

One-step Synthesis of (2-Amino-2-carboxyethylthio)dopas (Cys-dopas) from Dopa and Cysteine by Hydrogen Peroxide in the Presence of Iron-EDTA Complex

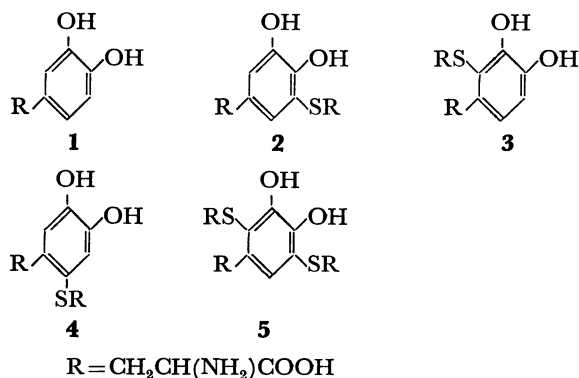
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Synopsis. 5-(2-Amino-2-carboxyethylthio)dopa, 2-(2-amino-2-carboxyethylthio)dopa, and 2,5-bis(2-amino-2-carboxyethylthio)dopa were synthesized from dopa and cysteine by oxidation in a neutral buffer with H_2O_2 in the presence of iron-EDTA complex. H_2O_2 , iron salt, and EDTA were essential for the reaction. Ultimate oxidizing species seem to be hydroxyl and superoxide radicals.

(2-Amino-2-carboxyethylthio)dopas (represented as Cys-dopas in the following; **2**–**5**) arise in melanocytes by the addition of cysteine to dopaquinone formed by tyrosinase oxidation of dopa (3,4-dihydroxyphenylalanine; **1**).¹ They are precursors of pheomelanin, yellow to reddish-brown pigment of feathers and hair. Increased amounts of these catechols, especially **2**, have been found in the urine of melanoma patients.² We have recently shown that **2** is selectively toxic to a variety of human tumor cells *in vitro* and possesses antitumor activity against murine L1210 leukemia and B16 melanoma.³



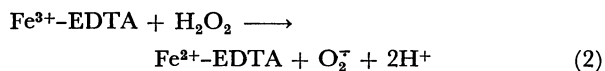
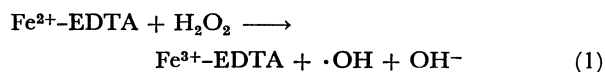
Three methods have been reported for the preparation of Cys-dopas (**2**–**5**). 1) Tyrosinase-catalyzed oxidation of dopa in the presence of cysteine gives Cys-dopas (**2**–**5**) in a high total yield;⁴ however, this method is not suitable for a large-scale preparation. 2) Multi-step chemical synthesis of **2** and **3** involving nucleophilic addition of cysteine to *N*-acetyldopaquinone ethyl ester requires expensive silver oxide as oxidant.^{5,6} 3) Reaction of dopa with cystine in boiling aqueous HBr yields Cys-dopas (**2**–**4**) with the 6-isomer (**4**) being the major product (6–7% yield).⁶ This paper presents a more convenient method for a large-scale preparation of **2**, **3**, and **5** by oxidation of a mixture of dopa and cysteine with H_2O_2 and Fe^{2+} -EDTA complex.

The oxidation, performed on an analytical scale, was followed by TLC on cellulose (in 1-propanol–1 M HCl,† 3:2) and the increase of absorbance at 300 nm where Cys-dopas (**2**–**5**) show strong absorption⁶ while **1** does not. In some instances the products were

also analyzed by column chromatography. Under the standard conditions (Expt 1 in Table 1), **2**, **3**, and **5** were obtained in 13.9, 3.1, and 4.2%, respectively, while **4** was found only in a trace amount (much less than 1%). The relative yields of the Cys-dopa isomers (**2**–**4**) paralleled those obtained with the tyrosinase oxidation,⁴ suggesting that the present reaction also proceeds *via* dopaquinone. Most of the cysteine was consumed as cystine in addition to Cys-dopas. Sum of the yields of **1**–**5** approached nearly 100%, indicating that the reaction did not give pigments or ill-defined products.

Effects of pH, concentrations of H_2O_2 , iron salts, and EDTA, and addition of radical scavengers were summarized in Table 1. The optimum pH of the reaction was around pH 7 (Expts 2–4). Omitting any one of H_2O_2 , iron salts, and EDTA resulted in no formation Cys-dopas (Expts 5, 8, 15). Almost identical yields of Cys-dopas were obtained when $FeCl_3$ was used in place of $FeSO_4$ (Expt 13), while $CuSO_4$ had no catalytic activity (Expt 14). Doubling the amount of H_2O_2 (and cysteine) did not improve the yield of **2**, but it led to a great increase in the yield of **5** (Expts 21, 22).

Reaction of H_2O_2 with Fe^{2+} -EDTA complex produces hydroxyl radical ($\cdot OH$) (Reaction 1) which is a powerful oxidizing agent.⁷ The resulting Fe^{3+} -EDTA complex may be reduced back by H_2O_2 with the formation of superoxide radical ($O_2^{\cdot -}$) (Reaction 2).⁷



Scavengers of hydroxyl radical, D-mannitol and formate,⁸ inhibited the formation of Cys-dopas by 20 and 17%, respectively (Expts 18, 19). Addition of superoxide dismutase also caused a 21% inhibition of the reaction (Expt 20). These results suggest that not only $\cdot OH$ (Reaction 1) but also $O_2^{\cdot -}$ (Reaction 2) participated in the iron-EDTA-catalyzed oxidation of dopa with H_2O_2 . We have recently shown that superoxide radical generated in hypoxanthine-xanthine oxidase system can mediate the formation of Cys-dopas from dopa and cysteine.⁹

When **2** was used in place of **1**, a 6.3% yield of **5** was obtained with a 93.6% recovery of **2**.

Based on these results, the following conditions were established for the preparation of **2**, **3**, and **5** on a preparative scale. The reaction mixture consisted of dopa, cysteine (added in 5 portions), $FeSO_4$, EDTA·2Na, and H_2O_2 (added in 5 portions) at a molar ratio

† 1 M = 1 mol dm⁻³.

TABLE 1. EFFECTS OF pH, CONCENTRATIONS OF H_2O_2 , IRON SALTS, AND EDTA, AND RADICAL SCAVENGERS ON THE FORMATION OF Cys-dopas FROM DOPA AND CYSTEINE^{a)}

Expt No.	Conditions	$A_{300}-A_{340}^b)$	Yield/%			
			1	2	3	5
1	Complete system (pH 7.0) ^{a)}	0.117	77.0	13.9	3.1	4.2
2	pH 5.0	0.036				
3	pH 6.0	0.096				
4	pH 8.0	0.064				
5	H_2O_2 omitted (Fe^{2+} or Fe^{3+})	0.000				
6	H_2O_2 (1 mmol; in 50 portions)	0.054				
7	H_2O_2 (1 mmol; in one portion)	0.091				
8	Fe^{2+} omitted	0.000				
9	Fe^{2+} (0.01 mmol)	0.048				
10	Fe^{2+} (0.05 mmol)	0.083				
11	Fe^{2+} (0.2 mmol) ^{c)}	0.162	68.4	16.5	3.5	4.1
12	Fe^{2+} (1.0 mmol)	0.109				
13	Fe^{3+} (0.1 mmol)	0.127	74.8	13.9	3.0	4.0
14	Cu^{2+} (0.1 mmol) ^{d)}	0.000				
15	EDTA omitted	0.000				
16	EDTA (0.2 mmol)	0.116	76.0	16.0	3.0	2.8
17	EDTA (5.0 mmol)	0.069				
18	L-Mannitol (10 mmol) added	0.079	ND ^{e)}	12.4	2.2	2.3
19	HCOONa (10 mmol) added	0.092	ND	11.7	2.2	3.7
20	SOD (5 mg) added	0.083	82.3	12.3	2.1	2.3
21	Complete system ^{f)}	0.144	70.5	18.6	3.4	7.4
22	Complete system ^{g)}	0.265	54.6	17.9	5.5	13.1

a) L-Dopa (1 mmol), L-cysteine (2 mmol), and $FeSO_4$ (0.1 mmol) were dissolved in water (100 ml), pH being adjusted to 7.0 with Na_2HPO_4 . Then, H_2O_2 (1 mmol) was added in 5 portions every 10 min and the mixture was vigorously stirred at room temperature (23–26 °C). The reaction was terminated 10 min after the final addition of H_2O_2 by adding 1 ml of 6 M HCl. The mixture was applied on a column (1.0 cm $\times$ 7 cm) of Dowex 50W-X2 and the products eluted with 3 M HCl after washing with 0.5 M HCl (100 ml). The products were separated by rechromatography on Dowex 50W-X2 (2.0 cm $\times$ 24 cm; equilibrated with 2 M HCl) as described in Ref. 4. b) Difference in absorbances at 300 and 340 nm. Cys-dopas (2–5) show strong absorptions at 300 nm where 1 shows no absorption. Aliquots of the reaction mixture were diluted 50-fold with 1 M HCl and the UV spectra recorded. c) Some formation of purple pigment was noted. d) The formation of cystine was greatly accelerated. e) Not determined. f) L-Cysteine (2 mmol) was added in 5 portions 1 min prior to the additions of H_2O_2 . g) L-Cysteine (4 mmol) and H_2O_2 (2 mmol) were added in 10 portions every 10 min.

of 1:2:0.1:0.2:1. Starting from 9.9 g of dopa and 12.1 g of cysteine, the reaction gave, in addition to 7.5 g (76%) of dopa recovered, 2.4 g (14%) of **2**, 0.5 g (3%) of **3**, and 0.9 g (4%) of **5** after chromatographic separation and crystallization. Thus, the present method appears to be the most convenient one for the large-scale preparation of **2**, **3**, and **5**.

Udenfriend and co-workers¹⁰⁾ have described a system, consisting of L-ascorbic acid, iron-EDTA complex, and either O_2 or H_2O_2 , and a buffer around neutral pH, which can hydroxylate various aromatic compounds. Our present system offered another example of chemical oxidation using H_2O_2 and iron-EDTA complex which mimics enzymic oxidation.

Experimental

Synthesis of Cys-dopas on a Preparative Scale. A solution of 9.86 g (50 mmol) of L-dopa, 3.72 g (10 mmol) of EDTA·

2Na, and 1.39 g (5 mmol) of $FeSO_4 \cdot 7H_2O$ in 5 l of water was adjusted to pH 7.0 with crystals of $Na_2HPO_4 \cdot 12H_2O$. To the vigorously stirred solution were added at 23 °C 2.42 g (20 mmol) of L-cysteine and 1 min later 860 μ l (10 mmol) of 11.6 M H_2O_2 . The additions of the same amounts of L-cysteine and H_2O_2 were repeated 5 times at 10-min intervals. The reaction was terminated 10 min after the final addition of H_2O_2 by adding 50 ml of 6 M HCl and 5 ml of mercaptoacetic acid. The mixture was evaporated *in vacuo* at 60 °C to ca. 300 ml and stored overnight in a refrigerator. The resulting precipitate of cystine and EDTA was filtered off and the filtrate was applied on a column (3.6 cm $\times$ 25 cm) of Dowex 50W-X2 (H^+ form, 200–400 mesh; equilibrated with water). Elution with 0.5 M HCl gave dopa (frs. 48–144; 20 ml per fraction) which was recovered as 7.48 g (75.9%) of crystals after evaporation and neutralization. Further elution with 3 M HCl afforded a mixture of **2**–**5** (frs. 20–140; 20 ml per fraction). The mixture was rechromatographed on a column (3.6 cm $\times$ 25 cm) of Dowex 50W-X2 (equilibrated and eluted with 3 M HCl) and fractions of 20 ml were collected and analyzed by UV. Fractions 104–128, 132–220, and 229–325 contained **3**, **2**, and **5**, respectively, which were evaporated to give HCl salts of the amino acids. They were crystallized from aqueous 1% $Na_2S_2O_5$ adjusted to pH 6 with CH_3COONa to afford 2420 mg (14.4%) of **2**, 520 mg (2.8%) of **3**, and 932 mg (4.0%) of **5**. These catechols were identified with the authentic samples⁶⁾ by comparison of chromatographic behaviors and UV spectra.

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