clarified that aloenin (**1**) and its metabolites were accumulated in the kidney and the liver to a level of about 60% of the administered sample in 24 h after feeding, and decreased rapidly in the next 24 h.

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18 TLC analyses were performed on a glass plate (0.25 mm thick) coated with silica gel (Merck Si gel 60 G) using 4 different solvent systems [(a) $CHCl_3$:MeOH = 5:1, (b) EtOAc:hexane = 1:9, (c) $CHCl_3$:MeOH = 19:1 and (d) $CHCl_3$:MeOH = 2:3]. HPLC analyses were carried out with 3 different systems [(a) JASCO WC-03-500 column (EtOAc:hexane = 7:3), (b) JASCO SV-02-500 column (H_2O:MeOH = 4:1) and (c) JASCO SV-02-500 column (H_2O:MeOH = 9:1)].

Electrochemical synthesis of cysteinyldopas[1]

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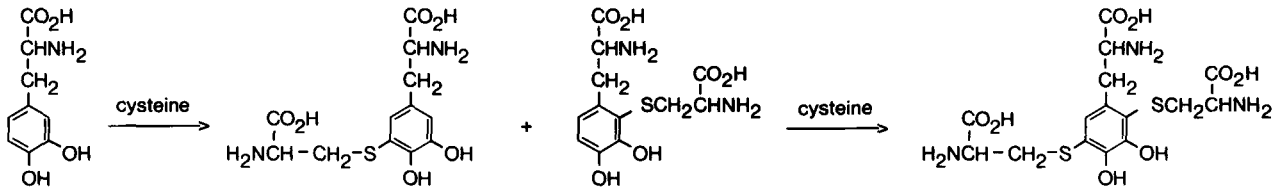
Summary. A fast and inexpensive method for the synthesis of mono- and dicysteinyldopas is described. Dopa is oxidized by electrochemical techniques, and cysteine is then added to give cysteinyldopas.

5-S-Cysteinyldopa is the major metabolite of dopa under the oxidative conditions present in the melanocyte. Quantitative analysis of 5-S-cysteinyldopa in the urine is diagnostically useful in cases of malignant melanoma metastases, and today this analysis is in widespread use[2]. Besides 5-S-cysteinyldopa (**I**), another isomer, 2-S-cysteinyldopa (**II**), and a further oxidation product 2,5-S,S-dicysteinyldopa (**III**) are excreted in large amounts in the urine of melanoma patients. The cysteinyldopa compounds are also the monomers from which the phaeomelanins are formed. The observed correlation between cysteinyldopa and melanoma has initiated extensive research in this field[3], and therefore a great demand for synthetic material has appeared.

2 methods for the synthesis of 5-S-cysteinyldopa have hitherto been published. Prota et al.[4] worked out a six-step synthesis with several sensitive steps. A simple enzymatic synthesis has also been published[5,6], but with the present great demand for 5-S-cysteinyldopa, the cost of the enzyme is unacceptably high. For that reason we have developed a simple and inexpensive electrochemical method which could be applied to synthesis on the gram scale.

Materials and methods. Dopa (2.5 mmoles) was oxidized in 150 ml 1 M sulfuric acid at a 100 cm^2 platinum anode in the presence of 5.0 mmoles cysteine under a nitrogen atmosphere at +10 °C. The anode potential was kept constant at +0.8 V vs saturated calomel electrode (SCE) using a potentiostat built in this laboratory. Due to filming on the electrode this high potential was found to be favourable. The platinum cathode was shielded by a porous glass membrane to prevent reduction of the dopaquinone formed. When a charge of 3 F per mole of dopa had been passed through the cell (about 2 h), the reaction was stopped. The yield of 5-S-cysteinyldopa was about 45% and the purification was performed by ion-exchange chromatography on a Dowex 50W-X4 column in H^+-form (20 × 1.8 cm)[5]. Under the given conditions the yield of 2-S-cysteinyldopa was 12% and of 2,5-S,S-dicysteinyldopa 8%. About 20% of dopa remained unreacted. The isolated compounds were identified by spectroscopic and chromatographic methods and compared with authentic material[5].

Results and discussion. The mechanism proposed for the formation of cysteinyldopas involves the oxidation of dopa to dopaquinone, and then a nucleophilic addition of cysteine gives the cysteinyldopas in their reduced form[4]. In a recent paper preliminary experiments indicate a more complex free radical mechanism, at least when the oxidation of dopa is performed by basic ferricyanide[7]. Besides the addition of cysteine a competing intramolecular addition of the nitrogen group of dopa, giving a 5.6-dihydroxyindoline derivative, takes place. Another intramolecular addition of the cysteine-nitrogen of the oxidized product, 5-S-cysteinyldopaquinone, giving a benzothiazine derivative, makes the protection of the nitrogen groups necessary. The simplest way to inactivate the nucleophilic properties of a



Dopa — **I** 5-S-cysteinyldopa — **II** 2-S-cysteinyldopa — **III** 2,5-S,S-dicysteinyldopa

nitrogen group, but not those of sulfhydryl group, is to run the reaction in strong acid and thus protonate the nitrogen atom.

The oxidation of dopa can be performed by several common oxidizing reagents, but the relatively poor stability of the product complicates matters. The electrochemical oxidation of catecholic compounds has been studied from a mechanistic point of view[8], but its preparative use has not been explored. We studied the cyclic voltamogram of dopa and 5-S-cysteinyldopa at pH 1, and found that both compounds have peaks with E_{pa}-values close to +0.7 V vs saturated calomel electrode on the first anodic sweep. Some excess of cysteine is advantageous for good yields of monocysteinyldopas which indicates a simultaneous oxidation of cysteine although the E_{pa}-value of cysteine is reported to be higher than +0.9 V in 1 M H_2SO_4[9]. Various types of anode electrode material were tested since the oxidation products of dopa are to some degree polymerized on the electrode surface even in strong acid, and we preferred the platinum electrode.

From our enzymatic studies on the oxidation of dopa in the presence of cysteine, we knew that in addition to 5-S-cysteinyldopa, 2-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa are also formed in substantial amounts. As 2,5-S,S-dicysteinyldopa is formed by an oxidation of the monocysteinyldopas followed by the addition of cysteine, an increased amount of 2,5-S,S-dicysteinyldopa is to be expected when an excess amount of charge, Q, is passed through the cell. The amounts of dopa, 5-S-cysteinyldopa, 2-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa were determined by HPLC[10] at different stages of the reaction. After the theoretical amount of charge (2 F per mole dopa) had been passed through the cell, a large amount of dopa (about 30%) was left. 1 F/mole of dopa more increased the yield of 5-S-cysteinyldopa by 10%. Further oxidation lowers the yields of monocysteinyldopas, but is advantageous if 2,5-S,S-dicysteinyldopa is desired.

The preparative electrochemical synthesis of cysteinyldopas is a fast and inexpensive method. It is simple to reproduce and large amounts of either mono- or dicysteinyldopa can easily be prepared.

1 Acknowledgment. This investigation has been supported by grants from the Swedish Cancer Society (project 626-B80-08XB and 626-B80-08P).

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Aminotransferase study of *Neurospora crassa ser-3* versus wild type ST4A

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Summary. The *Neurospora crassa* serine auxotroph *ser-3* and the wild type ST4A were compared with respect to their phosphoserine and serine aminotransferase activities. The results obtained indicate no deficiency of either of these enzymes in the mutant strain.

Three pathways for the biosynthesis of serine are known in microorganisms. The 1st pathway involves the glycolytic intermediate 3-phosphoglycerate which is oxidized to 3-phosphohydroxypyruvate followed by transamination to L-P-serine. In the last step, L-P-serine is hydrolyzed to L-serine[3]. The 2nd pathway is nonphosphorylated. It begins with glycerate formed by the dephosphorylation of 3-phosphoglycerate. The glycerate is then oxidized to hydroxypyruvate and transaminated to serine[4]. The 3rd pathway involves C_1 fragments, glycine and the enzyme transhydroxymethyltransferase (EC 2.1.2.1)[5].

In *Neurospora crassa* both phosphorylated and nonphosphorylated pathways for L-serine synthesis are functioning to some extent in the wild type strain Li-la[6]. Preliminary results obtained by Sojka using the same procedures described herein[7] suggested that the serine auxotroph of *N. crassa ser-3* (47903), was blocked in the phosphorylated pathway at the transamination step. The purpose of this study was to determine whether the parallel reaction in the nonphosphorylated pathway, transamination of hydroxypyruvate, was also affected, indicating that a common transaminase is used in the 2 pathways, or whether the defect affected a specific phosphoserine transaminase.

Materials and methods. Stock cultures of *N. crassa* wild type ST4A were maintained on Vogel's minimal medium N slopes[8] containing 2% (w/v) sucrose and 2% (w/v) agar; *ser-3* cultures were supplemented with 10 mM L-serine. To obtain a dense conidial suspension, 50 ml of sterile Westergaard and Mitchell medium[9] was added to 7-day-old cultures grown on 20 ml of agarized medium in 125-ml Erlenmeyer flasks. The conidial suspension was used to inoculate 700 ml of the same liquid medium contained in a 2-l Fernbach flask and the resultant culture was incubated with shaking for 48 h in a 32 °C waterbath. All remaining procedures were carried out in a 4 °C cold room. The mycelial pad was harvested, rinsed, weighed, and ground in

Assay conditions

	A	B	C
2-keto [5-^{14}C] Glutarate	0.046 µmole/ml	1.0	1.0
KH_2PO_4/KOH pH 8.0	1.0 M	0.5	0.5
Pyridoxal phosphate	10 µg/ml	0.2	0.2
L-Serine	1 mg/ml	0.0	2.0
O-L-Phosphoserine	20 µmoles/ml	2.0	0.0
Protein extract	0.4 mg/ml	1.0	1.0
Water	-	0.3	0.3

A, Concentration of solution used; B, volume used for L-phosphoserine aminotransferase assay; C, volume used for L-serine aminotransferase assay; total volume, 5.0 ml. Temperature of incubation of the reaction mixture, 32 °C; specific activity of 2-keto[5-^{14}C] glutarate, 10.3 Ci/mole.