conclusion that all common α-aminoacids, with the exception of proline and hydroxyproline, undergo oxidative deamination by the action of the model aminochrome, 4-methyl-5-(1'-pyrrolidyl)-1,2-benzoquinone(2), to give the same intensely greenish-blue phenoxazine dye 3. This new colour-forming reaction may form the basis for a new method for the detection, specifically, of α-aminoacids. Unlike ninhydrin, 2 is not capable to bring about the oxidative deamination of β-amino-acids and amines. As expected, the SH-containing aminoacid cysteine reacts differently with 2, giving a yellow condensation product, identified as 3-carboxy-5-hydroxy-8-methyl-7-(1'-pyrrolidyl)-2H-1,4-benzothiazine (7).

It has long been known that 1,2-quinones catalyze the oxidative deamination of certain aminoacids with the concomitant formation of deeply coloured pigments.[2-4] Studies of the oxygen consumptions and spectrophotometric characteristics of the reactions suggest that the process is initiated by the conjugate addition of the aminoacid to the quinone to give as a first intermediate an aminochrome[5] pigment of the type 1, which oxidizes the excess aminoacid with liberation of ammonia and a ketoacid.[6,7] However, even in the case of glycine, which is deaminated far more rapidly than other aminoacids, the ammonia evolved is much less than one mole, thus suggesting that the primary process is accompanied by some concomitant reaction in which the amino group originally present in the aminoacid enters into combination with the aminochrome intermediate 1, which acts as the oxidizing agent.



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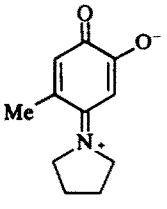

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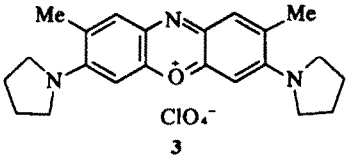
With a view to clarifying the nature of these secondary reactions we have undertaken a study of the oxidative deamination of α-aminoacids by the action of a stable model aminochrome, namely 4-methyl-5-(1'-pyrrolidyl)-1,2-benzoquinone (2), which was obtained in crystalline form by oxidation of a 1:1 mixture of 4-methylcatechol and pyrrolidine with silver oxide.

When an equimolecular solution of 2 and glycine in aqueous methanol was kept at 37°, a smooth



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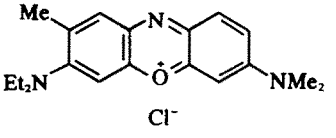

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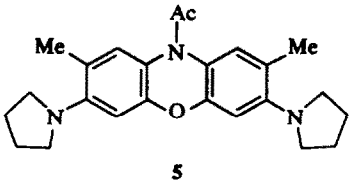

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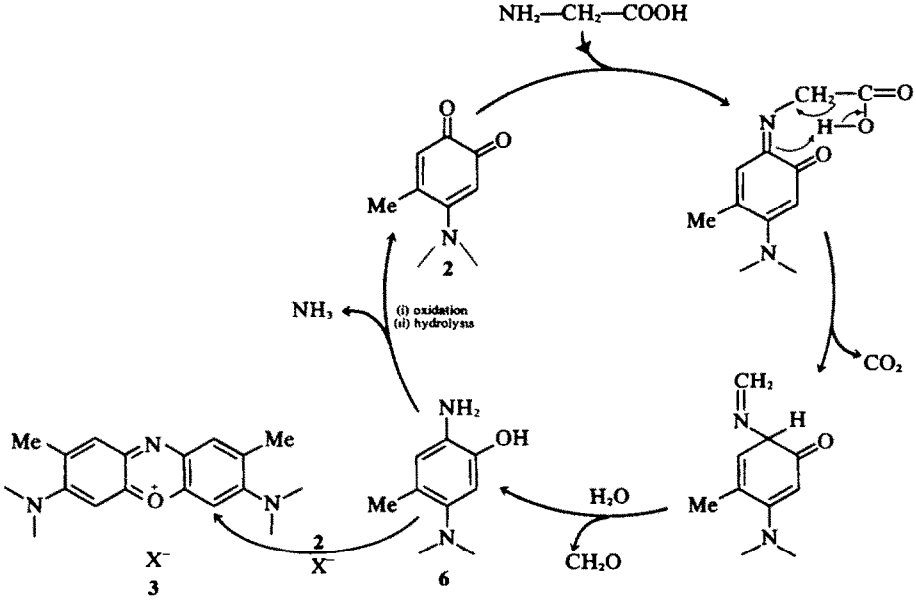
reaction took place, as shown by the formation of formaldehyde,* and by a colour change from red to greenish-blue. Within 3 h the visible absorption maximum of the aminochrome at 529 nm was completely replaced by a new and more intense maximum at 629 nm. Thus, as a result of the oxidative deamination of glycine by the action of **2**, a greenish-blue dye was formed. Preliminary experiments showed that this latter was basic in character, giving water-insoluble salts with certain inorganic acids, especially with perchloric acid which, when added to the reaction mixture, allowed the isolation of a microcrystalline perchlorate, $C_{22}H_{26}N_3O_5Cl$. This was then identified as the phenoxazonium perchlorate **3** on the basis of the following evidence.

The product shows redox properties and displays absorption maxima (MeOH) at 670,622fl, 310,262, and 248 nm (log ϵ 4·84, 4·63, 4·16, 4·36, and 4·37), indicating the presence of an aminophenoxazonium chromophore. Indeed, the spectrum resembles that of some commercial dyes, particularly that of Capri Blue GON (**4**). Also characteristic is the mass spectrum of the product, in which most of the total ion current is carried by two ionic species at *m/e* 348(20%) and *m/e* 349 (100%), corresponding to the phenoxazonium radical ion, $C_{22}H_{26}N_3O\dagger$, derived from **3** and to its reduced species, $C_{22}H_{27}N_3O^+$. The latter arises probably by reduction of the former ion by the moisture present in the mass spectrometer, as in the case of 1,2-quinones.[8] As the PMR spectrum could not be obtained owing to the exceedingly low solubility of the dye in organic solvents, evidence for the arrangement of substituents as in **3** was derived from the spectroscopic characteristics of the leucomonoacetate **5**, $C_{24}H_{29}N_3O_2$, $\nu_{C=O}$(CHCl$_3$) 1667 cm^{-1}, which was formed on reductive acetylation. Its PMR spectrum (CDCl$_3$) was remarkably simple showing, in addition to the N-Ac signal, two complex symmetrical multiplets at δ 1·95 and 3·19 for two pyrrolidyl residues, a 6 H singlet at δ 2·31 for two Ar-Me groups, and two singlets (two protons each) at δ 6·67 and 7·19, indicating two pairs of *para*-oriented aromatic protons, in agreement with the symmetrically substitution pattern in **5**.

The formation of the phenoxazonium cation of **3** from the oxidative deamination of glycine by the aminochrome **2** can be easily explained on the basis of a Strecker degradation leading to the aminophenol **6**. The subsequent fate of **6** depends upon the prevailing conditions; formation of the dye by condensation with **2** is favoured by the absence of oxygen and a 1:1 molar ratio of glycine and **2**, while for the catalytic deamination the presence of oxygen and a high ratio of glycine and **2** is required (Scheme 1). The latter sequence is a modification of that proposed by Trautner and Roberts[9] for the oxidation of α-aminoacids by 1,2-benzoquinone.

As expected, all common α-aminoacids, with the exception of proline and hydroxyproline, reacted similarly with the aminochrome **2** to give the same intensely greenish-blue phenoxazonium cation of **3** (UV and TLC evidence). This was also formed,

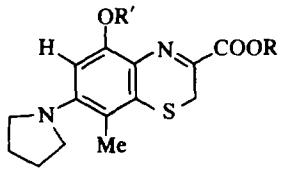
*The aldehyde was identified by its specific reaction with chromatopic acid.



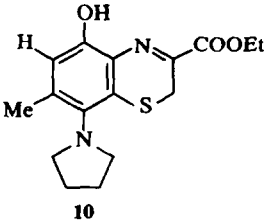
SCHEME 1

albeit in smaller yields, when a methanolic solution of 2 was allowed to react with an aqueous solution of ammonium acetate. Thus, the reaction of ammonia and α-aminoacids with 2 resembles that with ninhydrin both mechanistically and in the formation of an intensely coloured dye. It can be used for the detection of α-aminoacids on chromatograms giving green spots on a light red background. Unlike ninhydrin, the reaction is specific for α-aminoacids since with β-amino-acids (i.e. β-alanine and β-aminobutyric acid) and amines (i.e. n-propylamine, benzylamine, and aniline) oxidative deamination failed to occur, probably because the reaction stopped with the formation of the corresponding Schiff's bases (UV evidence).

As expected, the SH-containing aminoacid cysteine behaved differently with the aminochrome 2 resulting in the formation of a yellow product (7) with λ_{max} 410 nm. Attempts to isolate this compound from the reaction mixture were unsuccessful, owing to its instability under the usual chromatographic conditions. However, the corresponding product arising from the reaction of 2 and cysteine ethyl ester could be isolated in crystalline form and was identified as the 2H-1,4-benzothiazine derivative 8 on the following grounds. The compound, $C_{16}H_{20}N_2O_3S$, displayed absorption maxima (MeOH) at 428, 280 and 244 nm (log ε 4·06, 3·75, and 4·18), consistent with the presence of a 2H-1,4-benzothiazine chromophore.[10] On treatment with acetic anhydride, it gave a monoacetate 9, the IR spectrum of which showed no absorption in the NH/OH stretching region and two CO bands at 1770 (–OAC) and 1710 (conjugated COOEt) cm^{-1}. Consequently, two alternative structures 8 and 10 appeared possible for the condensation product. That the former was correct was deduced from its PMR spectrum ($CDCl_3$) showing in the aromatic region a 1 H sharp singlet at δ 6·30, shifted on addition of DCl to δ 7·21, indicating that the aromatic proton was located next to the basic pyrrolidyl residue as in 8. Thus, it appears that the reaction between the aminochrome 2 and cysteine follows a pathway which is essentially analogous to that previously observed for the condensation of β-aminothiols with 4-methyl-1,2-benzoquinone.[10,11]



7: R = R′ = H
8: R = Et; R′ = H
9: R = Et; R′ = Ac



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EXPERIMENTAL

M.ps were determined for samples with a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer infracord 137 E, UV spectra with a Perkin-Elmer 402 spectrophotometer and PMR spectra with a Perkin-Elmer R-12 A spectrometer. Chemical shifts are expressed in δ values (ppm) downfield from TMS as internal reference. Mass spectra and exact mass measurements were obtained by the direct insertion technique with an AEI-MS 902 spectrometer (70 eV) with the lowest source temp which produced a definite spectrum. Besides the molecular ion the most abundant ions in the mass spectrum (above *m/e* 100) are given with their relative intensities. TLC were carried out on Merck silica GF_{254} and all solvents used for development and elution were redistilled. Proportions given for mixed solvents are by volume.

4-Methyl-5-(1′-pyrrolidyl)-1,2-benzoquinone (2). To a soln of 4-methylcatechol (1·24 g) and pyrrolidine (0·71 g) in anhyd EtOH (300 ml) dried Na_2SO_4 (13 g) and dry alkali-free silver oxide (12 g) were added. The mixture was shaken vigorously in the stoppered flask for 15 min and filtered through a layer of anhyd Na_2SO_4. The clear red filtrate was diluted gradually with 300 ml anhyd Et_2O and cooled at − 15°. The purplish-black crystals which separated after scratching were collected, washed with a small volume of cold MeOH and dried, yielding 1·01 g of 2, homogeneous on TLC ($CHCl_3$–MeOH, 95:5), decomposing at 112°. (Found: C, 68·9; H, 7·0; N, 7·2. $C_{11}H_{13}N$ requires: C, 69·1; H, 6·8; N, 7·3%); *m/e* 193 (M + 2, 95), 192 (M + 1, 100), 191 (M^+, 10), 178 (6), 165 (14), 164 (33), 150 (14), 146 (5), and 132 (7%); λ_{max} (H_2O) 316 and 529 nm (log ε 3·97 and 3·55); λ_{max} ($H_2O - H^+$) 310 and 438 nm (log ε 4·12 and 3·26); ν_{max} ($CHCl_3$) 1640, 1575 cm^{-1}.

Reaction between the aminochrome 2 and glycine

Isolation of phenoxazonium perchlorate 3. A soln of glycine and 2 (3 mmoles each) in 50% aqueous MeOH (150 ml) was kept at 37° in a stoppered flask. After 4 h the greenish-blue mixture was evaporated under red. press; to two thirds of its original volume and extracted 3 times with EtOAc. The aqueous layer, diluted with water to 300 ml, was then warmed at 60° and treated with 20 ml of 1 N $NaClO_4$ dropwise. The microcrystalline ppt which deposited on cooling at 4° was collected by filtration, washed with water and dried over P_2O_5, *in vacuo* to give 3 (33% yield), homogeneous on TLC ($CHCl_3$–MeOH, 88:12), m.p. > 310 (Found: C, 58·2; H, 6·1; N, 9·6; Cl, 7·4. $C_{22}H_{26}N_3O_5Cl$ requires: C, 58·9; H, 5·8; N, 9·4; Cl, 7·9%).

In another experiment, carried out in the presence of atmospheric oxygen, the yield of 3 was less than 5% and during the reaction ammonia was evolved, as shown by sweeping it out with air and collecting it in an ethanolic soln of picric acid to give ammonium picrate.

Reductive acetylation of 3. To a soln of 3 (70 mg) in Ac_2O (10 ml) freshly dried Zn dust (300 mg) was added. The mixture was heated with occasional stirring at 100° for 1·5 h, filtered and evaporated *in vacuo*. The residue was extracted with EtOAc and the extract washed with

water and evaporated. Crystallization of the residue from EtOH gave 52 mg of **5**, as colourless prisms, m.p. 174–176°. (Found: C, 73·1; H, 6·9; N, 10·2. $C_{24}H_{29}N_3O_2$ requires: C, 73·6; H, 7·4; N, 10·7%); *m/e* 391 (M^+, 24), 349 (26), and 348 (100%).

Reaction of the aminochrome **2** *with other α-aminoacids and related compounds.* To test the behaviour of other α-aminoacids, β-amino-acids, and amines in the reaction with the aminochrome **2**, a soln of the latter (0·2 mmoles) and the functional reagent (0·2 mmoles) in 50% aqueous MeOH (5–10 ml) was left in a thermostat at 37° for 4 h. After addition of 1 N $NaClO_4$ (0·5 ml), the mixture was examined by UV spectroscopy and by TLC over silica (eluent: $CHCl_3$–MeOH, 88:12). In all experiments, provided that the oxidative deamination took place at all, the yield of **3**, determined spectrophotometrically, was over 25% of theoretical. In the case of ammonium acetate, the reaction was preferentially carried out in water at room temp, using a 5:1 molar ratio of ammonium acetate and aminochrome. After 24 h the yield of **3** was about 14% (based on **2**).

Reaction between **2** *and cysteine ethyl ester*

Isolation of the benzothiazine derivative **8**. To a well stirred soln of **2** (229 mg) in 0·5 M acetate buffer, pH 6·8, an aqueous soln of cysteine ethyl ester hydrochloride (370 mg) was added dropwise over a period of 30 min. After the addition the mixture was extracted with EtOAc, and the organic layer washed with water and evaporated to dryness *in vacuo*. Crystallization of the yellow oily residue (251 mg) from MeOH gave 92 mg of **8**, as yellow prisms, m.p. 74–76°. An additional 60 mg of the same material was obtained by evaporating the filtrate to dryness and by fractionating the residue by preparative TLC on silica with C_6H_6–Et_2O (90:10). (Found: C, 59·3; H, 6·7; N, 7·9; S, 9·5. $C_{16}H_{20}N_2O_3S$ requires: C, 60·0; H, 6·2; N, 8·7; S, 10·00%); *m/e* 320 (M^+, 100), 247 (55), 219 (11), and 149 (10%); ν_{max} ($CHCl_3$) 3350 and 1702 cm^{-1}; δ [$(CD_3)_2CO$] 1·34 (3H, t, J 7·0 Hz, CH_3—CH_2O—), 1·95 (4H, m, —$\underline{CH_2}$—CH_2—N—), 2·19 (3H, s, Ar—Me), 3·32 (4H, m, CH_2—$\underline{CH_2}$—N—), 3·65 (2H, s, S—CH_2—), 4·32 (2H, q, J 7·0 Hz, CH_3—$\underline{CH_2}O$—), 6·30 (1H, s, aromatic, shifted to 7·21 by addition of DCl), and 8·05 (1H, br, OH, removed by D-exchange).

Acetyl derivative of **8**. A mixture of **8** (220 mg), Ac_2O (5 ml), and pyridine (0·5 ml) was allowed to stand at room temp for 24 h. Removal of the volatile constituents left an oil, which was crystallized from EtOH to give 150 mg of **9**, as pale yellow prisms, m.p. 113–114; *m/e* 362 (M^+, 73), 320 (100), and 247 (42%); λ_{max} ($CHCl_3$) 258, 297 and 402 nm (log ε 4·11, 3·55, and 2·76); δ ($CDCl_3$) 1·37 (3H, t, J 7·0 Hz, $\underline{CH_3}$—CH_2—O—), 1·90 (4H, m, —$\underline{CH_2}$—CH_2—N—), 2·26 (3H, s, O—Ac), 2·33 (3H, s, Ar—Me), 3·25 (4H, m, —CH_2—$\underline{CH_2}$—N—), 3·56 (2H, s, S—CH_2—), 4·35 (2H, q, J 7·0 Hz, CH_3—$\underline{CH_2}$—O—), 6·53 (1H, s, aromatic).

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