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Synthesis and Melanogenesis of the DOPA Dimer

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It is generally accepted that, in the presence of tyrosinase, β-(3,4-dihydroxyphenyl)alanine (DOPA) yields melanin *via* cyclization to 5,6-dihydroxyindole.[1] Molecular orbital calculation[2-4] suggests that 5, 6-dihydroxyindole will polymerize oxidatively at the 4 and 7 positions, which correspond to the positions 2 and 5 of DOPA. Recently the presence of uncyclized units in DOPA melanin has been suggested by several groups of investigators,[5-7] This suggests the possibility that DOPA will polymerize at the position 2, 5, or 6 without any cyclization to 5,6-dihydroxyindole. Therefore, it is of interest to examine whether or not the dimer at the position 2, 5, or 6 of DOPA can be an intermediate of the melanogenesis of DOPA. One DOPA dimer, a novel bi-(amino acid), 5,5'-bi-[β-(3,4-dihydroxyphenyl)alanine](V), has thus been synthesized, and its melanogenesis has been examined.[8]

Elbs prepared 2,2'-diacetoxy-5,5'-diformyl-3,3'-dimethoxybiphenyl (I)[9,10] from dehydrodivanillin. The treatment of I and hippuric acid under reflux with fused sodium acetate in acetic anhydride yielded 5,5'-bi-[4-(4-acetoxy-3-methoxybenzylidene)-2-phenyloxazolone](II). Heating the oxazolone II under reflux with fused sodium acetate in methanol gave the unsaturated ester, 5,5'-bi-(methyl 4-acetoxy-α-benzamido-3-methoxycinnamate)(III). The oxazolone II showed IR absorptions at 1790 (oxazolone C=O) and 1766 (enol acetate C=O) cm^{-1}, while the unsaturated ester III showed absorptions at 1766 (enol acetate C=O) and 1720 (unsaturated ester C=O) cm^{-1}.

The catalytic hydrogenation of III with platinum oxide in acetic acid at atmospheric pressure and at room temperature afforded a saturated ester, 5,5'-bi-(methyl 4-acetoxy-α-benzamido-3-methoxydihydrocinnamate)(IV). When IV was hydrolyzed with hydrobromic acid and neutralized with ammonium hydroxide, the DOPA dimer(V) was produced as a colorless powder. Both the UV and the IR spectra, as well as the elemental analyses, favor the structure V for the DOPA dimer. Considering that the UV absorption spectrum of V is similar to that of monomeric DOPA (Fig. 1), it is probable that the two phenyl nuclei of V are almost perpendicular to one another.

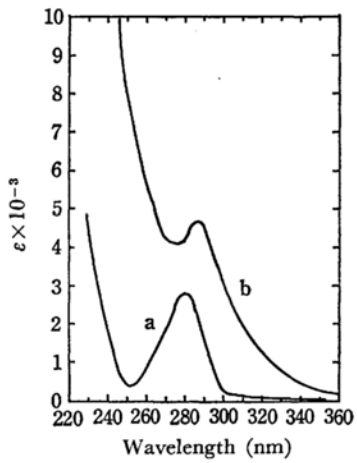
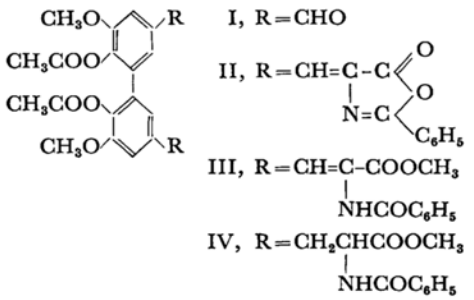


Fig. 1. Ultraviolet absorption spectra of DOPA and the DOPA dimer (V) in water.

a: DOPA b: DOPA dimer

Also, we succeeded in the direct synthesis of the DOPA dimer V from the unsaturated ester III by boiling them with hydroiodic acid and red phosphorus in acetic anhydride, as will be described in the Experimental section.



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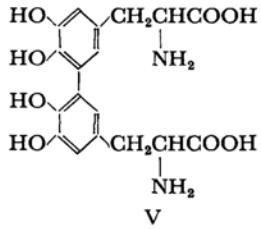

V

In the presence of atmospheric oxygen, it has been shown that DOPA and the DOPA dimer polymerized at about the same rate. In the presence of tyrosinase and atmospheric oxygen, however, the melanogenesis of DOPA was very much accelerated, while that of the DOPA dimer was accelerated only slightly. This suggests that the role of the DOPA dimer as an intermediate of enzymatic melanogenesis is rather improbable, though the possibility of a role in non-enzymatic melanogenesis can not be eliminated.

Experimental

All the melting points are uncorrected.

5,5'-Bi-[4-(4-acetoxy-3-methoxybenzylidene)-2-phenyloxazolone] (II). A mixture of 2,2'-diacetoxy-5,5'-diformyl-3,3'-dimethoxybiphenyl (I) (46 g), hippuric acid (46 g), and fused sodium acetate (24 g) in acetic anhydride (200 m*l*) was heated at 100°C for 2 hr with stirring. Into the reaction mixture ethanol (50 m*l*) was then gradually stirred, after which the mixture was kept overnight in an refrigerator. The solid substance which thus separated was filtered, and washed with a small portion of cold ethanol and then with boiling water. Treatment with chloroform-benzene gave the oxazolone II as a yellow powder (40 g); mp 287°C. Found: C, 66.13; H, 4.23; N, 4.33%. Calcd for $C_{38}H_{28}O_{10}N_2$: C, 67.85; H, 4.20; N, 4.17%. UV $\lambda_{max}^{CHCl_3}$ nm (ε) 380 (75000) and 402 (76000). IR ν^{KBr} cm^{-1} 1790, 1766, 1653 and 1593.

5,5'-Bi-(methyl 4-acetoxy-α-benzamido-3-methoxycinnamate) (III). The oxazolone II (20 g) and fused sodium acetate (9 g) in absolute methanol (250 m*l*) were refluxed for 3 hr, by which time the solid had dissolved completely. Then the solution was condensed to about one third of its initial volume; after cooling, it was poured into ice water (300 m*l*) and kept overnight in a refrigerator. The treatment of the precipitates with benzene gave III as a colorless amorphous powder (17.3 g); mp 141—145°C. Found: C, 65.62; H, 5.09; N, 3.81%. Calcd for $C_{40}H_{36}O_{12}N_2$: C, 65.21, H, 4.93; N, 3.80%. UV: λ_{max}^{EtOH} nm (ε) 225 (46600) and 295 (31000). IR ν^{KBr} cm^{-1} 3320, 1766, 1720, 1655 and 1585.

5,5'-Bi-(methyl 4-acetoxy-α-benzamido-3-methoxydihydrocinnamate) (IV). A mixture of III (2 g) in acetic acid (10 m*l*) and platinum oxide (0.05 g) was stirred with hydrogen at atmospheric pressure and room temperature for 8 hr. After the catalyst had then been filtered off, the solution was treated with active charcoal and evaporated under reduced pressure. There was thus obtained an amorphous powder of IV; mp 65—75°C. It was pure enough for the next step. Found: C, 63.32; H, 5.91; N, 3.53%. Calcd for $C_{40}H_{40}O_{12}N_2$: C, 64.85; H, 5.44; N, 3.78%. UV λ_{max}^{EtOH} nm (ε) 281 (4500). IR ν^{KBr} cm^{-1} 3400, 1770, 1753, 1653 and 1600.

5,5'-Bi-[β-(3,4-dihydroxyphenyl)alanine] (V). A solution of IV (20 g) in hydrobromic acid (47%, 100 m*l*) was refluxed for 3 hr. Then toluene (250 m*l*) was added, a Dean-Stark trap equipped with a stopcock outlet for the removal of water was inserted between the flask and the reflux condenser, and the mixture was refluxed until no more water was collected in the trap (about 8 hr). Then the hydrogen bromide salt of V, which had separated, was filtered off. It was brown and decomposed at 132°C. The salt dissolved in water (40 m*l*) was neutralized with ammonium hydroxide. The solution was evaporated to dryness. The crude DOPA dimer was boiled in water containing a little sulfur dioxide and was filtered while still hot. The filtrate was then allowed to evaporate slowly under reduced pressure to separate the pure DOPA dimer V as a colorless powder (yield 7.1 g); mp above 300°C. The color became brown and changed gradually at about 230°C from brown to black. Found: C, 55.06; H, 5.12; N, 7.08%. Calcd for $C_{18}H_{20}O_8N_2$: C, 55.10; H, 5.14; N, 7.14%. UV $\lambda_{max}^{H_2O}$ nm (ε) 287 (4700). IR ν^{KBr} cm^{-1} 3200 (broad), 1628 and 1598. The elemental analysis was carried out just after the sample had been dried at 100°C/10^{-3}—10^{-4} mmHg for 3 hr.

The DOPA dimer V was also obtained directly from III by the following reductive hydrolysis. A mixture of III (5 g), hydroiodic acid (d=1.7, 35 m*l*) and red phosphorus (3.5 g) was refluxed for 3 hr. After cooling, the unreacted phosphorus was filtered off, the solution was evaporated to dryness under reduced pressure, and water (50 m*l*) was added. The solution was extracted five times with ether (50 m*l*) in order to remove any benzoic acid and iodine, and then the aqueous solution was evaporated to dryness under reduced pressure. The residual solid was dissolved in water (10 m*l*), neutralized with ammonium hydroxide, and purified as has been described above. Yield, 1.2 g.

DOPA Melanin and DOPA Dimer Melanin. *(a) Melanogenesis by Autoxidation.* A solution of DOPA (250 mg) or the DOPA dimer (100 mg) dissolved in, respectively, 100 m*l* or 200 m*l* of a M/30 phosphate buffer at pH 6.9 was stirred in the presence of atmospheric oxygen at 25°C. Both the DOPA and DOPA dimer solutions became faint orange after one hour and then gradually turned dark over an 8-hr period. The relative rates of melanogenesis, based on the absorbancy of the solution (C=10^{-3} mol/*l*) at 550 nm (shoulder), were as follows: DOPA, 1.0 : 1.5 : 6.4 : 14.0, and the DOPA dimer, 1.0 : 1.9 : 6.6 : 13.4, after 1, 4, 8 and 16 hr respectively. Both the DOPA solution and the DOPA dimer solution produced melanin after 24 hr. Each solution was acidified, and black pigments were collected by centrifugation. The melanin thus produced was treated with concentrated hydrochloric acid at room temperature for 4 days, collected by centrifugation, washed with dilute hydrochloric acid, ethanol and finally acetone, and dried. DOPA melanin (yield, 100 mg). Found: C, 58.05; H, 3.14; N, 8.41%. DOPA dimer melanin (yield, 40 mg). Found: C, 55.00; H, 3.73; N, 7.23%.

(b) Enzymatic Melanogenesis. To a solution of DOPA (200 mg) or the DOPA dimer (200 mg) dissolved in, respectively, 100 m*l* or 500 m*l* of a M/30

phosphate buffer at pH 6.9, there was added mushroom tyrosinase (Nutritional Biochemicals Corp., 500 units/mg) (10 mg); a stream of air was then passed through for 24 hr at 35°C. The DOPA solution immediately turned red and then changed to purple within 10 min; black precipitates appeared soon after. The DOPA dimer solution changed gradually from brown to dark over an 8-hr period. After 24 hr each solution was treated as has been described above. DOPA melanin (yield, 150 mg). Found: C, 57.15; H, 3.03; N, 8.61.% DOPA dimer melanin (yield, 110 mg). Found: C, 55.16; H, 3.61; N, 7.71%.