Studies related to the Chemistry of Melanins. Part XIV.[1] The Alleged Formation of a *p*-Quinonoid Aminochrome by Oxidation of 2,4,5-Trihydroxyphenethylamine

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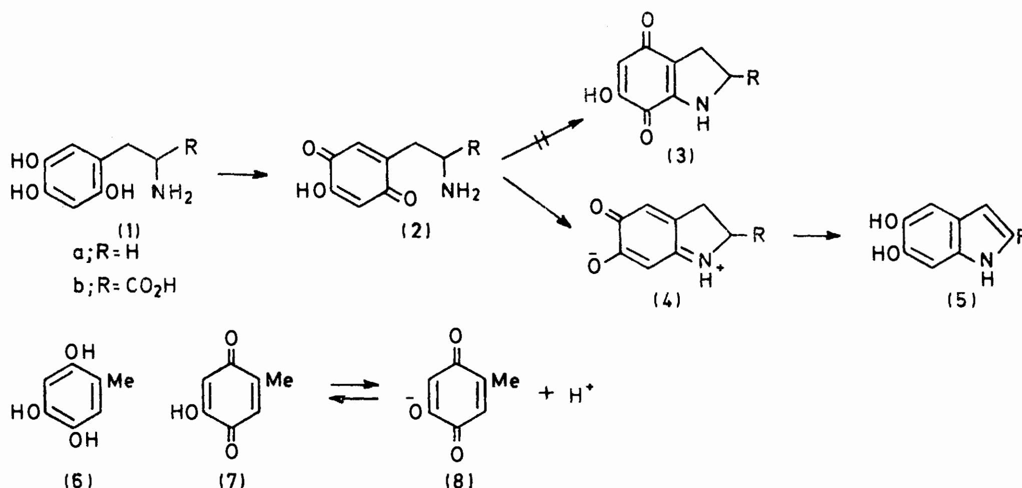
The oxidation product of 2,4,5-trihydroxyphenethylamine (1a) formulated by Senoh and Witkop as 2,3-dihydro-6-hydroxyindole-4,7-quinone (3a) is 2-(2-aminoethyl)-5-hydroxy-*p*-benzoquinone (2a) and undergoes cyclisation to yield a dopachrome-type product (4a) and thence 5,6-dihydroxyindole (5a).

SENOH and WITKOP[2] found that when an ethanolic solution of 2,4,5-trihydroxyphenethylamine (1a) is rendered alkaline by sodium hydroxide rapid aerobic oxidation occurs, as observed spectroscopically, yielding a relatively stable product which they formulated as the *p*-quinonoid aminochrome (3a). They drew attention to the difference between *o*- and *p*-quinonoid aminochromes, namely that whereas the former (*e.g.* adrenochrome and dopachrome) undergo easy base-catalysed isomerisation to dihydroxyindoles, their alleged *p*-quinonoid aminochrome (3a) was stable to base, which stability they attributed to its oxidation–reduction potential being too low for an internal hydrogen shift to occur.



The formulation of the oxidation product of (1a) as (3a) is surprising. Although intermolecular reactions between 1,4-benzoquinones and aliphatic primary or secondary amines generally result in diamination *via* 1,4-addition, similar intramolecular amination results in 1,2-addition (*i.e.* attack at the carbonyl group rather than at the C=C bond) (see ref. 3). The formation of compound (3a) would therefore be exceptional. Moreover Harley-Mason[4] has isolated 5,6-dihydroxyindole (5a) from the products of oxidation of (1a) by potassium hexacyanoferrate(III) in the presence of sodium hydrogen carbonate; and Baird[5] questioned the correctness of the formulation of (3a), without however reaching the correct conclusion as to the structure of the compound concerned.

A solution of 2,4,5-trihydroxyphenylalanine (1b) in 0.06M-phosphate buffer of pH 5.6 showed $\lambda_{max.}$ 292 nm. When hydrogen peroxide and horseradish peroxidase were added, the solution rapidly became red and the 292 nm peak was replaced by peaks at 270 and 490 nm. When the solution was kept at room temperature the 490 nm peak slowly shifted to 475 nm, while the 270 nm peak shifted to 300 nm, in accord with the formation of dopachrome (4b), for which Mason[6] gives $\lambda_{max.}$ (pH 5.6) 305 and 475 nm ($\varepsilon_{max.}$ 9 300 and 3 500). The peaks at 270 and 490 nm are therefore presumably due to the uncyclised quinone (2b) or its anion.

A solution of 2,4,5-trihydroxyphenethylamine (1a) in 0.06M-phosphate buffer of pH 5.6 showed $\lambda_{max.}$ 295 nm (given erroneously as 272 nm at pH 6.8 in ref. 7). When hydrogen peroxide and peroxidase were added, the

[1] Part XIII, F. Binns, J. A. G. King, S. N. Mishra, A. Percival, N. C. Robson, G. A. Swan, and A. Waggott, *J. Chem. Soc.* (*C*), 1970, 2063.
[2] S. Senoh and B. Witkop, *J. Amer. Chem. Soc.*, 1959, **81**, 6231.
[3] D. B. Baird, I. Baxter, D. W. Cameron, and W. R. Phillips, *J.C.S. Perkin I*, 1973, 832.
[4] J. Harley-Mason, *J. Chem. Soc.*, 1953, 200.
[5] D. B. Baird, Ph.D. Thesis, University of Cambridge, 1971.
[6] H. S. Mason, *J. Biol. Chem.*, 1948, **172**, 83.
[7] R. F. Chapman, A. Percival, and G. A. Swan, *J. Chem. Soc.* (*C*), 1970, 1664.

solution rapidly became red and the 295 nm peak was replaced by peaks at 270 and 495 nm. When the resulting solution was kept at room temperature its spectrum showed a change similar to that for (1b) but slower, and consequently less clearly defined. However the 495 nm peak shifted to 480 nm and the 270 nm peak decreased in intensity and moved towards longer wavelength. A similar, although much slower, oxidation occurred also when the solution of (1a) was kept at pH 5.6 in the presence of air, but in the absence of hydrogen peroxide and peroxidase. Such a solution which had been kept for 5 h and then acidified gave a peak at 292 nm. When a solution of dopachrome was rendered strongly acidic with concentrated hydrochloric acid the 305 and 475 nm peaks moved to 292 and 425 nm, respectively. The aminochrome formed by oxidation of dopamine had maxima at 298 and 485 nm, shifted on acidification to 293 and 418 nm, respectively. The Table shows that the initial product formed by oxidation of (1a) at pH 5.6 is the same as the compound formulated by Senoh and Witkop as the indolequinone (3a) obtained by oxidation of (1a) in alkaline solution. It is therefore proposed that this compound should be reformulated as 2-(2-aminoethyl)-5-hydroxy-*p*-benzoquinone (2a). Confirmation of this follows from the spectral behaviour of 2,4,5-trihydroxytoluene (6) exposed to air in alkaline solution, which corresponds closely to that of compound (1a). Moreover the spectra of 2-hydroxy-5-methyl-*p*-benzoquinone (7) [8] closely corresponded to those of the oxidation product of compound (1a).

The spectrum of 2-hydroxy-5-methyl-*p*-benzoquinone (7) in chloroform [9] resembles that of the same compound in acidified ethanol, rather than its spectra at pH 5.6 or higher. Thus, (7) being a comparatively strong acid (vinylogous carboxylic acid) the 262 and 390 nm peaks in acidic solution are presumably due to the hydroxy-quinone (7), whereas the spectra at pH 5.6 or higher with peaks at 268—270 and 470—495 nm must contain a substantial contribution from the anion (8). A similar situation prevails in the case of compound (2), although here a zwitterionic form is also possible. Cyclisation is presumably faster at pH 5.6 than at higher values, on account of the higher proportion of the hydroxy-quinone present, relative to its anion. Because of the low extinction coefficient associated with the long-wavelength band the presence of the hydroxy-quinone would be difficult to detect except in solutions in which this form is predominant.

U.v. data [$\lambda_{max.}$/nm (ε)]

Compound (1a)	in ethanol (Senoh and Witkop [2])	295 (4 700)			
	in buffer, pH 5.6 (Swan)	295 (4 400)			
Product of oxidation of (1a) in alkaline solution	in ethanol, pH 9 (Senoh and Witkop [2])		278 (11 200)		495 (1 800)
	in ethanol, pH 7.5 (Senoh and Witkop [2])		270 (10 800)		495 (2 250)
	in ethanol, pH 5 (Senoh and Witkop [2])		262 (16 300)	385 (1 100)	
	in alkaline ethanol (Swan)		272 (10 300)		495 (2 200)
	in acidic ethanol (Swan)		262 (14 000)	380	
Product of oxidation of (1a) at pH 5.6 (peroxidase)	at pH 5.6 (Swan)		270 (11 000)		495 (2 200)
	in alkaline solution (Swan)		273 (9 500)		495
	in acidic solution (Swan)		265 (14 000)	385 (900)	
Compound (1b) in buffer, pH 5.6 (Swan)		292			
Product of oxidation of (1b) at pH 5.6 (peroxidase)	at pH 5.6 (Swan)		270		490
	in alkaline solution (Swan)		268 (13 000)		495 (2 000)
	in acidic solution (Swan)		262 (17 000)	390 (700)	
Compound (6) in ethanol (Swan)		292			
Compound (7)	in ethanol (alkaline) (Swan)		268 (12 000)		495 (2 200)
	in buffer, pH 5.6 (Swan)		270 (10 000)		470 (2 700)
	in ethanol (acidic) (Swan)		262 (16 000)	390 (900)	
	in chloroform (Flaig *et al.*[9])		264 (19 000)	382 (660)	
Hydroxy-*p*-benzoquinone	at pH 5.1 (Dawson and Tarpley [10])		260 (8 000)		485 (2 000)
	at pH 5.4 (Mason [11])		260 (4 700)		480—485 (2 100)

The implication of this reinterpretation to the melanins [7] obtained in low yield by autoxidation of compounds (1a and b) seems to be that the main units present in these melanins are probably derived from 5,6-dihydroxyindole, the latter being formed by a process which at pH 8 is relatively inefficient.

EXPERIMENTAL

The horseradish peroxidase was Sigma type II. In a typical experiment a solution of 2,4,5-trihydroxyphen-

[8] J. Thiele and E. Winter, *Annalen*, 1900, **311**, 341.

[9] W. Flaig, J.-C. Salfeld, and E. Baume, *Annalen*, 1958, **618**, 117.

[10] C. R. Dawson and W. B. Tarpley, *Ann. New York Acad. Sci.*, 1963, **100**, 937.

[11] H. S. Mason, *J. Biol. Chem.*, 1949, **181**, 803.

ethylamine hydrochloride (5 mg) in Sörensen's 0.06M-phosphate buffer (pH 5.6; 50 ml) was prepared. To this solution (3 ml) were added a solution of peroxidase (1 mg) in water (0.3 ml) and 0.1M hydrogen peroxide (0.2 ml), in a spectrometer cell. The formation of the quinone was rapid. For measurements in the u.v. range, the solution was diluted 1 : 5 with buffer solution.

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